BIOCHEMICAL AND MOLECULAR MECHANISMS OF RESISTANCE TO *Helicoverpa armigera* (Hubner) IN WILD RELATIVES OF CHICKPEA

GOLLA SIVA KUMAR

M.Sc. (Ag.)

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(ENTOMOLOGY)

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BY

GOLLA SIVA KUMAR

M.Sc. (Ag.)

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CHAIRPERSON: Dr. P. RAJASEKHAR

DEPARTMENT OF ENTOMOLOGY
SRI VENKATESWARA AGRICULTURAL COLLEGE, TIRUPATI
ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY
GUNTUR – 522 509, A.P.

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DECLARATION

I, **Mr. GOLLA SIVA KUMAR,** hereby declare that the thesis entitled "**BIOCHEMICAL AND MOLECULAR MECHANISMS OF RESISTANCE TO *Helicoverpa armigera* (Hubner) IN WILD RELATIVES OF CHICKPEA"** submitted to the **Acharya N.G. Ranga Agricultural University**, for the degree of **Doctor of Philosophy in Agriculture** is the result of original research work done by me. I also declare that no material contained in this thesis has been published earlier in any manner.

Place: **(GOLLA SIVA KUMAR)**

Date : **ID. No: TAD/2013-09**

CERTIFICATE

Mr. GOLLA SIVA KUMAR has satisfactorily prosecuted the course of research and that thesis entitled **"BIOCHEMICAL AND MOLECULAR MECHANISMS OF RESISTANCE TO *Helicoverpa armigera* (Hubner) IN WILD RELATIVES OF CHICKPEA"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any university.

Place :

Date :

Dr. P. RAJASEKHAR
(Chairperson)
Principal Scientist (Entomology) & Head
Agricultural Research Station
Nellore - 524 004, A.P.

CERTIFICATE

This is to certify that the thesis entitled **"BIOCHEMICAL AND MOLECULAR MECHANISMS OF RESISTANCE TO *Helicoverpa armigera* (Hubner) IN WILD RELATIVES OF CHICKPEA"** submitted in partial fulfilment of the requirements for the degree of **'Doctor of Philosophy in Agriculture'** to the Acharya N.G. Ranga Agricultural University, Guntur is a record of the bonafide original research work carried out by **Mr. GOLLA SIVA KUMAR** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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Chairperson	:	**Dr. P. RAJASEKHAR** Principal Scientist (Entomology) & Head Agricultural Research Station Nellore - 524 004, A.P.	_____
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Member	:	**Dr. B. RAVINDRA REDDY** Assistant Professor Department of Statistics and Maths S.V. Agricultural College Tirupati - 517 502, A.P.	_____
External-Examiner of final viva-voce	:	**Dr. D.S. RAJAVEL** Professor (Entomology) Tamil Nadu Agricultural University Regional Research Station, Kovilangulam, Aruppukottai - 626 107, Tamil Nadu	_____

Date of final viva-voce:

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Siva Kumar Golla...

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
i.e.	:	That is
viz.,	:	Namely
°C	:	Degree centigrade
cm	:	Centimetre
mm	:	Millimetre
m	:	Metre
m^2	:	Squre meter
et al.,	:	And others
g	:	Gram
mg	:	Milligram
ml	:	Milli litre
ml l^{-1}	:	Millilitre per litre
mg g^{-1}	:	Milligram per gram
kg ha^{-1}	:	Kilogram per hectare
ppm	:	Parts per million
HPLC	:	High Performance Liquid Chromatography
GC-MS	:	Gas Chromatography Mass Spectrometry
BA*p*NA	:	Nα-benzoyl-L-arg-p-nitroanilide
SAAPF*p*NA	:	Succinyl-ala-ala-pro-phe-pnitroanilide
L*p*NA	:	Leucine-p-nitronilide
SDS-PAGE	:	Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis
SSR	:	Simple Sequence repeats
NS	:	Non Significant
SE	:	Standard Error of mean
LSD	:	Least Significant Difference
ANOVA	:	Analysis of Variance
DMRT	:	Duncan's Multiple Range Test
r	:	Correlation coefficient
M	:	Molarity
N	:	Normality
pH	:	Potential of hydrogen ion concentration
rpm	:	Revolutions per minute
nm	:	Nano metres

Name of the Author	:	**GOLLA SIVA KUMAR**
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ABSTRACT

The present studies on "Biochemical and molecular mechanisms of resistance to *Helicoverpa armigera* (Hubner) in wild relatives of chickpea" were carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, during 2014-16. A total of 20 accessions (15 wild relatives and five varieties of cultivated chickpea) were used to evaluate the mechanism of resistance to *H. armigera*. Under field conditions, during post-rainy seasons 2014-15 and 2015-16, all genotypes of wild relatives of chickpea recorded less number of *H. armigera* larvae, low visual leaf damage rating and per cent pod damage compared to cultivated chickpea.

The genotypes IG 70012, PI 599046, IG 70022, PI 599066, IG 70006, IG 70018 (*Cicer bijugum*), ICC 506EB, ICCL 86111 (resistant checks), IG 72933, IG 72953 (*C. reticulatum*) IG 69979 (*C. cuneatum*) and IG 599076 (*C. chrossanicum*) showed high levels of antixenosis for oviposition of *H. armigera* under multi-, dual- and no-choice cage conditions.

Studies on detached leaf assay revealed that the genotypes IG 70012, IG 70022, IG 70018, IG 70006, PI 599046, PI 599066 (*C. bijugum*), IG 69979 (*C. cuneatum*), PI 568217, PI 599077 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*) showed less damage rating and low larval weights compared to susceptible checks. Larval survival was greater on the wild relatives than on the cultivated chickpea. Detached pod assay studies revealed that all wild relatives of chickpea exhibited less damage rating, lower per cent pod damage and lower percentage of weight gained by third-instar larva compared to cultivated chickpea.

Survival and development of *H. armigera* on artificial diet impregnated with lyophilized leaf powders revealed that all wild relatives of chickpea genotypes showed high levels of antibiosis to *H. armigera* compared to cultivated chickpea in terms of lower larval survival, per cent pupation and adult emergence, decreased larval and pupal weight, prolonged larval and pupal developmental periods and reduced fecundity.

Among morphological characters, glandular and non-glandular trichomes showed negative association with oviposition under multi-choice and no-choice conditions. Glandular trichomes had significant negative association with damage rating, whereas non-glandular trichomes had significant positive association with damage rating and larval weight but negative association with larval survival in detached leaf assay. Pod wall thickness showed significant negative association with damage rating and per cent pod damage in detached pod assay.

HPLC finger prints of leaf organic acids revealed a negative association of oxalic acid with oviposition, while malic acid showed positive and significant association with oviposition under multi- and no-choice conditions. Oxalic acid and malic acid had significant and negative correlation with larval survival in detached leaf assay, which indicates that higher amounts of these acids in cultivated chickpea resulted in reduced larval survival compared to wild relatives.

The flavonoid compounds *viz*., chlorogenic acid, ferulic acid, naringin, 3, 4-dihydroxy flavones, quercetin, naringenin, genestein, formononetin and biochanin A identified through HPLC finger prints exhibited negative effects on survival and development of *H. armigera* reared on artificial diet impregnated with lyophilized leaf powders. Proteins and phenols showed negative effect, while tannins and total soluble sugars showed positive effect on survival and development of *H. armigera* reared on artificial diet with lyophilized leaf powders of wild relatives of chickpea.

Zymogram analysis revealed presence of 3 to 7 trypsin inhibitor (TI) isoforms in all 20 genotypes. The genotypes, IG 70018, IG 70012, IG 70006, IG 70022, PI 599066, IG 72933, IG 72953 and IG 69979 showed higher inhibitory activity of *H. armigera* gut (HG) proteases, while genotypes PI 510663, PI 599109, PI 568217 and ICCW 17148 showed low inhibitory activity under *in vitro* conditions. Studies on hemagglutination of lectins revealed that wild relatives of chickpea genotypes showed more agglutination even at less concentration. Schiff's base staining of lectins revealed that only one isoform with a molecular weight of 29 kDa was observed in wild relatives of chickpea.

GC-MS profile peaks of leaf surface chemicals identified with hexane extracts showed 56 peaks in all genotypes. Correlation studies with detached leaf assay and oviposition preference indicated presence of feeding and oviposition repellents as well as phagostimulants and oviposition attractants. A total of 107 GC-MS profile peaks were identified with methanol extracts. Correlation studies indicated that methanol extracts had higher amount of phagostimulants and oviposition repellents than antifeedants and oviposition attractants.

The 26 SSR markers used for assessing genetic diversity of wild relatives of chickpea detected a total of 186 alleles with an average of 7.15 alleles per marker. PIC values varied from 0.21 (CaM2064) to 0.89 (CaM0958, ICCM0249 and TAA58). Gene diversity varied from 0.24 (CaM2064) to 0.90 (CaM0958, ICCM0249 and TAA58). The average observed heterozygosity was 0.20.

The dendrogram based on UPGMA showed that cultivated chickpea showed a closer genetic relation with the *C. reticulatum*, while, the species *C. microphyllum*, *C. judaicum*, *C. bijugum* and *C. pinnatifidum* were placed in other cluster. The other species *C. cuneatum* was placed in separate cluster indicated that it is distantly related to species in other two clusters.

Chapter ~ I

Introduction

Chapter I

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop after dry beans and peas produced in the world. Average annual chickpea area in the world is 14.8 million ha with a production of 14.23 million tonnes, of which Asia accounts for 88 per cent of area and 84 per cent of production (FAO STAT, 2014). In India, it is cultivated on 6.67 million hectares with an annual production of 5.3 million tonnes with an average yield of 844 kg ha^{-1} (CMIE, 2011). Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh together account for 91% of production and 90% of area under chickpea.

Chickpea is an important source of protein for millions of people in developing countries and has the highest nutritional compositions of any dry edible grain legume. In addition to high protein content, chickpea is also rich in fiber and minerals, and its lipid fraction is high in unsaturated fatty acids (Williams and Singh, 1987). Chickpea contains higher amounts of carotenoids such as β-carotene than genetically engineered "golden rice" (Abbo *et al*., 2005). Chickpea can fix up to 140 kg nitrogen ha^{-1} and meet up to 80% of its nitrogen requirement through symbiotic nitrogen fixation (Saraf *et al*., 1998). Substantial amounts of nitrogen remain in the soil following the cultivation of chickpea crop, which is beneficial to subsequent crops. Chickpea crop residues add much needed organic matter for the maintenance of soil health, long term fertility, and sustainability of the ecosystems. The productivity of chickpea is 0.8 t ha^{-1} and it continues to be far below the potential yield of over 5.0 t ha^{-1} and the realizable yield of 2.5 t ha^{-1} on the farmer's fields.

Pod borer, *Helicoverpa armigera* (Hubner), beet army worm, *Spodoptera exigua* (Hubner), *Fusarium* wilt, root rots, *Ascochyta* blight, *Botrytis* gray mold and drought are some of the major constraints to increase the productivity of chickpea (Chen *et al*., 2011). Nearly 60 insect species are known to feed on chickpea, of which black cut worm, *Agrotis ipsilon* (Hfn.), leafminer, *Liriomyza cicerina* (Rondani), aphid, *Aphis craccivora* Koch, pod borer, *H. armigera*, leaf eating caterpillar, *S. exigua*, bruchid, *Callosobruchus chinensis* L. and termite, *Microtermes obesi* (Holm.) are the major pests worldwide (Reed *et al*., 1987), of which the legume pod borer, *H. armigera* is the most important biotic constraint in

chickpea production. It causes an estimated loss of US$325 million on chickpea, and over US$2 billion on different crops in the semi-arid tropics, despite application of insecticides costing over US$500 million annually (Sharma, 2005). The average losses due to pod borer damage on chickpea vary from 25 to 30 per cent, and under certain situations, there may be a complete loss of the crop inspite of several rounds of insecticidal applications (Sarwar *et al*., 2009). The larvae feed on seedlings, leaves, flowers and pods.

The development of crop cultivars resistant or tolerant to *H. armigera* has a major potential for use in integrated pest management. More than 14000 chickpea germplasm accessions have been screened for resistance to *H. armigera* at ICRISAT, Patancheru, India under field conditions (Lateef and Sachan, 1990). Several germplasm accessions (ICC 506 EB, ICC 10667, ICC 10619, ICC 4935, ICC 10243, ICCV 95992 and ICC 80817) with resistance to *H. armigera* have been identified, and varieties with moderate levels of resistance have been released for cultivation (Lateef, 1985 and Narayanamma *et al*., 2007). However, only moderate levels of resistance are available in the cultivated germplasm of chickpea and thus there is a need to identify wild relatives as a source of resistance so as to transfer to cultivated chickpea and increase the levels of resistance.

Some of wild relatives of chickpea *viz*., *Cicer bijugum*, *C. pinnatifidum*, *C. judaicum*, and *C. reticulatum* have shown very high levels of resistance to *H. armigera* (Sharma *et al*., 2004, 2005a, b, 2006). Progenies obtained from *C. echinospermum* and *C. reticulatum* showed consistently low levels of damage (10% or less) due to pod borers (Mallikarjuna *et al*., 2007). These wild relatives of chickpea may have different mechanisms of resistance than the cultivated types, which could be used in crop improvement to diversify the basis of resistance to the pest.

Plant-insect interactions are dependent on nutritional levels of plant tissues during different growth forms of the insect and chemical and mechanical defenses of the plant (Cates, 1980). Trichome density and trichome exudates play an important role in the ovipositional behavior and host selection process of insect herbivores (Bernays and Champman, 1994). Chickpea trichome exudates contain acidic chemicals such as malic acid, oxalic acid and succinic acid. Oxalic acid has an antibiotic effect on the larvae of pod borer, *H. armigera*, which results in reduced

pod damage (Yoshida *et al*., 1995). A dense mat of non-glandular trichomes prevents the small larvae from feeding on the plant (Peter and Shanower, 1998).

The chemical basis of resistance to *H. armigera* has been attributed to acid exudates which can be used as marker for resistance, though the quantity of exudates and resistance levels vary across locations with environment (Rembold, 1981 and Rembold and Winter, 1982). Malic and oxalic acids in cultivated chickpea exert antifeedant and antibiotic effects on *H. armigera* (Narayanamma *et al*., 2013). The wild relatives of chickpea also contain flavonoids and isoflavonoids. The levels of total extractable flavonoid and isoflavonoid contents exhibit different levels of resistance and susceptibility to insect pest. These flavonoids and isoflavonoids in the wild relatives of chickpea have shown antibiosis effect towards *H. armigera* (Simmonds and Stevenson, 2001 and Steveson *et al*., 2005). Protease inhibitors and lectins are also important defensive mechanisms in grain legumes (Shukla *et al*., 2005). Any interference in the activity of digestive enzymes by enzyme inhibitors of host plant can result in poor nutrient utilization and developmental retardation (Jongsma and Bolter, 1997 and Gatehouse and Gatehouse, 1999). There is a wide variation in protease inhibitory activity in wild relatives of chickpea compared to cultivated chickpea (Harsulkar *et al*., 1999). Chickpea and snowdrop lectins have shown marked antibiotic effects on *H. armigera* by reducing survival and development (Shukla *et al*., 2005). A basic understanding of the interactions between the secondary metabolites in wild relatives of chickpea and *H. armigera* is important to develop appropriate strategies to develop chickpea cultivars with high levels of resistance to *H. armigera*.

Modern plant breeding and agricultural systems have narrowed the base for the genetic diversity of cultivated chickpea (Robertson *et al*., 1997). Therefore, it is necessary to explore wild relatives having varied genetic diversity. The effectiveness of improvement in any crop depends upon the extent and nature of phenotypic and genotypic variation present in different traits of the broader population. Genetic diversity among the parents is a prerequisite for ensuring the chance of improved segregate selection for various characters (Dwevedi and Gaibriyal, 2009). Criteria for the assessment of genetic variability can include morphological traits (Upadhaya *et al*., 2007) and molecular markers (Sharma *et al*., 1995). DNA molecular markers have more advantages than phenotypic markers, since they are free of environmental

influences when determining genetic variability (Virk *et al*., 1995 and Serret *et al*., 1997). Molecular markers have proved as valuable tools for the characterization and assessment of genetic variability within and between species and populations (Talebi *et al*., 2008). Hence, the diversity available in different traits of the wild *Cicer* is very valuable (Heslop-Harrison, 2002).

Most of the wild relatives of chickpea showing resistance to *H. armigera* have not yet been characterized for different mechanisms of resistance such as oviposition preference, antifeedant and antibiosis effect on larvae. Therefore, measurement of different resistance mechanisms in wild relatives of chickpea against *H. armigera* is highly important, where these characters could be used as source for development of cultivars with high and stable resistance to this pest. Therefore, the present studies have been planned with the following objectives.

OBJECTIVES OF INVESTIGATION:

1. Identify wild relatives of chickpea with diverse mechanisms (antixenosis and antibiosis) of resistance to *H. armigera*.
2. Identify morphological, physiological and biochemical components associated with resistance to *H. armigera*.
3. Assess genetic diversity of wild relatives of chickpea exhibiting resistance to *H. armigera* by using biological, morphological, biochemical and molecular markers.

Chapter ~ II

Review of Literature

Chapter II

REVIEW OF LITERATURE

Chickpea (*Cicer arietinum* L.) is an important grain legume of the semi-arid tropics and one of the major components of human diet. It is grown in about 50 countries with an estimated 95 per cent of the cultivated area in the developing countries. Chickpea production is particularly important in the countries of South Asia and accounts for about 71 per cent of global area devoted to the crop.

Chickpea yields remained stagnant for the past two to three decades due to various biotic and abiotic factors such as, pod borers, *Helicoverpa armigera*, *Spodoptera exigua*, *Fusarium* wilt, *Aschochyta* blight, *Botrytis* gray mold, drought and low temperatures of which *H. armigera* (Hubner) is the key pest. The damage caused by this pest on chickpea ranged upto 84.4% with an average of 7% under different farming systems (Lateef, 1992). It has long been recognized that plant resistance perhaps is the most effective and economic option for pest management, particularly under subsistence farming conditions in the semi-arid tropics. The levels of resistance in the cultivated chickpea germplasm have been found to be low to moderate (Lateef, 1985., Lateef and Sachan, 1990 and Sharma, 2001). Wild relatives of crops are useful source of genes for resistance to biotic and abiotic stress factors (Croser *et al*., 2003). Therefore, there is a potential for exploiting the wild relatives of chickpea with different mechanisms as source of resistance to increase the level and diversify the basis of resistance to *H. armigera* in cultivated germplasm.

2.1 ORIGIN AND TAXONOMY OF CHICKPEA

The *Cicer* genus belongs to the family Leguminoseae, subfamily Papilionaceae and tribe Cicereae. It encompasses 9 annual and 34 perennial species. Most of these species are found in West Asia and North Africa covering Turkey in the North to Ethiopia in the South and Pakistan in the East to Morocco in the West. Of the nine annual species, *C. arietinum* is the only cultivated species. The eight other annual species of chickpea are wild which includes, *C. reticulatum, C. echinospermum, C. pinnatifidum, C. judaicum, C. bijugum, C. cuneatum, C. chorassanicum* and *C. yamashitae*. Van der Maesen (1987) classified the *Cicer* species into four sections based on their morphological characteristics, life cycle and geographical

distribution. Eight annual species except *C. chorassanicum* were placed in section Monocicer, whereas *C. chorassanicum* and perennial species *C. incisum* were placed in section Chamaecicer, 23 perennial species in section Polycicer and seven woody perennial species in section Acanthocicer. It is considered to be one of the 'founder crops' of the 'Neolithic revolution' in the near East around 10,000 years ago (Lev-Yadun *et al*., 2000 and Zohary and Hopf, 2000). Earlier, cultivated chickpea (*C. arietinum*) was considered to have originated from the Southern Caucasus and Northern Persia (Iran) regions (Van der Maesen, 1972). However, with the discovery of the wild progenitor *C. reticulatum* by Ladizinsky (1975), present day South-Eastern Turkey is considered as the most likely origin of cultivated chickpea (Ladizinsky, 1995). This is consistent with the very limited distribution of the *C. reticulatum* wild progenitor species and of the closely related *C. echinospermum* in South-Eastern Turkey (Ladizinsky, 1975 and Berger *et al*., 2003).

2.2 GENE POOLS (GP) OF CHICKPEA

Based on the concepts of primary, secondary and tertiary gene pools, crops have improved consistency and comparability at both inter and intraspecific levels. Harlan and De Wet (1971) included all the variants of the cultigen in the primary gene pool together with those wild and weedy taxa which cross freely and produce fertile hybrids with the cultigen. The secondary gene pool included those species which can be crossed with the cultigen often with some difficulty, but the resulting hybrids are partially fertile. The tertiary gene pool includes species which are cross-incompatible with the crop, or whose hybrids with the crop are totally sterile. Using the Harlan and De Wet (1971) gene pool concept, the chickpea gene pool may be characterized as follows:

Cultigen= GP 1a	GP 1b	GP 2	GP 3
Cicer arietinum	*C. reticulatum* *C. echinospermum*	*C. bijugum* *C. judaicum* *C. pinnatifidum*	Other *Cicer* species

Using the classification proposed by Harlan and De Wet (1971), a modification of the classification is proposed for chickpea gene pools based on its crossability of wild relatives with cultigens (Mallikarjuna *et al*., 2011). The primary gene pool consists of cultivated species and landraces. The secondary gene pool consists of the progenitor species, *C. reticulatum* and *C. echinospermum*, a species

that is crossable with *C. arietinum* but with reduced fertility of the resulting hybrids and progenies. The tertiary gene pool consists of all other annuals and perennial *Cicer* that are not crossable with cultivated *C. arietinum*. The species in secondary and tertiary gene pools could be effectively exploited for genetic enhancement of chickpea by overcoming pre and post fertilization barriers or through genetic transformation route.

2.3 INCIDENCE OF POD BORER, *H. armigera* IN CHICKPEA

The Pod borer, *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) is a polyphagous and devastating pest of many important crop plants and responsible for heavy economic losses to agriculture. It is a highly adaptive pest and infests more than 300 plant species throughout the world (Rajapakse and Walter, 2007). In chickpea, it is the key biotic constraint which at times causes 90 to 95% damage, under severe infestation losses may leads upto 100% inspite of several rounds of insecticidal applications (Sarwar *et al*., 2009). The knowledge on the seasonal abundance of *H. armigera* will certainly be helpful in formulating the pest management strategies.

Rao *et al*. (2001) observed pod borer damage on chickpea at the flowering stage *i.e.* 38 days after sowing (two larvae/10 plants) whereas the peak incidence was recorded at 87 days old crop (20 larvae/10 plants) during the month of January.

The later sown crop of chickpea suffered most from the *H armigera* and yielded less than earlier sown crop. There was higher incidence of *H. armigera* in the crop sown at 10th November and later date, maximum being recorded on crop sown at 20th November. The grain yield was also decreased as the sowing was delayed, indicating direct correlation with pest incidence (Singh *et al*., 2002).

Seasonal incidence of *H. armigera* on cv. ICCC 37 revealed that oviposition was highest during the second fortnight of December. The pest incidence started at 15 DAS (7.30 larvae/20 plants), then gradually increased until first peak at 29 DAS (60.30 larvae/20 plants), second peak at 57 DAS (85.50 larvae/20 plants) and the third peak at 85 DAS (74.30 larvae/20 plants) (Suganthy *et al*., 2003).

Altaf *et al*. (2008) reported that, in chickpea highest pod damage of 22.82 and 27.36% was observed in October and the lowest damage of 11.76 and 20.16% was observed in November during 2003-04 and 2004-05, respectively.

Hossain *et al*. (2008a) observed that the incidence of pod borer, *H. armigera* started in 2nd to 4th week of January and further reported that both the early (October 15 to November 01) and late sown (December and onwards) crops exhibited higher pod borer damage and produced lower yields. But mid sown (November 08 to 30) crops recorded less pod borer damage and produced higher yield.

The incidence of the *H. armigera* in chickpea commenced from first fortnight of February with mean larval population of 0.05 per plant. The larval populations started increasing and reached its maximum of 12.97 mean larvae per plant during 4th week of March (12th SW). The population was in significant positive correlation with both minimum and maximum temperature (r= 0.71 and 0.82, respectively) whereas, it had negative correlation (r= -0.66) with morning and afternoon relative humidity. The rainfall, wind velocity and the sunshine hours showed positive correlation with larval population but it was nonsignificant (Reddy *et al*., 2009).

Yadav and Jat (2009) reported that, the infestation of *H. armigera* on chickpea started in the second fortnight of November and reached its peak by the end of February. The larval population of the pest occurred throughout the crop growth period and maximum population was recorded at pod formation and grain developmental stages.

Carryover of *H. armigera* on different crops revealed that the activity first appeared in second fortnight of July on sunflower and cotton and remained active up to end of the September. Second peak activity of *H. armigera* was observed on pigeonpea from last week of September to January month during this period it migrated to chickpea and sorghum crops during second week of October upto February. Third peak of *H. armigera* was noticed on chilli and tomato crops during March to May (Jadhav *et al*., 2010).

Larval population of *H. armigera* in different chickpea genotypes ranged from 0.33 to 4.33 per meter row from 1st week of March to 3rd week of April, whereas the pod damage varied from 7.40 to 14.20% (Nadeem *et al*., 2010).

Zafar *et al*. (2013) observed that *H. armigera* population was built up in sunflower crop from April 12 to April 27 in terms of egg count. The larval population increased continuously from April 12 to May 01 and a tremendous decrease was observed thereafter. Maximum temperature showed significant

positive correlation (r = 0.51) with the egg counts, whereas relative humidity and rainfall had significant negative correlation (r = -0.52 and -0.47, respectively).

The incidence of *H. armigera* started in the second week of December with a mean population of 0.90 and 0.60 larvae/plant and touched its peak with a mean of 1.80 and 1.90 larvae/plant in the 7th SW and 6th SW during *rabi*, 2012-13 and 2013-14, respectively. The *H. armigera* population exhibited significant positive correlation with mean temperature and negative but nonsignificant with mean relative humidity (Yadav *et al*., 2016).

2.4 SCREENING OF CHICKPEA GENOTYPES FOR RESISTANCE TO *H. armigera*

Larval population was high on Phule G 5 (26.33 larvae/5 plants) and lowest on Chaffa (14.32 larvae/5 plants), pod damage was lower (9.55%) on chaffa, whereas PG 81-1-1 showed higher pod damage (18.49%), ICCC 4 gave more grain yield (1250 kg ha^{-1}) as compared to Chaffa (722 kg ha^{-1}) (Bhatt and Patel, 2001).

Singh *et al*. (2002) reported that GL-769 showed the highest pod infestation (13.08 and 12.70%) while PBG-1 showed the highest grain yield (1403.27 and 1414.27 kg ha^{-1}) during the 1999 and 2000 seasons, respectively.

Rajput *et al*. (2003) reported that data on larval population, percentage damage and yield components was highly variable, showing the percentage larval attack severity from 1.00 to 50.00, pod damage from 8.5 to 90% and 23.33 to 1920 g grain yield of the sampling unit. Data revealed that the genotype C-727 was relatively resistant against *H. armigera* infestation in chickpea.

Maurya and Ujagir (2004) evaluated chickpea germplasm against pod borer, *H. armigera* and reported that oviposition ranged from 3.00 to 27.60 eggs/10 plants and larval count ranged from 25.00 to 71.30 larvae/10 plants. The cultivar ICC 10464 showed highest pod damage (87.50%), and the entries ICC 11180, ICC 2171 and ICC 11175 produced high seed yields (38.90, 38.90 and 33.30 kg ha^{-1}, respectively). Whereas, Deepak and Ujagir (2005) observed that the ICCV 93929, ICCV 96029, ICCV 96030 and ICCV 2 were resistant and ICCV 10, ICCV 97115, ICCV 97119 and ICC 16381 were tolerant to *H. armigera*.

Gowda and Sharnabasappa (2005) screened 20 chickpea genotypes against *H. armigera* in 2000-01 and 2001-02 and reported that the genotype, BGD-237

recorded the lowest pod damage of 11.86 and 10.84%, respectively with a rating of five. The genotypes JAKI-5226 and BK-36 showed high pod damage, with a pest susceptibility rating of seven. Pooled data indicated that BGD-237 had the lowest pod damage, while JAKI-5226 recorded highest damage.

Twenty five chickpea genotypes were screened for resistance to *H. armigera* under field conditions. The mean pod damage ranged from 20.37% in ICCL 87311 to 34.27% in ICC 12492. ICCL 87311 and ICCV 2 suffered damage ratings of five, and whereas ICCL 79033, ICCL 80129, ICCL 12746, ICC 12479, ICC 12480, ICCL 87314, IG 362 and Annigeri had a damage rating of six, and suffered less damage than ICC 506EB. ICC 9854 and ICC 12490 had grain yield of 1391 and 1483 kg ha^{-1} respectively, and were superior to ICC 506EB indicating tolerance to *H. armigera* damage (Sanap and Jamadagni, 2005).

A total of 184 chickpea genotypes were evaluated for resistance to pod borer and a large variation was observed in pod damage (30.87 to 70.65%). Forty five genotypes were moderately resistant with infestation ranging from 34.05 to 51.65%, except IPC 96-3 and FG 1235 with mean infestation of 30.85 and 30.95%, respectively and were resistant (Kaur *et al*., 2005).

Wakil *et al*. (2005) reported that among the 27 genotypes of chickpea, none of the genotypes showed complete resistance to *H. armigera*. The lowest pod infestation was recorded in CM-4068/97 (12.71%) and the maximum infestation was 38.83% (cv.93127). Similarly, the number of larvae per plant ranged from 1.27 (Paidar-91) to 5.40 (C-44).

Chandraker *et al*. (2006) evaluated several chickpea cultivars for resistance to gram pod borer, *H. armigera* and reported that the genotype BGD-74 had the minimum pod damage (6.64%) and highest yield of 1433 kg ha^{-1}.

The maximum larval population of *H. armigera* was found in BG 273 (38.19 larvae/5 plants) and the minimum (15.59 larvae/5 plants) was observed in DCP 92-3. The maximum pod damage was seen in JG 11 (20.60%) whereas, the highest grain yield (23.00 q ha^{-1}) was produced by BG 256 in spite of higher larval population i.e. 30.0 larvae/5 plants and hence BG 256 was classified to be tolerant (Singh and Yadav, 2006).

Hossain *et al*. (2007) screened 14 chickpea lines and six released varieties and reported that the genotypes ICCV 95138, ICCV 96020, ICCV 95939, ICCL 87315 and ICCV 98936 were the most promising lines against *H. armigera* with respect to lowest pod damage (7.94%) and pest susceptibility rating (4.00). Pod damage was highest (33.60%) in ICC 4918 and highest yield (1886.00 kg ha^{-1}) was recorded in ICCV 95138.

Among 25 chickpea genotypes, ICC 16374 (5.05%) and ICCL 7903 (5.90%) recorded less pod damage compared to the resistant genotype, ICC 506EB (6.35%). Pest susceptibility rating scale (PSRS) of five was recorded on the genotype ICC 16374. The remaining genotypes were highly susceptible compared to ICC 506EB and PSRS ranged from 6 and 9. The mean grain yield ranged from 1024 kg ha^{-1} in RIL 115 to 2548 kg ha^{-1} in ICCC 37 (Patil *et al*., 2007).

Among 207 chickpea genotypes evaluated for resistance to *H. armigera*, the genotypes ICC 1882 and ICCC 1422 gave the best performance with 24.33 and 25.04% pod damage and 3.3 mean pest resistance susceptibility rating (PRSR) compared to checks, C 235, PBG 1 and L 550 with pod damage ranged from 39.33 to 45.96% and PRSR of 4.5, 4.8 and 6.0, respectively (Kooner and Cheema, 2008).

Shafique *et al*. (2008) evaluated 13 kabuli chickpea recombinants and reported that CH 70/02, CH 75/02, CH 83/02 and CH 86/02 were found highly resistant against *H. armigera*, and CH 62/02, CH 64/02 and CH 66/02 possessed intermediate resistance, while CH 60/02, CH 63/02, CH 67/02 and CH 68/02 were least resistant. In another study, the desi chickpea genotype CH 16/02 exhibited better resistance against *H. armigera* with lower larval population, pod damage and higher grain yield (Shafique *et al*., 2009).

The incidence of pod borer on genotypes, IC 269317, IC 268855, IC 269218 and IC 269347 ranged from 11.24 to 14.23% as compared to 21.36, 21.53, 23.94 and 35.04% on the checks, PBG 1, L 550, GPF 2 and infester, respectively. The mean pest resistance rating was three on the promising genotypes whereas it was four on check varieties and six on the infester. Genotype IC 269347 recorded higher yield comparable to check varieties (Cheema *et al*., 2010).

Deshmukh *et al*. (2010) reported that the chickpea genotypes BG-372, HC-1, SAKI-9516, Vijay and Avrodhi were found to be less susceptible to *H. armigera*, and recorded lower larval population (1.07 to 1.32 larvae/plant), with lower damage to pods (11.41 to 14.16%) and higher grain yield (1187 to 1375 kg ha^{-1}).

Among the 30 chickpea lines evaluated for resistance to *H. armigera*, the lowest larval population was recorded in genotypes EC 583318, ICC 4958, ICCVX 960186-1, ICCVX 960-28 and ICCVX 960183-69, which were comparable with the tolerant check (ICC 506). Whereas, more larval population was recorded on ICC 4973, ICC 1356 and ICC 14402 which were comparable with the susceptible checks (ICC 3137 and ICCC 37). Characteristically, the genotypes had deep green colour, small leaflets and more hairy were less preferred by the pest (Mulwa *et al*., 2010).

Incidence of *H. armigera* on chickpea was recorded at vegetative, flowering and podding stages. The genotype ICC 730103 showed minimum infestation and produced higher yield (1383.84 kg ha^{-1}), whereas JG 62 recorded poor yield (479.27 kg ha^{-1}) with higher infestation (Ravikant, 2010).

Nadeem *et al*. (2011) reported that chickpea genotypes CH 73/02, CH 76/02 and CC 21/100 showed more resistance to *H. armigera*, whereas CH 72/02, CH 77/02 and CH 80/02 showed moderate resistance and CH 79/02, B 17/03, CH 65/02 were least resistant. CH 73/02 was highly resistant showing lowest pod damage (8.20%) and increase in grain yield (77.80%) over the check.

Based on larval population, percentage of pod damage and yield components of chickpea, the genotypes CM 2100/96 and CM- 4068/97 were relatively resistant while 96051 and PBC-2000 lines were susceptible to pod borer, *H. armigera* (Sarwar *et al*., 2011).

Kumar *et al*. (2013) reported that among 50 chickpea genotypes evaluated for resistance to *H. armigera*, genotypes DGP 15, GIG 0312, ICCL 87315, ICCV 7, RIL 115, ICC 29, ICC 12470, ICCV 10 and PG 23 with rating scale of 1 and pod damage ranged from 5.50 to 8.50% were moderate between resistant and susceptible. Seven genotypes (NDGS 32, ICCC 37, RIL 27, DCP 8, BDNG 9-3, Udai and ICC12479) had shown pod damage ranging from 20 to 23% and rating scale of 3 and were placed under susceptible and remaining 34 genotypes with pod damage of 10.50 to 19% and rating scale of 2, were graded as moderately resistant.

Considering overall performance, the genotypes CM-24-2/02, CM-210/01, CH-53/99, and CC-94/99 proved to be most stable for lessening *H. armigera* larval density, pod damage and enhancing grain yield. This was almost certainly due to high potential of resistant chickpea genotypes for pest tolerance and yield enhancement (Sarwar, 2013).

Among 28 chickpea genotypes evaluated for resistance to *H. armigera*, genotypes Atmore and Flip03-139c were recorded higher resistance than the Mattama, Hawata, Selwa, Wad Hamed, Jabel Marra, Flip03-127c and Flip04-9c, which showed moderate resistance. The cv. Hawata recorded the highest seed yield (1482 kg ha^{-1}) followed by Atmore (1276 kg ha^{-1}) and Shandi (1246 kg ha^{-1}) (Ali and Mohamed, 2014).

Shankar *et al*. (2014) reported that chickpea genotypes ICC 10393, ICCL 86111, ICC 12475, RIL 25, RIL 20 and ICCV 10 recorded lower larval density of pod borers, *H. armigera* and *S. exigua* and leaf damage during vegetative, flowering and podding stages compared to susceptible check ICC 3137. Pod damage was significantly lower and grain yield was significantly greater in these genotypes than that of ICC 3137, thus these genotypes can be used for improving chickpea to pod borer resistance for sustainable crop production.

Based on the observations on larval population and pod damage, chickpea genotypes RSG 963, ICCL 86111 and DCP-92-3 were identified as less susceptible against the *H. armigera* which were at par with the resistant check ICCC 37. The genotypes, CSJ-479, DCP-92-3 and GPF-2 recorded significantly higher grain yields i.e., 1923.67, 1372.68 and 1356.47 kg ha^{-1}, respectively. Mean loss in grain yield due to damage across genotypes was 29.62%. As per the 'maximin-minimax' method five genotypes namely GPF-2, CSJ- 479, ICCC 37, DCP-92-3 and ICC 3137 were rated as susceptible high yielding i.e. tolerant to *H. armigera* (Ghugal and Shrivastava, 2015).

2.5 MECHANISMS OF HOST PLANT RESISTANCE TO *H. armigera*

Insect populations must be able to overcome the host plant resistance in order that they can maintain their ability to feed on that host. The ability to evolve resistance to host plant defences depends upon additive genetic variation in larval performance and adult host choice preference (Cotter and Edwards, 2006).

2.5.1 Antixenosis Mechanism of Resistance

Green *et al*. (2002) observed feeding non-preferrence in pigeonpea cultivated species *Cajanus cajan* and wild relative *C. scarabaeoides* to *H. armigera* larvae and reported that the first and second instar larvae preferred to feed upon *C. cajan* than *C. scarabaeoides* and on flowers rather than pods or leaves of *C. cajan*. First and second instar larvae preferred pods with trichomes removed than pods with trichomes when fed on *C. scarabaeoides*.

Kumari *et al*. (2006) studied a diverse array of pigeonpea genotypes and suggested that the genotypes ICPL 187-1, ICP 7203-1, ICPL 88039, T 21, ICPL 84060, and ICPL 332 exhibited antixenosis for oviposition under no, dual and multi-choice conditions compared to susceptible check, ICPL 87 which was highly preferred for oviposition.

Gopalaswamy *et al*. (2008) reported that no differences were observed in the oviposition on the inflorescences of the transgenic pigeonpeas with *cry1Ab* or *SBTI* genes compared to non-transgenic plants and further suggested that transgenic plants have no influence on the oviposition and feeding preferences of *H. armigera*.

The accessions of wild relatives of pigeonpea, ICPW 1 (*C. acutifolius*), ICPW 13 and 14 (*C. albicans*), ICPW 159 and 160 (*C. sericeus*), ICPW 68 (*C. platycarpus*), ICPW 83, 90, 94, 125, 137, 141 and 280 (*C. scarabaeoides*), ICPW 207 (*Paracalyx scariosa*) and ICPW 210 (*Rhynchosia aurea*) showed high levels of antixenosis for oviposition under no, dual and multi-choice conditions (Sujana *et al*., 2008).

2.5.2 Antibiosis Mechanism of Resistance

Antibiosis mechanism was studied against *H. armigera* on chickpea genotypes, ICCX 730041, ICC 10817, ICC 79048 (less susceptible), C 235 (moderately susceptible) and K 850, ICC 1403 and ICC 3137 (highly susceptible). The larval survival ranged from 77 to 90%, larval and pupal weight, 333 to 436 mg and 231 to 310 mg, respectively, adult longevity, 8 to 10 and 10 to 12 days for males and females, respectively (Srivastava and Srivastava, 1990).

Antibiosis effect of short duration pigeonpea genotypes on *H. armigera* revealed that larval and pupal weights were significantly higher, larval developmental period significantly shorter and adult lifespan significantly longer when larvae were reared on pods compared with flowers or leaves. Lowest larval

and pupal weight, longest larval developmental period, and shortest adult lifespan were observed when larvae were reared on leaves. Larvae reared on ICPL 87 had the shortest larval developmental time, the highest larval and pupal weights, and the longest adult lifespan. Larvae reared on ICPL 86012 had the lowest larval weight and longest larval period (Sison and Shanower, 1994).

Antibiosis in chickpea genotypes revealed that pupae of *H. armigera* from the larvae reared on ICC 506 and ICCV 7 weighed less than those reared on susceptible controls, Annigeri and ICC 3137. Fewer eggs were recorded on ICC 506 compared to susceptible control when observed for antixenosis for oviposition (Cowgill and Lateef, 1996).

Shanower *et al*. (1997) observed antibiosis mechanism in terms of lower larval survival, growth and fecundity of *H. armigera* on pods of cultivated pigeonpea and two wild species, *C. scarabaeoides* and *C. platycarpus* and reported that *C. scarabaeoides* had high antibiosis levels, whereas *C. platycarpus* had intermediate levels and *C. cajan* showed lower levels of resistance to *H. armigera*.

The larval, pupal and total developmental periods of *H. armigera* were longest when they fed on diet with lyophilized powders of chickpea genotype, NIFA-95 (16.90, 10.00 and 26.90 days, respectively) and shortest on CMNK-440-9 (14.63, 9.33 and 23.90 days, respectively). Larval weight and pupal recovery were lowest on NIFA-95 (60.95 and 30.00%, respectively) (Khattak *et al*., 2002).

Sreelatha (2003) recorded lower larval and pupal weights and prolonged larval and pupal periods on leaves, pods, and artificial diet impregnated with lyophilized leaves and pods of resistant chickpea genotypes ICC 12475, ICC 12476, ICC 12477, ICC 12478, ICC 12479, ICC 12490, ICC 12491 and ICC 12495 as compared to that of the susceptible genotypes ICC 12426, ICC 3137, ICC 4973 and ICC 4962.

Based on leaf feeding, larval survival, and larval weights in the detached leaf assay, the wild relatives of chickpea accessions IG 69941, IG 70002, IG70003, IG 70009, IG 70019, IG 70022, ICC17125, ICC 17122, ICC 17156, IG 70006, and ICC 17187 (*C. bijugum)*, IG 69995 and IG 70030 *(C. judaicum*) and IG 69988, IG 69999 IG 70021, IG 70025, and IG 70028 (*C. pinnatifidum)* showed low leaf feeding, low larval weights, and low host suitability index against *H. armigera* (Sharma *et al*., 2004).

Sharma *et al*. (2005a) evaluated antibiotic effect of wild relatives of chickpea for *H. armigera* and reported that accessions ICC 17257, IG 70002, IG 70003, IG 70012, (*C. bijugum*), IG 69948 (*C. pinnatifidum*), IG 69979 (*C. cuneatum*), IG 70032, IG 70033, IG 70038, and IG 72931 (*C. judaicum*) showed lower leaf feeding, larval weight and poor host suitability index as compared to the cultivated chickpeas. Based on percentage weight gain by the larvae, accessions IG 70003, IG 70022, IG 70016, IG 70013, IG 70012, IG 70010, IG 70001, IG 70018, and IG 70002 (*C. bijugum*), IG 69979 (*C. cuneatum*) and IG 72953 (*C. reticulatum*) showed high levels of resistance to *H. armigera*. There was no pupation and adult emergence when the larvae were reared on accessions of *C. pinnatifidum* (IG 69948 and IG 70039), and *C. judaicum* (IG 69980, IG 70032, IG 70033 and IG 72931).

Wild relatives of chickpea *C. reticulatum* genotypes, IG 69960, IG 72934, and IG 72936 showed significantly lower leaf feeding than the cultivated genotypes. Larval weights were lower or comparable with that on *C. judaicum* (IG 70032) and *C. bijugum* (IG 70019) in *C. reticulatum* accessions IG 72933, IG 72934, IG 72936, and IG 72953. Prolonged larval and total developmental periods were observed on *C. reticultatum* accessions compared with those on ICCC 37 (Sharma *et al*., 2005b).

Sharma *et al*. (2005c) standardized cage technique to screen chickpeas for resistance to *H. armigera.* Leaf feeding by the larvae was lower on ICC 506 than on ICCC 37 when the seedlings were infested with 20 neonates per five plants at seedling emergence or 10 neonates per three plants at the flowering stage. Maximum pod damage was observed when the plants were infested with six larvae of third instar per three plants in the greenhouse, and with eight larvae per plant under field conditions. Larval weights were lower on ICC 506 than on ICCC 37 across growth stages and infestation levels. At the podding stage, percentage of reduction in grain yield was greater on ICCC 37 and Annigeri than on ICCV 2 and ICC 506.

Sharma *et al*. (2006) observed antibiosis effect of wild relatives of chickpea against *H. armigera* in terms of reduction in leaf feeding, larval survival and larval weights when the larvae were fed on the leaves of *C. microphyllum* accessions ICC 17146, ICC 17236, ICC 17240 and ICC 17248. Under natural infestation, accessions belonging to *C. microphyllum*, *C. canariense* and *C. macracanthum* suffered a damage rating of less than 2.0 compared to 4.0 in *C. judaicum* accession and 8.5 to 9.0 in the cultivated chickpeas.

Antibiosis effect in terms of low larval weights was observed in *H. armigera* reared on ICC 12476, ICC 12478 and ICC 506EB and weight gained by third instar larvae was also low on genotypes ICC 12476, ICC 12477, ICC 12478, ICC 12479 and ICC 506EB at podding stage. Non-preference for oviposition and antibiosis were also expressed in F_1 hybrids based on ICC 12476, ICC 12477, ICC 12478, ICC 12479 and ICC 506EB indicating that ovipositional non-preference and antibiosis were influenced by parent genotype (Narayanamma *et al*., 2007).

High levels of antibiosis were observed in terms of lower larval weights and prolonged larval and pupal periods and delayed postembryonic development when the larvae of *H. armigera* were reared on leaves, pods and artificial diet impregnated with lyophilized leaf or pod powder of wild relatives of pigeonpea, *C. acutifolius* (ICPW 1), *C. cajanifolius* (ICPW 29), *C. sericeus* (ICPW 160), *P. scariosa* (ICPW 207), *C. scarabaeoides*, *R. aurea* and *C. albicans* (Sujana *et al*., 2008).

Studies on survival and development of *H. armigera* on chickpea revealed that four resistant genotypes resulted in lower larval survival, pupation, adult emergence and fecundity when compared to susceptible check. A similar trend was also observed for larval survival and development when using F_1 hybrids based on four resistant genotypes suggesting that antibiosis mechanism of resistance was transferred to the progeny from the resistant parents (Narayanamma *et al*., 2008).

Devi (2008) studied the survival and development of *H. armigera* on chickpea genotypes. The larval and pupal weights, pupation, adult emergence and fecundity were significantly lower on ICC 506EB (45.49 mg, 235.20 mg, 34.00%, 63.75% and 533.20, respectively) as compared to C 235 and L 550. The larval period was longer on ICC 506EB (21.85 days) compared to L 550 (18.93 days).

Reduced larval and pupal weights and prolonged larval and pupal periods were observed as a result of antibiosis in *H. armigera* reared on intact leaves, pods and artificial diet impregnated with lyophilized leaves or pods powders of pigeonpea genotypes ICPL 332, ICPL 84060, ICP 7035, ICPL 88039 and T 21. Incorporation of 10 g of lyophilized leaf or pod powder in 300 ml of artificial diet resulted in maximum differences in survival and development of *H. armigera* larvae on the resistant (ICPL 332) and susceptible (ICPL 87) genotypes (Kumari *et al*., 2010).

2.6 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERS ASSOCIATED WITH RESISTANCE TO *H. armigera*

Plant-insect interactions are dependent on nutritional levels of plant tissues during different growth forms of the insect and chemical and mechanical defences of the plant (Cates, 1980). Combination of information related to morphological and biochemical mechanisms provides more reliable information for host plant resistance.

2.6.1 Morphological Characters Associated with Resistance

Kanchana *et al*. (2005) studied the effect of morphological and biochemical parameters of selected chickpea varieties against *H. armigera* and indicated that increased pod length, pod width and protein content had positive correlation with pod damage.

Among gamma radiated genotypes of chickpea, minimum larval population was observed on Hassan-2k (40 krad of gamma radiation dose) while maximum was recorded on NIFA-95 (10 krad). Percent damage was highest in Hassan-2k (10 krad) and lowest in Pb-91 (20 krad). Maximum yield was recorded on Hassan-2k (30 krad). Trichome density and length were negatively correlated with *H. armigera* infestation (Shahzad *et al*., 2005).

Girija *et al.* (2008) reported least pod damage by *H. armigera* in chickpea genotype ICCL 87317 than ICC 86102, ICCV 95992, ICC 96752 and ICC 12494. Tolerant genotypes had higher number of trichomes and thicker pod husk and hence exhibited significantly less damage.

Influence of pod morphological traits on pod borer resistance in chickpea revealed that pod trichomes length and density, pod wall thickness, pod length, breadth and area of respective genotypes showed a significant negative correlation with pod borer damage, whereas number of pods per plant exhibited a positive association (Hossain *et al*. 2008b).

Sharma *et al*. (2009) reported that oviposition non-preference was an important component of resistance to *H. armigera* in wild relatives of pigeonpea where glandular trichomes (type A) on the calyxes and pods were associated with susceptibility to *H. armigera*, while the non-glandular trichomes (type C and D) were associated with resistance.

Shabbir *et al*. (2014) observed that chickpea genotypes which had higher trichome length and density and pod wall thickness were more resistant to pod borer, *H. armigera* infestation.

2.6.2 Biochemical Characters Associated with Resistance

The nature of plant derived allelochemicals or secondary metabolites involved in the different stages of insect-plant interactions, from habitat selection to host acceptance is varied (Simmonds, 2001).

2.6.2.1 Leaf Organic Acids

The trichomes of chickpea have a basal cell, long vacuolate stalk cells and a terminal cluster of dense secretory head cells (Schnepf, 1965 and Lazzaro and Thomson, 1989). The continuous vacuolar tubular system in these trichomes functions to rapidly deliver solute from the base of the trichome to the secretory head cells. The trichomes secrete hydrochloric acid, oxalic acid, malic acid, and calcium (Lauter and Munns, 1986 and Lazzaro and Thomson 1995). The secretions from these trichomes appear to protect the plants from herbivory (Srivastava and Srivastava, 1989).

Rembold *et al*. (1989) reported that the chickpea leaf exudates had malate and oxalate as the main components and the varieties with the high amount of malic acid were resistant to *H. armigera* and *Liriomyza cicerina*.

Srivastava and Srivastava (1989) reported that ICC 3137, K 850 and ICC 1403 were susceptible to *H. armigera* with more number of eggs and larvae than the resistant chickpea genotypes. Low amount of acidity in the leaf extracts was associated with susceptibility to *H. armigera*.

Patnaik and Senapati (1995) reported that egg and larval counts of pod borer, *H. armigera* were negatively correlated with increasing concentration of acid exudates of chickpea. Low densities of eggs (0.70 to 1.60/10 plants) and larvae (3.40 to 4.00/10 plants) were associated with high acidity (24.20 to 25.30 milli equivalents) in the cultivars, PDE 2-1, PDE 2-3, PDE 3-2 and PDE 7-2, while PDE 5-3 and Annigeri-1, which had a low acid content (13.50 to 15.10 milli equivalents) in their leaves harboured more eggs (≥2.70/10 plants) and larvae (≥5.90/10 plants).

Among leaf surface substances present in chickpea, oxalic acid was responsible for the growth inhibition of *H. armigera* larvae, while malic acid had no effect on growth rate (Yoshida *et al*., 1995). Malic acid stimulated oviposition at a concentration of 0.6 μmol/cm^2, but inhibited it at 3.4 μmol/cm^2, whereas oxalic acid showed neither stimulation nor inhibition of oviposition at 0.25 to 1.7 μmol/cm^2. Malic acid on the leaves stimulated oviposition during the vegetative and flowering stages, while during podding stage, there was no significant correlation between either egg density or pod damage and malic acid levels (Yoshida *et al*., 1997).

Peter and Shanower (1998) reported that chickpea trichome exudates contain acidic chemicals such as malic acid, oxalic acid and succinic acid. Oxalic acid has an antibiosis effect on the larvae of pod borer, *H. armigera*, which results in reduced pod damage. A dense mat of non-glandular trichomes in these species prevents the small larvae from feeding on the plant.

Citric and oxalic acid concentrations in chickpea were lower in resistant genotypes than the susceptible genotypes while, malic acid was higher in the resistant genotypes than the susceptible genotypes suggested that high level of malic acid may be selection criteria for *Ascochyta* blight resistance (Cagirgan *et al*., 2011).

Narayanamma *et al*. (2013) reported that the amounts of malic acid were negatively correlated with leaf feeding by *H. armigera* larvae at flowering and maturity and with pod damage. Oxalic acid showed a negative association with leaf damage, whereas the amounts of acetic acid were negatively correlated with larval weights and damage rating at the flowering and maturity stages. Citric acid levels were negatively associated with damage rating at the flowering stage.

Oxalic and malic acids present in chickpea leaves did not influence the biological activity of *Bt* toxin *Cry1Ac* towards *H. armigera* larvae. However, very high concentrations of the organic acids reduced the amounts of *Cry1Ac* in the midgut of *H. armigera* larvae. Organic acids reduced the amount of protein in the brush boarder membrane vesicles (BBMV) of insects reared on diets with *Cry1Ac*, possibly because of reduced size of the larvae (Devi *et al*. 2013). The antifeedant effects of the acid exudates resulted in reduced leaf feeding, larval survival and weights and hence might reduce the efficacy of *Bt* sprays or *Bt*-transgenic chickpeas, although the combined effect of plant resistance based on organic acids and *Bt* had a greater effect on survival and development of *H. armigera* than *Bt* alone (Devi *et al*. 2014).

2.6.2.2 Flavonoids

The behavioural response of flavonoid, rutin (quercetin-3-O-rhamnosyl glucoside) varied depending on the concentration tested and the age of insect. Rutin at concentrations greater than 10^{-3} M deterred final stadium larvae of *H. armigera* and *H. zea* from feeding, but at concentrations less than 10^{-4} M it stimulated feeding in final stadium larvae (Blaney and Simmonds, 1983).

Morimoto *et al*. (2000) reported that four flavonoids, 5-hydroxy-3,6,7,8,4'-pentamethoxyflavone, 5-hydroxy-3,6,7,8-tetramethoxyflavone, 5,6-dihydroxy-3,7-dimethoxyflavone, and 4,4',6'-trihydroxy-2'-methoxychalcone, that were isolated from cudweed, *Gnaphalium affine* had strong antifeedant activity against the *S. litura*.

Isoflavanoids (judaicin 7-O-glucoside, 2-methoxy judaicin, judaicin and maackiain) present in wild relatives of chickpea had shown antifeedant activity towards the larvae of *H. armigera* when incorporated into a diet. Isoflavonoids decreased the weight gained by early stadia larvae of *H. armigera* more than they did in later stadia. Maackiain and judaicin were found to be most potent (Simmonds and Stevenson, 2001).

Green *et al*. (2003) revealed that methanol extracts contained four phenolic compounds, isoquercitrin, quercetin, quercetin-3-methyl ether and stilbene (3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid) from the pod surface of pigeonpea stimulated feeding of fifth instar larvae of *H. armigera*.

Beninger *et al*. (2004) identified a flavanone (3, 4, 5-trihydroxyflavanone 7-*O*-glucuronide) and two phenolic acids (chlorogenic acid and 3,5-*O*-dicaffeoylquinic acid) from *Chrysanthemum morifolium* and reported that these phenolic substances reduced larval growth of the cabbage looper and gypsy moth when incorporated into artificial diet at 10 to 1000 ppm.

Ateyyat *et al*. (2012) revealed aphicide activity of three flavonoids (quercetin dehydrate, rutin hydrate and naringin) on apple woolly aphid in cut shoot bioassay and reported that mortality in nymphs was more than adults and further increased with an increasing concentration of 100, 1000 and 10,000 ppm. However, rutin hydrate was more toxic than quercetin dehydrate and naringin.

Flavonoids such as chlorogenic acid, caffeic acid and protocatechuic acid were more toxic to *H. armigera* larvae. Larval growth and development were

significantly reduced in *H. armigera* larvae fed on a diet with groundnut leaf lectin (GLL) and ConA. The digestive enzyme activities of the larvae were significantly reduced in flavonoid treated diets (War *et al*., 2013).

Induction of flavonoids in response to insect feeding observed through HPLC fingerprinting in *H. armigera* and *Aphis craccivora* infested and uninfested groundnut genotypes and reported that more number of compounds like chlorogenic acid, syringic acid, quercetin, caffeic acid, vanillic acid and ferulic acid were observed in insect infested plants, especially in the resistant genotypes (War *et al*., 2016).

2.6.2.3 Protease Inhibitors

Plant protease inhibitors (PIs) are a group of the reserve storage proteins present in seeds, which can also be a part of the constitutive and inducible array of defense strategies against feeding by insect pests (Blanco-Labra *et al*., 1995). Chickpea seeds are known to contain inhibitors of proteases and their properties have been studied in detail by Belew and Eaker (1976), Smirnoff *et al*. (1979) and Saini *et al*. (1992). Varietal differences regarding trypsin and chymotrypsin inhibitors in chickpea have been reported by Sastry and Murray (1987).

Giri *et al*. (1998) reported that there was a progressive increase in PI activity throughout seed development. The amount of PI activity increased several fold when seeds were injured by *H. armigera* feeding. Seven different trypsin inhibitory (TI) bands were present in seeds at the time of maximum *H. armigera* attack. Chickpea PIs showed differential inhibitory activity against *H. armigera* gut proteinases (HGPs), trypsin and chymotrypsin. *In-vitro* and *in-vivo* proteolysis of TIs indicated that the chickpea PIs were prone to proteolytic digestion by *H. armigera* gut proteinases either by production of inhibitor-insensitive proteinases or by secretion of proteinases.

The wild species exhibited diversity in TI isoforms with respect to both number and activity as compared to cultivated chickpea but none of the species offered complete protection against pod borer by inhibiting gut proteinases. Highest inhibition was exhibited by *C. bijugum* (36%) followed by *C. echinospermum* and *C. arietinum* (cv. Vijay) (33%). Among the seed organs, TI and HGPI activities were highly localized in the embryoaxis as compared to the cotyledons in immature and mature seeds (Patankar *et al*., 1999).

Larvae of *H. armigera* reared on a diet containing non-host PIs showed growth retardation, reduction in total and trypsin like proteinase activity and the production of inhibitor-insensitive proteinases, further, trypsin inhibitor activity bands were detected in all of the host and non-host plants, but HGP inhibitor activity bands were present only in non-host plants except cotton in the host plant group (Harsulkar *et al*., 1999).

Patankar *et al*. (2001) reported that larvae of *H. armigera* reared on chickpea indicated >2.5-3 fold protease activity compared with those fed on the other host plants. Higher protease activity in the larvae fed on chickpea was probably because of higher protein amount in the food or hyper production of proteases in response to the ingested protease inhibitors.

Tomato flowers accumulated about 300 and 1000 times higher levels of TI while 700 and 400 times higher levels of HGPI as compared to leaves and fruits, respectively. Tomato PIs inhibited about 50 to 80% HGP activity of *H. armigera* larvae and were found to be highly stable to insect proteinases. *H. armigera* larvae fed on artificial diet containing tomato PIs revealed adverse effect on larval growth, pupae development, adult formation and fecundity (Damle *et al*., 2005).

Of the two proteases from midgut of *H. armigera*, HGP-1 was not only insensitive to a PI from chickpea but was also able to degrade it, and it was capable of hydrolyzing a synthetic substrate of elastase. Whereas, HGP-2 activity was inhibited over 50% by same PI from chickpea and it was inhibited by a synthetic trypsin inhibitor also (Telang *et al*., 2005).

A progressive decline in larval weight, growth, survival and adult emergence as well as extension of larval period was observed in *H. armigera* fed on diet supplemented with increasing concentrations of chickpea TIs (Kansal *et al*., 2008).

Hivrale *et al*. (2013) reported that *H. armigera* larvae fed on diet containing partially purified PIs from *Albizia lebbeck* seeds showed reduced larval growth and survival. Higher activities of HGP isoforms observed in the midgut of control larvae and were inhibited in the midgut of larvae fed on diet with PI and also some HGP isoforms were induced in the larvae fed on diet with PI. Aminopeptidase activities were significantly increased in the midgut of larvae fed on diet PI as compensatory effect of inhibitory proteinases.

Lomate and Hivrale (2013) observed significant reduction in the growth and survival of *H. armigera* larvae fed on diet incorporated with the combination *Cry1Ac* and protease inhibitor, phenylmethylsulfonyl fluoride (PMSF) compared to *Cry1Ac* and PMSF alone. Serine proteinase activities were significantly declined in the larvae reared on diet with PMSF.

Jamal *et al*. (2015) observed that purified protease inhibitor from the seeds of *Butea monosperma* (BmPI) exhibited inhibitory activity of trypsin and total gut proteolytic enzymes of *H. armigera* and bovine trypsin. BmPI supplemented artificial diet caused dose dependent mortality and reduction in growth and weight where fertility and fecundity of *H. armigera* declined and larval and pupal period extended.

2.6.2.4 Lectins

Lectins are carbohydrate binding proteins (or glycoproteins) of nonimmune nature and bind reversibly to specific mono or oligosaccharides (Goldstein *et al*., 1980 and Van Damme *et al*., 1998). They have been reported to affect survival and development of insect pests (Ferry *et al*., 2004). They bind to the glycan receptors present on the surface lining of the insect gut (Pusztai and Bardocz, 1996) and interfere with the formation and integrity of the peritrophic membrane of the midgut (Harper *et al*., 1998). The harmful effects of lectins on insects resulted in reduced larval weight and size, increased mortality, feeding inhibition, delayed developmental time, pupation and adult emergence and reduced fecundity (Vasconcelos and Oliveira, 2004).

Lectins from wheat, castor and camel's foot tree fed to neonate of European corn borer, *Ostrinia nubilalis* larvae incorporated into the artificial diet recorded 50% weight loss in surviving larvae, whereas lectins from castor, pokeweed *(Phytolacca americana*) and green marine algae (*Codium fragile*) inhibited larval growth by more than 40% when fed to neonates of Southern corn rootworm, *Diabrotica undecimpunctata howardi* (Czapla and Lang, 1990).

Murdock *et al*. (1990) observed dose dependent response of lectins on cowpea weevil, *Callosobruchus maculatus* through artificial seed method and reported that for every 1% increase in dose of peanut agglutinin lectin there was a 0.49 day delay in developmental time whereas, for every 1% increase in dose of

wheat germ agglutinin (WGA) there was a 1.47 day delay in developmental time and also for every 0.1% increase WGA there was 2.79% increase in mortality.

Mannose specific lectin, concavalin A (ConA) from jackbean, recorded decreased larval survival, weight and retarded development when fed to tomato moth (*Lacanobia oleracea*) in artificial diet and transgenic potato but had only a small effect on larval weight, whereas in peach-potato aphids (*Myzus persicae*), ConA reduced aphid size, retarded development and reduced fecundity, but had little effect on survival (Gatehouse *et al*., 1999).

Feeding bioassays using artificial diet revealed that *Listera ovata* agglutinin (LOA) and *Galanthus nivalis* agglutinin (GNA) had detrimental effects to larval survival, weight, feeding inhibition, pupation, adult emergence and fecundity against *Maruca vitrata* (Machuka *et al*., 1999).

Larvae of *H. armigera* reared on diet with lectins from chickpea, garlic, fieldbean and pigeonpea recorded reduced larval and pupal weight, pupal period, pupation and adult emergence (Arora *et al*., 2005). Larval survival and fecundity of *H. armigera* were adversely affected as a result of feeding with lectin intoxicated diet with soybean agglutinin, jackfruit lectin, wheat germ agglutinin and pea lectin (Gupta *et al*., 2005). Larval survival, pupal weight, pupation and adult emergence percentage of *H. armigera* was lower in artificial diet impregnated with snowdrop and chickpea lectins and soybean trypsin inhibitor (Shukla *et al*., 2005).

Macedo *et al*. (2007) reported that *Bauhinia monandra* leaf lectin (BmoLL) recorded 50% mortality in Mexican bean weevil (*Zabrotes subfasciatus*) and cowpea weevil (*C. maculatus*) when incorporated into artificial diet, whereas, in case of Mediterranean flour moth (*Anagasta kuehniella*) larvae it did not decrease the survival, but decreased 40% in weight.

Gaidamashvili *et al*. (2009) reported that lectin from *Dioscorea batatas* (DB1) strongly bound to gut epithelia, brush border and membrane structures of *H. armigera* larvae although DB1 had no or marginal inhibitory effects on gut proteolytic and glycolic enzymes (Ohizumi *et al*., 2009). The insecticidal properties of the DB1 may be determined by subsequent toxic effects to the midgut of larvae.

Mannose specific lectin from *Hippeastrum* hybrid (*Amaryllis*) (HHA) bulbs affected larval growth resulted in development retardation and larval weight decrease

in *Spodoptera littoralis*. The toxic effect was due to HHA interaction with the brush border of midgut cells and that further interferes with normal nutrient absorption in the midgut of *S. littoralis*, thereby affecting larval growth (Caccia *et al*., 2012).

GNA (*Galanthus nivalis* agglutinin) retarded larval and pupal weight, larval and pupal developmental time, adult longevity, and adult emergence of *Spodoptera exigua* in dose dependent manner when incorporated in artificial diet (Naghdi and Bandani, 2012).

2.6.2.5 Proteins, Phenols, Tannins and Sugars

Chhabra *et al*. (1990) found that crude fibre content, non-reducing sugars and starch were associated with resistance to *H. armigera* in cultivar GL 645, while a high percentage of cellulose, hemi cellulose and lignin in the pod wall were thought to inhibit pod damage.

Grayer *et al*. (1992) reported that a strong negative relationship between the concentration of procyanidin (condensed tannin) in the leaf bud petioles of groundnut genotypes and fecundity of the aphid *A. craccivora*. The aphids fed on genotype with highest amount of procyanidin produced significantly fewer offspring than aphids reared on genotypes with low procyanidin levels.

The chickpea genotypes, desi 3108, GL 1002 and LCG 3580 were least susceptible to the *H. armigera*. The mechanism of resistance revealed that chemical components such as malic acid, sugar, crude fibre, cellulose and lignin in the plant parts were responsible for the level of incidence of the pest (Chhabra *et al*., 1993).

The chickpea cultivar 96052 was the most resistant (8.10% mean damage) to *H. armigera* with relatively higher amounts of lignin and reducing sugars, non-reducing sugars, cellulose, hemi cellulose, ash and silica (Afzal *et al*., 2001).

Rupalighodeswar *et al*. (2003) reported that grain and pod shell tissues of chickpea cultivars tolerant to the *H. armigera* found to contain significantly higher total phenolics, chlorogenic acid, silica, malic acid and higher activities of polyphenol oxidase and peroxidase whereas, susceptible cultivars had higher crude protein and sugars. High total phenolics, chlorogenic acid, silica, malic acid and peroxidase activity were seemed to be desirable biochemical characters in enhancing the tolerance of chickpea against pod borer infestation.

Expression of resistance to *H. armigera* was associated with low amounts of sugars and high amounts of tannins and polyphenols in wild relatives of pigeonpea (Sharma *et al*., 2009).

2.6.2.5 Methanol and Hexane Extracts of Leaf Surface Chemicals

Green *et al*. (2002) reported that solvent extraction of pod surfaces affected the feeding preference of *H. armigera* in wild and cultivated pigeonpea as the larvae preferred unextracted pods of *C. cajan*, the extracted pods of *C. scarabaeoides* (first and second instar) or the unextracted pods of *C. scarabaeoides* (fourth and fifth instar). Glass fibre disc bioassays showed that the methanol, hexane and water extracts from the pod surface of *C. cajan* stimulated the feeding of fifth instars.

Acetone extracts from pods of *C. cajan* and *C. platycarpus* had a significant feeding stimulant effect on *H. armigera* larvae whereas extracts from pods of *C. scarabaeoides* had no effect. Water extract of C. *scarabaeoides* pods had a significant antifeedant effect, whereas extracts from *C. cajan* and *C. platycarpus* pods had no effect (Shanower *et al*., 1997).

Feeding preference of *H. armigera* larvae revealed that methanol washed pods of wild relatives of pigeonpea were less preferred for feeding than the unwashed pods, but the hexane washed pods were preferred more than the unwashed pods which suggested that methanol extracted the phagostimulants from the pod surface, while hexane removed the antifeedants (Sujana *et al*., 2012).

2.7 Genetic Diversity in Cultigen and Wild Relatives of Chickpea

Chickpea is a diploid with 2n=2x=16 having a genome size of approximately 740 Mbp. It is a highly self-pollinated crop with an outcrossing rate of less than one per cent. The knowledge of genetic relationships between the cultivated chickpea and its wild relatives is a prerequisite to track the evolution of cultivated species and also to determine the close relatives which can be exploited for introgression of useful traits into the cultigen in plant breeding programmes. Systematic collection and evaluation of wild species for useful traits has revealed presence of a diverse gene pool for tolerance to the biotic and abiotic stresses (Singh *et al*., 2008).

DNA markers have been used widely for fingerprinting of plant genomes, genetic diversity analysis and to understand the evolutionary relationships among crop species. Among the different classes of molecular markers, SSRs have been

proven useful for a variety of applications in plant genetics and breeding because of their reproducibility, multi allelic nature, codominant inheritance, relative abundance and genome wide coverage (Gupta and Varshney, 2000). In case of chickpea, several hundred SSR markers have been developed (Winter *et al*., 1999, 2000., Choudhary *et al*., 2006, 2009., Sethy *et al*., 2006a and Nayak *et al*., 2010).

Huttel *et al.* (1999) evaluated the ability of SSRs in detecting intraspecific variation in chickpea. Sixteen SSR loci detected 2 to 4 alleles at intraspecific level out of 22 loci tested. Two SSR loci, CaSTMS10 and CaSTMS15 detected 25 and 16 alleles among 63 accessions of *C. arietinum* from different geographic locations, reflecting gene diversity values of 0.937 and 0.922, respectively.

The sequences flanking microsatellite sites were generally conserved within species and also often in closely related species (Gupta and Varshney, 2000). The flanking sequences of microsatellite loci of cultivated chickpea were found to be conserved in related annual species also. The highest degree of conservation was observed in *C. reticulatum* (92%) and *C. echinospermum* (83%), whereas lowest was observed in *C. cuneatum* (50%) (Choumane *et al.*, 2000).

Sudupak *et al*. (2002) used RAPD markers to investigate genetic relationships among the *Cicer* species. The dendrogram contained two main clusters, one of which comprised accessions of the four perennial species (*C. montbretii, C. isauricum, C. anatolicum* and *C. incisum*) together with the accessions of the three annual species (*C. pinnatifidum, C. judaicum* and *C. bijugum*), and the other cluster included the remaining three annual species (*C. echinospermum, C. reticulatum* and *C. arietinum*). It was observed that among perennial species *C. incisum* was the most similar to annuals, and *C. reticulatum* was the closest annual species of chickpea.

AFLP based grouping of *Cicer* species revealed two clusters, one of which included three perennial species, *C. montbretii*, *C. isauricum* and *C. anatolicum*, while the other cluster consisted of two sub clusters of which one included one perennial, *C. incisum*, along with three annuals from second crossability group (*C. bijugum*, *C. pinnatifidum* and *C. judaicum*) and the other one comprised three annuals from the first crossability group (*C. arietinum, C. echinospermum* and *C. reticulatum*) (Sudupak *et al*., 2004).

Udupa *et al*. (2004) studied dynamics of microsatellite evolution in chickpea by using di and tri nucleotide repeat (TA)n and (TAA)n, respectively, and based on polymorphism they observed that the two loci did not evolved in complete independence. Below a threshold level they evolved independently and above that threshold level if one allele increased in size the other closely linked locus decreased and *vice versa*, without change in the overall ratio.

Shan *et al*. (2005) characterized geographic patterns of genetic variation in wild annual *Cicer* germplasm using AFLP markers and revealed that maximum genetic diversity of *C. reticulatum, C. echinospermum, C. bijugum* and *C. pinnatifidum* was found in South-Eastern Turkey, while Palestine was the centre of maximum genetic variation for *C. judaicum*.

Sethy *et al*. (2006a) reported that out of the 74 functional microsatellite markers developed, 25 polymorphic markers were used to analyse the intraspecific genetic diversity within 36 geographically diverse chickpea accessions. The 25 primer pairs amplified at single loci produced a minimum of two and maximum of 11 alleles. A total of 159 alleles were detected with an average of 6.4 alleles per locus. Cloning and sequencing of size variant alleles at two microsatellite loci revealed that the variable numbers of AG repeats in different alleles were the major source of polymorphism.

Sethy *et al.* (2006b) cloned microsatellite sequences from *C. reticulatum* and developed 11 SSR markers to analyse 29 accessions representing all nine annual *Cicer* species. Efficient marker transferability (97%) of the *C. reticulatum* was observed as compared to microsatellite markers developed from cultivated species. Based on cluster analysis all the accessions (except two *C. judaicum* accessions) distinguished from one another and revealed intra and interspecific variability. An annual *Cicer* phylogeny was depicted which established higher similarity between *C. arietinum* and *C. reticulatum*. In the study, placement of C. *pinnatifidum* in the second crossability group and its closeness to *C. bijugum* was supported. Two species *C. yamashitae* and *C. chorassanicum* were grouped distinctly and seemed to be genetically diverse from members of first crossability group.

Choudhary *et al*. (2009) identified 246 SSR motifs from which 183 primer pairs were designed and 60 validated as functional markers. Genetic diversity analysis across 30 chickpea accessions revealed ten markers to be polymorphic

producing a total of 29 alleles and an observed heterozygosity average of 0.16 thereby exhibiting low levels of intraspecific polymorphism. However, the markers exhibited high cross species transferability ranging from 68.3 to 96.6% across the six annual *Cicer* species and from 29.4 to 61.7% across the seven legume genera.

Genetic variation among species of *Cicer* using RAPD markers revealed that dendrogram included three clusters. Cluster I included *C. arietinum, C. reticulatum* and *C. echinospermum*. Within this group, *C. reticulatum* accessions were clustered closest to the *C. arietinum* and *C. yamashitae*. The second cluster was separated from the other clusters. Cluster III included *C. judaicum, C. pinnatifidum* and *C. cuneatum* (Talebi *et al*., 2009).

The 15 microsatellite markers used to characterize chickpea cultivars showed a high level of polymorphism, a total of 154 different alleles were detected, with a mean of 10.3 alleles per locus. The polymorphic information content (PIC) value ranged from 0.455 to 0.897. All the markers, with the exception of TA130, TA135 and TA144 were considered to be informative (PIC>0.7), indicating their potential usefulness for cultivar identification. A subset of markers (TA186, TA200, TA106, TA113, TA117 and TA30) was sufficient to identify all the cultivars studied (Castro *et al*., 2011).

Naghavi *et al*. (2012) estimated genetic diversity of chickpea germplasm from Iran using 16 microsatellite loci. The number of alleles per microsatellite locus ranged from 8 to 29, with an average of 19.31 per locus. A high level of genetic diversity in the Northern area (*He* = 0.76*)*, even with a limited number of available landraces compared with the other three regions, might confirm the Northern Persia as part of the chickpea centre of origin. Cluster analyses based on molecular data showed that the Northern area was separated clearly from the other three regions.

The genetic diversity of 23 chickpea accessions was characterized using nine microsatellite markers which generated a total of 122 alleles. The number of alleles (*Na*) per locus varied from 9 to 20. The observed heterozygosity (*Ho*) ranged between 0.05 and 0.43 (average 0.13) whereas both the expected heterozygosity (*He*) and PIC ranged from 0.71 to 0.90 (average 0.83). Total genetic variation found within accessions was 62% while the remaining 38% was found among accessions (Torutaeva *et al*., 2014).

The genetic diversity estimates of chickpea using STMS markers revealed that 31 STMS primers generated a total of 153 loci (an average of 4.94 loci per primer) out of which 129 loci were found to be polymorphic and 24 loci were monomorphic. The value of PIC varied from 0.128 to 0.783. Percentage of polymorphic loci was 50.98, 58.82 and 96.73 for susceptible, resistant and miscellaneous genotypes, respectively. The overall Nei's gene diversity (0.238) and Shannon's information index (0.372) indicated high degree of genetic polymorphism among the genotypes (Aggarwal *et al*., 2015a).

Aggarwal *et al*. (2015b) assessed genetic diversity of 125 cultivars of chickpea and revealed that out of 40 ISSR primers, 26 primers generated 213 polymorphic loci. On average, nine loci per marker were found. The average PIC was 0.72, ranging from 0.26 to 0.91. Genetic diversity analysis in terms of Shannon's index and Nei's gene diversity revealed higher values for miscellaneous cultivars compare to resistant and susceptible cultivars, indicating more variability among these cultivars.

Chapter ~ III

Material and Methods

Chapter III
MATERIAL AND METHODS

The present investigations on the "Biochemical and molecular mechanisms of resistance to *Helicoverpa armigera* (Hubner) in wild relatives of chickpea" were conducted during 2014-16 post-rainy seasons at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana state (latitude 17.53^{o} N, longitude 78.27^{o} E and altitude of 545 m). Procedures followed and materials used in these studies are presented hereunder.

3.1 EXPERIMENTAL MATERIAL

3.1.1 Plant Material

A total of 20 accessions (15 wild relatives and five varieties of cultivated chickpea) used in the present study were presented in Table 3.1. Of the 15 accessions of wild relatives of chickpea, six accessions belong to *Cicer bijugum*, two accessions belong each to *C. judaicum*, *C. pinnatifidum* and *C. reticulatum*, and one accession belong each to *C. chrossanicum, C. cuneatum* and *C. microphyllum.* Five cultivars belonging to cultivated chickpea (*C. arietinum*), JG 11 (Commercial cultivar), KAK 2, ICC 3137 (Susceptible checks) and ICCL 86111, ICC 506EB (Moderately resistant checks) were included to evaluate the relative resistance or susceptibility to *H. armigera*. The crop was raised under field conditions, during 2014-15 and 2015-16 post rainy seasons at ICRISAT, Patancheru (Plate 1). Each entry was sown in a two row plot, each with 2 m long and there were two replications in a randomized complete block design. The seeds of the wild relatives were scarified at one end with a scalpel to enhance water absorption and faster germination then soaked in water for 24 h and treated with thiram (3 g per kg of seed) before sowing. The seeds of cultivated chickpeas were sown without scarification. The trial was planted with a spacing of 60 cm between the rows and 30 cm between plants in deep black Vertisols. Normal agronomic practices were followed for raising the crop. The plants were irrigated occasionally and weeding operations were carried out as and when needed. Under glasshouse conditions, plants were raised in plastic pots (30 cm diameter, 30 cm deep) (Plate 2). The pots were filled with a potting mixture of black soil, sand, and farmyard manure (2:1:1). The seeds were scarified, treated with thiram (3 g per kg of seed), and placed in a

Table 3.1. Wild relatives of chickpea genotypes evaluated for resistance to pod borer, *H. armigera*

S. No.	Genotype	Species
1	IG 599076	*C. chrossanicum*
2	IG 69979	*C. cuneatum*
3	IG 70006	*C. bijugum*
4	IG 70012	*C. bijugum*
5	IG 70018	*C. bijugum*
6	IG 70022	*C. bijugum*
7	IG 72933	*C. reticulatum*
8	IG 72953	*C. reticulatum*
9	PI 510663	*C. pinnatifidum*
10	PI 568217	*C. judaicum*
11	PI 599046	*C. bijugum*
12	PI 599066	*C. bijugum*
13	PI 599077	*C. judaicum*
14	PI 599109	*C. pinnatifidum*
15	ICCW 17148	*C. microphyllum*
16	JG 11 (C)	*C. arietinum*
17	KAK 2 (S)	*C. arietinum*
18	ICC 3137 (S)	*C. arietinum*
19	ICCL 86111 (R)	*C. arietinum*
20	ICC 506 EB (R)	*C. arietinum*

C-Commercial cultivar, S- Susceptible check, R- Resistant check

Plate 1. Wild relatives of chickpea genotypes grown under field condition

Plate 2. Wild relatives of chickpea genotypes grown under glass house condition

Petri dish for 24 h soaking for germination. After germination, the seeds were sown in the soil and watered immediately. Three to five seedlings were raised in each pot and there were three pots for each accession. The pots were arranged in a completely randomized design. The glasshouse was cooled by desert coolers to maintain the temperature at 28±5 ºC and relative humidity >65%. The plants were watered as and when needed.

3.1.2 *Helicoverpa armigera* Culture

The larvae of *H. armigera* used in the bioassays were maintained in the laboratory at ICRISAT, Patancheru. The *H. armigera* larvae were reared on chickpea based artificial diet (Armes *et al*., 1992) at 27±2 ºC and the composition of diet were presented in Table 3.2 and 3.3. The neonates were reared for 5 days in groups of 200 to 250 in 200 ml plastic cups having a 2 to 3 mm layer of artificial diet on the bottom and sides of the cup. Thereafter, the larvae were transferred individually to six cell-well plates (each cell-well measured 3.5 cm in diameter and 2 cm in depth) to avoid cannibalism. Each cell-well had a sufficient amount of the artificial diet (7 ml) to support larval development until pupation. The pupae were removed from cell-wells, sterilized with 2% sodium hypochlorite solution and kept in groups of 50 in plastic jars containing moistened vermiculite. Upon emergence, 10 pairs of adults were released in an oviposition cage (30x30x30 cm). Adults were provided with 10% sucrose or honey on a cotton swab for feeding. Liners having a rough surface were provided as a substrate for egg laying. The liners were removed daily and the eggs were sterilized in 2% sodium hypochlorite solution. The liners were then dried and placed inside the plastic cups. After 4 days, the liners were removed. Freshly emerged neonate larvae were used for bioassays using detached leaf assay and diet impregnation assay.

3.2 IDENTIFICATION OF WILD RELATIVES OF CHICKPEA WITH DIVERSE MECHANISMS OF RESISTANCE TO *H. armigera*

3.2.1 Screening for Pod Borer Resistance under Multi-choice Field Conditions

Under multi-choice field conditions all fifteen accessions of wild relatives of chickpea including five cultivars were screened to evaluate their relative resistance or susceptibility to pod borers. The crop was raised during post rainy seasons, 2014-15 and 2015-16 under rain fed conditions as described earlier.

Table 3.2. Artificial diet composition for rearing of *H. armigera* larvae

Ingredients	**Quantity**
Chickpea flour	75.0 g
L-ascorbic acid	1.175 g
Sorbic acid	0.75 g
Methyl -*p*- hydroxy benzoate	1.25 g
Aureomycin	2.875 g
Yeast	12.0 g
Formaldehyde (40%)	1.0 ml
Vitamin stock solution	2.5 ml
Water	112.5 ml
Agar-agar solution	
Agar-agar	4.325 g
Water	200 ml

Table 3.3. Composition of vitamin stock solution (500 ml).

Ingredients	**Quantity**
Nicotinic acid	1.528 g
Calcium pantothenate	1.528 g
Riboflavin	0.764 g
Aneurine hydrochloride	0.382 g
Pyridoxine hydrochloride	0.382 g
Folic acid	0.382 g
D-Biotin	0.305 g
Cyano cobalamine	0.003 g
Water	500 ml

Data were recorded on visual damage rating of the plants on 1 to 9 scale (1= <10% and 9= >80% area damaged), larval incidence and oviposition of pod borers, *H. armigera* and *Spodoptera exigua*, number of cocoons in case of parasitoid, *Campoletis chlorideae* in randomly selected five plants in each genotype in fortnight intervals from 15 days after emergence to maturity of the crop. Percentage of pod damage was recorded at the time of harvesting in each genotype. The data were subjected to ANOVA for observing the significant differences between the genotypes.

3.2.2 Antixenosis Mechanism of Resistance

Oviposition preference to *H. armigera* in wild relatives of chickpea was studied under controlled conditions (temperature 27±2 ^{o}C, relative humidity 70% and photoperiod 12 h) using no-, dual- and multi-choice cage conditions (Kumari *et al*., 2006) (Plate 3).

Under no-choice condition, three to five twigs of test genotype (l0 cm long) were kept in a conical flask placed in cage (30 x 30 x 30 cm). The twigs were kept in a conical flask filled with water to keep them in a turgid condition. A cotton swab was wrapped around the twigs to keep them in an upright position. Five pairs of newly emerged male and female moths were released in each cage. The moths were provided with 10% sucrose solution in a cotton swab as food. Fresh twigs were provided for oviposition everyday. Likewise, three replications were maintained and observations on oviposition were recorded for three consecutive days after two days after releasing moths.

Under dual-choice condition, conical flasks with twigs of the test genotype and susceptible check (ICC 3137) were kept inside the wooden cage (30 x 30 x 30 cm) as a choice to the female moths for oviposition between test entry and susceptible check. Three replications were maintained in completely randomized design and all experimental details were similar to that of no-choice condition.

Oviposition non-preference under multi-choice condition was studied by keeping the twigs of all the 20 genotypes together in a large cage (80 x 70 x 60 cm). Fifty pairs of newly emerged moths were released into the cage. The twigs were arranged in completely randomized block design. Three replications were maintained and all other experimental details were same as above.

3a. Multi-choice cage condition

3b. No-choice cage condition

3c. Dual-choice cage condition

Plate 3. Oviposition preference for *H. armigera* towards wild relatives of chickpea

Data were subjected to ANOVA under no-choice and multi choice conditions and the data for dual-choice test was subjected to paired t-test using GENSTAT 14.0 version. The significance of differences between the treatments was measured by F-test at P=0.05.

3.2.3 Antibiosis Mechanism of Resistance to *H. armigera* in Wild Relatives of Chickpea

Different experiments *viz.* detached leaf assay, detached pod assay and diet incorporation assay were conducted to evaluate antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea.

3.2.3.1 Detached leaf assay

The plants grown in the field and in the greenhouse were used in the bioassays conducted under controlled condition in the laboratory (27±2 ºC temperature, 65 to 75% RH and photoperiod of 12 h). Agar-agar (3%) was boiled and poured in a slanting manner into plastic cups (4.5 x 11.5 cm diameter) with a thickness of 2.5 cm on one side of the plastic cup. The solidified agar-agar was used as a substratum for holding a chickpea branch. A terminal branch with 3 to 4 fully expanded leaves and a terminal bud was cut and immediately placed inside the cup in a slanting manner into agar-agar medium (Sharma *et al*., 2005d) (Plate 4). Care was taken to see that the chickpea branches did not touch the inner walls o f the cup. Ten neonate *H. armigera* larvae per replication were released on the chickpea leaves and covered with a lid to keep the chickpea leaves in turbid condition. The experiment was conducted in CRD with three replications. The experiment was terminated when more than 80 per cent of the leaf area was consumed in the susceptible control or when there were maximum differences between the resistant and susceptible checks (generally at 5 to 6 days after releasing the larvae). The test genotypes were evaluated for leaf feeding visually on 1 to 9 scale (1= <10% and 9= >80% area damaged). The number of larvae survived after the feeding period was recorded and weights of the larvae were recorded three hours after terminating the experiment. The data were expressed as percentage of larval survival and mean weight of the larvae.

Plate 4. Detached leaf assay

Plate 5. Detached pod assay

3.2.3.2 Detached pod assay

Relative resistance or susceptibility of wild relatives of chickpea to pod borer was evaluated with detached pod assay by using third instar larvae of *H. armigera* (Sharma *et al*., 2005a) (Plate 5). Detached inflorescences with pods were cut with the blades, and immediately placed in a slanting manner into 3% agar-agar medium in a plastic cup (4.5 x 11.5 cm diameter). There were five replications for each accession in a completely randomized design. A single third instar larva was released on chickpea branches with 4 to 6 pods in each plastic cup. Data were recorded on initial weight of the larva, weight of the larva after the feeding period, and percentage pods damaged after when there were maximum differences between the resistant and susceptible checks (generally at 4 to 5 days after releasing the larva). The percentage of weight gained by the larvae was computed as follows:

$$\text{Weight gained (\%)} = \frac{\text{(Final weight of the larva} - \text{Initial weight of the larva)}}{\text{Initial weight of the larva}} \times 100$$

Data on detached leaf and pod assay were subjected to ANOVA by using GENSTAT 14.0 version. The significance of differences between the treatments was measured by F-test at P=0.05, whereas the treatment means were compared using the least significant difference (LSD) at P=0.05.

3.2.3.3 Diet incorporation assay

The antibiosis component of resistance to *H. armigera* in wild relatives of chickpea was evaluated by rearing the neonate larvae on artificial diet impregnated with lyophilized leaf powders (Narayanamma *et al*., 2008) (Plate 6). The chickpea terminals or branches with tender green leaves were collected from the plants grown in the field and the glass house at full vegetative growth stage of the plant and placed in an icebox and eventually frozen at -20 ^{o}C (REMI, Model-RQF 425, Japan). The leaves were freeze dried in a lyophilizer (Modulyo D, Thermo Savant, Japan) at -45 ^{o}C temperature and pressure of 436 mbar for 3 to 4 days to avoid changes in chemical composition of the leaves. The leaves were then powdered and stored in a dessicator till used.

Dried powder of chickpea leaves (20 g) was incorporated into the artificial diet as a replacement for part of the chickpea flour for rearing of *H. armigera* (Table 3.4). The neonate larvae were released individually on the diet into the 25 cell-well

Table 3.4. Composition of artificial diet used for diet incorporation assay

Ingredients	**Quantity**
Chickpea flour	55 g
Chickpea lyophilized leaf powder	20 g
L-ascorbic acid	1.175 g
Sorbic acid	0.75 g
Methyl *p*-hydroxy benzoate	1.25 g
Aureomycin	2.875 g
Yeast	12 g
Formaldehyde (40%)	1.0 ml
Vitamin stock solution	2.5 ml
Water	112.5 ml
Agar-agar solution	
Agar-agar	4.325 g
Water	200 ml

H. armigera larvae rearing on artificial diet with lyophilized leaf powders

H. armigera pupae collected into a jar with vermiculite

H. armigera adults released in oviposition cage

Eggs laid by *H. armigera* adults on liners

Plate 6. Diet incorporation assay

plate with a fine camel hairbrush, each treatment was replicated thrice (25 larvae in each replication). The larvae were obtained from the insect culture maintained on chickpea flour based diet (Armes *et al*., 1992) in the laboratory at ICRISAT, Patancheru. The cell-wells were maintained at 27±2 ^{o}C temperature, 65 to 75% relative humidity and 12 h photoperiod after releasing neonates onto the diet. Data were recorded on larval survival and weights on 10th day after releasing the larvae into artificial diet. Pupal weights were recorded one day after pupation. Pupae from each replication were sterilized with 2% sodium hypochlorite solution and placed in a plastic jar containing moist vermiculite. Data were also recorded on larval and pupal periods. The adults were collected from the jars, and three pairs of adults that emerged on the same day on a particular genotype were placed inside a plastic cage and the numbers of eggs laid were counted. Percentage of larval survival on tenth day, and pupation and adult emergence were computed in relation to number of neonate larvae released in each replication. The data were subjected to ANOVA by using GENSTAT 14.0 version to test the significance of differences between treatments by F-test and the treatment means were compared by least significant difference at P=0.05.

3.3 MORPHOLOGICAL CHARACTERS OF WILD RELATIVES OF CHICKPEA IN RELATION TO EXPRESSION OF RESISTANCE TO *H. armigera*

Data were recorded on morphological characters such as trichome density on leaves and pod wall thickness. Data recorded on trichome density was correlated with oviposition of *H. armigera* and pod wall thickness was correlated with damage rating and weight gained percentage of larvae in detached pod assay for assessment of non-preference for oviposition and for feeding, respectively in different genotypes of wild relatives of chickpea.

3.3.1 Trichome Density

Trichome density in different wild relatives of chickpea genotypes were measured in accordance with Jackai and Oghiakhe (1989). The leaves were cut with scissor and were placed in acetic acid and alcohol (2:1) in stoppered glass vials (10 ml capacity) for 24 h to clear the chlorophyll and subsequently transferred into lactic acid (90%) as a preservative (Maiti and Bidinger, 1979). The presence of trichomes was recorded in minimum of 15 leaves from each accession and there were three

replications. The leaf sections were mounted on a glass slide in a drop of lactic acid and examined under a stereomicroscope (Zeiss. Inc., Thornwood, NY) at 10X magnification and expressed as number of trichomes/10X microscopic field.

3.3.2 Pod Wall Thickness

Pod wall thickness was measured using the vernier calipers for ten random pods per genotype for each replication likewise three replications were maintained. Three measurements in each pod were taken and averaged to compute pod wall thickness and represented in mm.

3.4 BIOCHEMICAL CHARACTERIZATION OF WILD RELATIVES OF CHICKPEA IN RELATION TO EXPRESSION OF RESISTANCE TO *H. armigera*

Different biochemical components such as proteins, phenols, total soluble sugars and tannins in lyophilized leaf powder of wild relatives of chickpea were estimated through spectrophotometric methods, whereas flavonoids in lyophilized leaf powders and leaf organic acids in fresh leaves were quantified through HPLC fingerprinting. Lectin and protease inhibitor activities were characterized from seeds of different genotypes.

3.4.1 Estimation of Proteins

The protein content was estimated as per Lowry *et al*. (1951) in different wild relatives of chickpea.

3.4.1.1 Preparation of reagents: Alkaline copper solution (reagent C) was prepared by mixing 50 ml of reagent A (2% sodium carbonate in 0.1 N sodium hydroxide) with 1 ml of reagent B (0.5% copper sulphate in 1.0% sodium potassium tartrate). Standard stock solution was prepared by dissolving 50 mg of bovine serum albumin in 50 ml of distilled water, from this 10 ml of the stock solution was diluted to 50 ml to obtain working standard with concentration of 200 µg of protein per ml.

3.4.1.2 Procedure: The sample (500 mg) was weighed and ground in a pestle and mortar in 5 to 10 ml of the sodium phosphate buffer. The sample was centrifuged, and the supernatant used for protein estimation. The sample extract of 0.1 ml was taken in test tubes along with working standards of 0.2, 0.4, 0.6, 0.8 and 1 ml whereas zero served as a blank. The volume in all tubes was made up to 1 ml with

distilled water. Reagent C (5 ml) was added to each tube including the blank. The solution was mixed well and allowed to stand for 10 min. To this solution 0.5 ml of Folin-Ciocalteu (FC) reagent (mixed with equal volume of water) was added and mixed well. The solution was incubated at room temperature in the dark for 30 min. The blue colour developed was read at 660 nm. The amount of protein was calculated from the standard curve and expressed in percentage.

3.4.2 Estimation of Phenols

The phenol content in wild relatives of chickpea genotypes were estimated as per the method presented by Bray and Thorpe (1954).

3.4.2.1 Principle: Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue) at 650 nm.

3.4.2.2 Preparation of reagents: 80 ml of ethanol was made upto 100 ml with distilled water to obtain 80% ethanol. Sodium carbonate (20%) was prepared by adding 20 g sodium carbonate in 100 ml of distilled water. Catechol (100 mg) was dissolved in 100 ml of distilled water and diluted 10 times for working standard, from the working standard different concentrations was taken from 0.1 to 1.0 ml.

3.4.2.3 Procedure: Lyophilised leaf sample of 500 mg was ground with 80% ethanol in a mortar and pestle. Centrifuged the homogenate at 10,000 rpm for 20 minutes, saved the supernatant and re-extracted the residue with five times volume of 80% ethanol. Supernatants were pooled and evaporated to dryness later residue was dissolved in a known volume of distilled water (5 ml). Different aliquots of the sample 0.2 to 2ml was pipetted out into the test tubes, made up the volume to 3 ml with distilled water and added 0.5 ml of FC reagent. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each test tube and mixed thoroughly. The tubes were placed in a boiling water bath for one minute and absorbance was recorded after cooling to room temperature at 650 nm. A standard curve was prepared using different concentrations of catechol. The concentration of the phenols in the test samples were obtained from the standard curve of catechol and expressed as mg per gram of sample.

3.4.3 Estimation of Tannins

Tannins in wild relatives of chickpea genotypes were estimated by vanillin hydrochloride method (Burns, 1971).

3.4.3.1 Principle: The vanillin reagent will react with any phenol that has an unsaturated resorcinol or pholoroglucinol nucleus and forms a coloured substituted product which is measured at 500nm.

3.4.3.2 Preparation of Reagents: Vanillin-hydrochloride reagent was prepared by adding equal volumes of 8% hydrochloric acid in methanol and 4% vanillin in methanol. The solution was prepared just before use. A stock solution was prepared by dissolving 1 mg of catechin in 1 ml of methanol. Then the stock solution was diluted to ten times to obtain final concentration of 100 µg/ ml.

3.4.3.3 Procedure: Extraction of tannins was done by homogenising one gram of lyophilised leaf powder in 50 ml of methanol and kept for continuous swirling for 20 to 28 h. After 28 h, centrifuged the contents and collected the supernatant. Pipetted out 1 ml aliquot of the sample into a test tube and added 5 ml of vanillin hydrochloride reagent. Mixed the contents and incubated it at room temperature for 20 min. Absorbance was recorded at 550 nm. A standard graph was prepared from the known concentrations of the catechin. From the standard graph, the amount of catechin was calculated as per the absorbance values and expressed as mg catechin equivalents per gram of sample.

3.4.4 Estimation of Total Soluble Sugars

Estimation of total carbohydrates in different wild relatives of chickpea genotypes was done as per the method developed by Hedge and Hofreiter (1962).

3.4.4.1 Principle: Carbohydrates dehydrated by concentrated H_2SO_4 to form furfural. Furfural so formed, condenses with the Anthrone to form a blue-green colored complex, which is colorimetrically measured at 630 nm.

3.4.4.2 Preparation of reagents: 2.5 N HCl was prepared by adding 21.4 ml of commercial HCl (11.7 N) to 78.6 ml of distilled water. Anthrone reagent was prepared fresh by dissolving 200 mg of anthrone in 100 ml of ice cold 95% H_2SO_4. Standard glucose stock was prepared by dissolving 100 mg of glucose in 100 ml of

distilled water and 10ml of stock was diluted to 100 ml to prepare working standard. It was stored in refrigerated condition after adding few drops of toluene.

3.4.4.3 Procedure: A sample of 100 mg was taken into boiling tube, after adding 5ml of 2.5 N HCl it was hydrolyzed in boiling water bath for three hours. After the sample was cooled to room temperature neutralised with solid sodium carbonate until effervescence ceases and volume in the flask was made up to 100 ml with distilled water. The sample was spun down once at 8000 rpm for 15 min in a centrifuge. The supernatant was collected and aliquots of 0.5 and 1.0 ml were used for estimation. The standards were prepared by using the 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard where as zero served as blank. The volume made up to 1 ml in all tubes including sample tubes with distilled water. Then 4 ml of anthrone reagent was added and kept on boiling water bath for 8 min. Absorbance was recorded at 630 nm. The amount of carbohydrate present in the sample tube was calculated by using standard graph and expressed in percentage.

3.4.5 Estimation of Organic Acids in Leaf Exudates

A standard protocol as suggested by Narayanamma *et al*. (2013) was followed for collection and analysis of organic acids from chickpea leaves.

3.4.5.1 Preparation of chemicals: Standards were prepared with two replicates of oxalic acid and malic acid by mixing 10 mg in 10 ml of water to get concentrations of 1000 ppm. Mobile phase of 25 mM KH_2PO_4 of pH 2.5 with H_3PO_4 was prepared, for this 6.805 g of KH_2PO_4 was weighed and taken in a 2 litre conical flask and mixed with 1 litre of millipore water until KH_2PO_4 was completely dissolved. Then 4 ml of H_3PO_4 was added and made up the volume to 1.8 L. The pH was adjusted to 2.5 by adding H_3PO_4 drop by drop, and finally made up the volume to 2 litres.

3.4.5.2 Extraction of leaf organic acids: The chickpea leaf samples were collected early in the morning (before 9 AM) in 15 ml centrifuge tubes containing 5 ml millipore water. The tubes were labelled for each genotype, and weight of the tube and water was recorded (initial weight). First fully expanded leaf from the plants was excised with scissors at random and placed in the respective tubes containing double distilled millipore water for 10 to 15 min. The weight of the tube with water and leaves was recorded (final weight) to compute fresh weight of the leaves.

The leaf exudates extracted in water were filtered through 0.22 µm hydrophilic PVDF millipore millex-HV filters using a 2.5 ml syringes. Sample solution of 2 ml was taken in syringe from the centrifuge tubes. The needle was removed from the syringe and attached to millipore filter to dispense 1.5 ml of the filtrate into the HPLC vials. Three replications were maintained for each sample and organic acids in the leaf exudates were quantified by HPLC.

3.4.5.3 HPLC procedure/protocol: After priming, the mobile phase was run for 1 h, the vials containing the leaf exudates of different chickpea genotypes were arranged in a carousel. The HPLC fingerprints were generated by using Atlantis dc-18 column (4.6 x 250 mm, 5µm). The sample retention time was recorded with a photodiode detector. Chromatographic separation was done with a flow rate of 0.8 ml min^{-1} using mobile phase and the injected volume of each sample was 20 µl with 20 min run time per sample (Plate 7).

Based on the standards retention time and peak areas, different organic acids present in the samples were identified and quantified. From the known concentrations of the standards, a linear curve was plotted against concentration on X-axis, and the absorbance on Y-axis. From the linearity curve, unknown concentrations of different organic acids from the leaf samples of different genotypes were plotted, and the amounts estimated. Amounts of organic acids present in a sample were expressed in mg g^{-1} fresh or dry weight basis.

3.4.6 Estimation of Flavonoids in Wild Relatives of Chickpea

Flavonoids were extracted by the method of Hahn *et al*. (1983) with slight modifications and analyzed using HPLC fingerprints.

3.4.6.1 Extraction of flavonoids: Lyophilized leaf sample (100 mg) was weighed and homogenized in 5 ml of HPLC grade methanol with mortar and pestle. Homogenized samples were centrifuged at 8000 rpm for 20 min and supernatant was collected. Hexane was added three times the volume of supernatant for partition in separation funnel and methanol phase was collected. This process was repeated for three times. Collected methanol phase was concentrated to volume of 2 ml in rotavapor. Concentrated samples were filtered through 0.22 µm millipore filter and injected into HPLC.

Plate 7. HPLC used for estimation of leaf organic acids and flavonoids

3.4.6.2 HPLC procedure/protocol: The samples and standards (20 µl) were chromatographed singly and in mixtures on a Waters Sunfire C_{18} column (4.6 X 250 mm) with 5 µm pore size. Waters High Performance Liquid Chromatography (HPLC) 2695 separations module (alliance) system consisting of a PCM 11 reciprocating piston pump and a 2996 photodiode array detector in the range of 190 to 800 nm was used in a gradient elution mode. Multistep gradient solvent system of 2% acetic acid in milliporewater (A) and 2% acetic acid in acetonitrile (B) was used for separation (Table 3.5) (Plate 7).

Table 3.5. Solvent system for separation of flavonoids through HPLC

Running time (min)	2% Acetic acid (A%)	Acetic acid-acetonitrile (B%)
0.00	95.00	5.00
10.00	95.00	5.00
17.50	85.00	15.00
31.00	85.00	15.00
41.00	50.00	50.00
45.00	50.00	50.00
50.00	85.00	15.00
55.00	95.00	5.00

3.4.7 Estimation of Midgut Protease Activity in *H. armigera*

3.4.7.1 Preparation of chemicals

Standard stocks of Nα-benzoyl-L-arg-p-nitroanilide (BA*p*NA) (0.1 M), N-succinyl-ala-ala-pro-phe-pnitroanilide (SAAPF*p*NA) (10 mg/ml) and Leucine-p-nitronilide (L*p*NA) (10 mM) (Sigma-Aldrich) were prepared in dimethyl sulfoxide.

3.4.7.2 Extraction of *H. armigera* midgut proteases

Larvae subjected to detached pod assay on different genotypes of wild relatives of chickpea were collected from the pods for the estimation of proteinase activity in their midgut after the termination of the assay. Midguts were removed by dissecting the larvae and kept frozen at -80 ^{o}C till used. The isolated midguts were homogenized in one volume of 0.1 M glycine-NaOH buffer (pH 10.0) in dounce homogenizer. The homogenate was centrifuged at 12,000 rpm for 15 min at 4 ^{o}C and the supernatants were used as enzyme source. Protein concentration in supernatants was quantified according to Lowry's method using BSA as a standard protein (Lowry *et al.*, 1951).

3.4.7.3 Total protease activity assay

Total protease activity was determined by azo-caseinolytic assay using 1% azocasein as a substrate (Visweshwar *et al*., 2015). Gut extract (100 µl) was mixed with 500 µl of 1% azocasein in 0.1 M glycine-NaOH buffer (pH 10.0) and incubated for 30 min at 37 ºC. The reaction was stopped by adding 200 µl of 5% trichloroacetic acid (TCA) and the sample was centrifuged at 12,000 rpm for 15 min. An equal volume of 1 N NaOH was added to the supernatant and absorbance was read at 450 nm. Specific activity was expressed as an increase in optical density/min/mg gut protein.

Units (U) = ΔABS/ Incubation time (min) × mg of protein

3.4.7.4 Specific proteolytic activity assay

Trypsin, chymotrypsin and aminopeptidase activities were estimated using enzyme specific substrates BA*p*NA, SAAPF*p*NA and L*p*NA, respectively (Visweshwar *et al*., 2015). The 1 ml of reaction mixture containing 50 µl of enzyme extract, 2 mM of substrate in 0.1 M glycine-NaOH buffer (pH 10.0) was incubated for 20 min at 37 °C. The reaction was stopped by adding 300 µl of 30% acetic acid. The samples were centrifuged at 10,000 rpm for 10 min and absorbance was read at 410 nm. Specific enzyme activity corresponds to the hydrolysis of 1 µmol substrate/min/mg of gut protein.

$$\text{Units (U)} = \frac{\text{OD} \times \text{dilution factor} \times \text{total reaction volume}}{\varepsilon_{mm} \times \text{mg of protein} \times \text{time (min)}}$$

Where, ε_{mm} is the extinction co-efficient for the liberated pNA, i.e., 8.8 at 410 nm.

Dilution factor = Total reaction volume (ml)/ Volume of enzyme (ml)

3.4.8 Protease Inhibitors (PI) in Wild Relatives of Chickpea against *H. armigera*

3.4.8.1 Extraction of PIs from seeds

Matured seeds of wild relatives of chickpea were washed with water, dried and ground to a fine powder. The seed powder was defatted with hexane and depigmented with acetone in six washes. Solvent was filtered off and the seed powder was air dried. The seed powders were homogenized using pestle and mortar in 0.1 M sodium phosphate buffer (pH 7.0) and kept overnight at 4 ºC for extraction of PIs with intermittent shaking. The suspension was centrifuged at 12,000 rpm for

20 min at 4 ºC and the supernatant was used as source of PIs. Protein content of the extract was determined by using BSA as a standard protein (Lowry *et al*., 1951).

3.4.8.2 Detection of PIs by dot-blot method

A simple, rapid and sensitive technique, called X-ray film method was used for the estimating serine protease inhibitor activity (Pichare and Kachole 1994). Porcine trypsin and chymotrypsin solutions were prepared in 0.1 M Tris-HCl buffer, pH 8.0 to obtain a final concentration of 0.1 mg ml^{-1}. Three varying concentrations of the enzyme and inhibitor 3:1, 1:1, and 1:3 (v/v), were prepared. The volume of the reaction mixture was adjusted with Tris-HCl buffer for trypsin and chymotrypsin. The final volume was made upto 20 µl, and then spotted onto a strip of X-ray film. Spots were incubated for 20 min on X-ray film depending on the extent of gelatin hydrolysis. The film was washed with warm water. When the inhibitor is present, the trypsin/chymotrypsin did not degrade the gelatin on the X-ray film. When the inhibitor was absent, a clear zone formed at the site of sample application on the X-ray film.

3.4.8.3 Extraction of *H. armigera* Gut Proteases (HGPs)

The late third or the early fourth instar larvae were collected from homogenous culture of *H. armigera* and they were used for extraction of HGPs. Larval midguts were isolated by dissecting the larvae and homogenized in one volume of 0.1 M glycine-NaOH buffer (pH 10.0) and centrifuged at 12,000 rpm for 15 min at 4 ºC and the supernatant was used as source of gut proteinases.

3.4.8.4 *H. armigera* Gut Protease Inhibitory (HGPI) Assays

Protease inhibitory assays were performed by mixing and pre-incubation of a suitable amount of seed extract as a source of inhibitor (20 μl) and HGPs extract (50 μl) for 30 min at 37 ºC prior to the addition of substrate (Udamale *et al*., 2013). The residual trypsin, chymotrypsin, and total gut protease activities were estimated by using the substrates BA*p*NA, SAAPF*p*NA, and azocasein, respectively.

One PI unit was defined as the amount of inhibitor that causes inhibition of one unit of proteinase activity under the given assay conditions. In all the inhibitory assays, protease activity of the suitable control was performed without mixing the seed extract and HGP inhibitory units per gram sample (U g^{-1}) was calculated with respect to their activity in the control.

3.4.8.5 Electrophoretic visualization of HGPs

Visualization of HGPs isoforms was carried out on non-reducing, denatured Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Laemmli, 1970). Composition of SDS-PAGE has shown hereunder (Table 3.6.)

Table 3.6. Composition for 10% SDS-PAGE system

	Resolving gel (10%)	**Stacking gel (5%)**
Distilled water (ml)	4.10	2.85
Buffer (ml)	2.60	1.25
Acrylamide (30%) (ml)	3.40	0.90
Sodium dodecyl sulfate (10% SDS) (µl)	100	60
Ammonium per sulphate (10%) (µl)	150	80
TEMED (N,N,N,N- tetramethyl ethylene diamine) (µl)	12	18

3.4.8.5.1 Preparation of Chemicals: Lower buffer (1.5 M Tris-HCl, pH 8.8) used in resolving gel was prepared by dissolving 18.5 g of tris-(hydroxymethyl) aminomethane in 100 ml distilled water and pH was adjusted to 8.8 using HCl. Upper buffer (0.5 M Tris-HCl, pH 6.8) used in stacking gel was prepared by dissolving 3 g of tris (hydroxymethyl) aminomethane in 50 ml distilled water and pH was adjusted to 6.8 using HCl. Acrylamide (30%) was prepared by dissolving 29.2 g acrylamide and 0.8 g bis-acrylamide (N,N-methylenebisacrylamide) in distilled water and made upto 100ml. Loading dye consisted of 0.2% bromophenol blue, 4% SDS and 20% glycerol in 0.1M Tris-HCl buffer, pH 6.8. Ammonium persulphate (10%) was prepared fresh. Staining solution was prepared by mixing 40 ml of methanol, 10 ml acetic acid and 0.2 g Coomassie Brilliant Blue R-250 (CBB) and made upto 100 ml with distilled water. Destaining solution was prepared with same composition of staining solution except the addition of CBB. Tank running buffer was prepared by dissolving 0.25 M tris-(hydroxymethyl) aminomethane, 0.2 M glycine, 0.1% SDS in distilled water.

3.4.8.5.2 Procedure: For the gel casting, vertical slab gel electrophoresis was used (115×110×2 mm). Ten per cent resolving gel was used for polymerization. The wells in the stacking gel (5%) was poured on top with 50 µl of HGPs (mixed with 15 µl of loading dye) and electrophoresis was carried out at 100 V at a constant current of 25 mA. After 2.5 h, when the tracking dye front reached the bottom, , the gel was removed and washed for 10 min with 2.5% Triton X-100 to remove SDS and then

incubated in 2% casein in 0.1 M glycine-NaOH, pH 10, for 1 h, and the gel was then stained with Coomassie Brilliant Blue R-250 for 5 min. HGPs bands were visualised as white bands with dark blue background after destaining.

3.4.8.6 Electrophoretic Visualization of Trypsin Inhibitors (TIs)

Trypsin inhibitor (TIs) isoforms were detected by using 10% polyacrylamide gel incorporated with 1% gelatine (Felicioli *et al*., 1997). After electrophoresis, the gels were transferred to 0.01% porcine trypsin in 0.1M Tris-HCl buffer, pH 8.0, and incubated for 1 h with constant shaking for gelatine hydrolysis. After that the gels were washed with distilled water then stained with Coomassie Brilliant Blue R-250 and destained. Protease inhibitor isoforms were detected as dark blue bands against white background due to the complex of nonhydrolyzed gelatine with staining.

3.4.9 Detection of Lectin Activity in Wild Relatives of Chickpea

3.4.9.1 Hemagglutination Assay

The seeds of wild relatives of chickpea were homogenized in pestle and mortar in 0.1 M sodium phosphate buffered saline (PBS) (0.15 M NaCl), pH 7.4, and the suspension was centrifuged at 10,000 rpm. The supernatant was subjected to ammonium sulphate precipitation (70%). The protein pellet was collected by centrifugation and resuspended in PBS and dialyzed extensively against the same.

A modified method for Banerjee *et al*. (2011) was followed for preparation of erythrocyte suspension. Human blood (O+ve) was collected into a syringe (2 ml) and the blood was immediately transferred to microfuge tube containing a pinch of EDTA and it was centrifuged at 5,000 rpm for 10 min at 4 ^{o}C. Subsequently, the erythrocyte solution was prepared by repeated washing with PBS and spun at 5,000 rpm for 10 minutes at 4 ^{o}C. After each cycle, the supernatant was carefully removed. The erythrocytes obtained in this manner were found to be free from leucocytes and cell debris. The pellet was resuspended in PBS and obtained final concentration of 2% erythrocyte suspension.

Agglutination activity of the lectins isolated from wild relatives of chickpea was assayed by the hemagglutination technique as described by Sultan and Swamy (2005) with slight modification. The suspension of human erythrocytes (50 µl) in PBS was mixed with serially diluted samples of the lectin extract (10 to 50 µl) in a

96 well microtitre plate and incubated at room temperature for 1 h. Then hemagglutination was observed with the unaided eye.

3.4.9.2 Electrophoretic visualization of lectins

Nondenaturing-polyacrylamide gel electrophoresis (Native-PAGE) was performed with a discontinuous buffer system for the detection of lectins. Basic gel electrophoresis (10% PAGE system) was carried out in the Davis buffer system (Davis, 1964) followed by periodic acid-Schiff staining as modified by Doerner and White (1990).

3.4.9.2.1 Preparation of Schiff's reagent: Schiff's reagent was prepared as per method by Kodousek (1969). Rosaniline hydrochloride (basic fuchsin) of 1g was ground and dissolved in 10 ml absolute ethanol in a 250 ml flask and shaken gently for some time after that 186 ml of cold distilled water was added. Pure sodium metabisulphite (5 g) was added followed by 3.4 ml of concentrated HCl. The dye was precipitated by metabisulphite but redissolved quickly with the addition of acid. Finally, 0.25 g sodium dithionate was added which resulted in immediate decolourization of red solution to a light yellowish shade. After stirring with approximately 2 g of activated pulverized charcoal for about 3 min and subsequent filtration, a perfectly colourless solution was obtained. The volume was adjusted to 200 ml with 0.2 N HCl. As the reagent is very sensitive, it was stored at 4 ^{o}C immediately.

3.4.9.2.2 Periodic acid-Schiff's staining: After electrophoretic run, the gels were transferred and incubated in 7.5% acetic acid for 30 min and then with 1% periodic acid for 20 min, then followed by three washings of 15% acetic acid for 15 min. After that the Schiff's reagent was added and incubated for 30 min. The Schiff's reagent was removed and the gels were washed in 7.5% acetic acid about six times for 1 h. Reddish-pink bands of stained glycoprotein would then be visible.

3.4.10 Estimation of Leaf Surface Chemicals through GC-MS using Methanol and Hexane Extracts

Leaf surface chemical present in different wild relatives of chickpea were identified with Gas Chromatography-Mass Spectrometry (GC-MS) using methanol and hexane as suitable solvent system in relation to differential levels of expression of resistance to *H. armigera*.

3.4.10.1 Extraction of leaf surface chemicals

The chickpea leaf samples were collected early in the morning (before 9 AM) in 15 ml centrifuge tubes containing 2 ml of solvent methanol or hexane. The tubes were labelled for each genotype and first fully expanded leaf from the plants was excised with scissors at random and placed in the respective tubes containing methanol or hexane for 10 to 15 min. The leaves were removed from the tubes and the solvent extracts were filtered through 0.22 µm millipore filter and injected into GC-MS.

3.4.10.2 GC-MS protocol/procedure

GC-MS measurements were obtained with GC-MS QP 2010Ultra equipped with an autosampler AOC 20 *i* series (Plate 8). The following conditions were used: ion source temp. 240°C, column CBP 5, 25 m x 0.2 mm i.d., 0.25µm film thickness column (Shimadzu, Kyoto, Japan), carrier gas helium at constant flow of 1 ml min^{-1}, temperature program: 50°C (2 min), 50 to 280°C (10 min), 280°C (10 min); injection temperature: 250°C, interface temperature: 280°C, solvent cut time 3 min, splitless injection, mass range of m/z 20 to m/z 600. Data acquisition and evaluation run were with GC Solutions 4.1. Identification of a selected set of metabolites was based on the measurements of reference compounds in WILEY and NIST library.

3.5 GENETIC DIVERSITY OF WILD RELATIVES OF CHICKPEA EXHIBITING RESISTANCE/SUSCEPTIBILITY TO *H. armigera* USING SSR MARKERS

A total of 26 SSR markers were selected based on linkage map reported by Winter *et al*. (2000) to assess the genetic diversity of the wild relatives of chickpea. SSR markers usually consist of di or tri nucleotide sequence repeats. These are also known as the microsatellite markers, they are co-dominant in nature, and distributed throughout the genome.

3.5.1 Extraction of DNA from the Seedlings of Wild Relatives of Chickpea

The genotypes of wild relatives of chickpea were grown in small plastic cups in the glasshouse after scarification and soaking of the seed for 24 hrs. Sampling of the plant material was done at ten days after seedling emergence. The extraction of DNA from the sampled material was done using CTAB method (Mace *et al*., 2003)

Plate 8. GC-MS used for estimation of leaf surface chemicals through hexane and methanol extracts

with slight modifications. The procedure adopted for 96 well plate DNA extraction was as follows.

3.5.1.1 Reagents required

1. 3% CTAB (Cetyl Trimethyl Ammonium Bromide) buffer having 10 mM Tris, 1.4 M NaCl, 20 mM EDTA and 3% CTAB. The pH was adjusted to 8.0 using HCl. Just before use, mercaptoethanol (0.17%) was added.
2. Chloroform-isoamyl alcohol mixture (24:1) stored in the dark at room temperature
3. Ice-cold isopropanol
4. RNase-A (10 mg/ml) dissolved in solution containing 10 mM Tris (pH 7.5) and 15 mM NaCl stored at -20°C; working stocks were stored at 4°C.
5. Phenol-chloroform-iso-amyl alcohol mixture (25:24:1)
6. 3 M sodium acetate (pH 5.2)
7. Ethanol (absolute and 70%)
8. $T_1E_{0.1}$ buffer (10 mM Tris and 1 mM EDTA)
9. $T_{10}E_1$ buffer (0.5 M Tris and 0.05 M EDTA)

3.5.1.2 DNA sample preparation

Steel balls (4 mm in diameter and 3 numbers per extraction tube), pre-chilled at -20°C for about 30 minutes, were added to the 12 x 8 well strip extraction tubes with strip caps (Marsh Biomarket, USA) that were kept on ice. Before initiation of DNA extraction, 3% CTAB buffer was preheated on a water bath at 65°C (Precision Scientific model: shaking water bath 50). The leaf samples of genotypes were collected from the glasshouse grown plants by cutting them into small pieces (approximately 30 mg). The samples were then transferred to extraction tubes fitted into a 96- tube box.

3.5.1.3 Grinding and extraction

To each extraction tube containing the leaf sample and pre chilled steel balls, 450 µl of preheated 3% CTAB buffer was added. Grinding was carried out using a Sigma Geno-Grinder (Spex Certiprep, USA) at 500 strokes per minute for 5 min. It was repeated until the leaf strip pieces were sufficiently macerated. After the first round of grinding, the boxes were checked for leakage by taking them out from the

Geno-Grinder and shaken for proper mixing of leaf tissue with buffer. After proper grinding, the box with the tubes was fixed in a locking device and incubated at 65 °C in a water bath for 20 minutes with occasional shaking.

3.5.1.4 Solvent extraction

Chloroform: isoamyl alcohol (24: 1) mixture of 450 µl was added to each tube, tubes were inverted twice and the samples centrifuged at 6200 rpm for 10 minutes (Sigma centrifuge 4K15C with QIAGEN rotor model NR09100: 2 x 120 g). After centrifugation, the aqueous layer (approximately 300 µl) was transferred to a fresh tube (Marsh Biomarket).

3.5.1.5 Initial DNA precipitation

To the each tube containing aqueous layer, 0.7 volumes (approximately 210 µl) of cold isopropanol (kept at -20°C) was added. The solution was carefully mixed and the tubes were kept at -20°C for 10 min. The samples were centrifuged at 6200 rpm for 15 minutes, and the supernatant decanted under the fume hood and pellets were dried.

3.5.1.6 RNase A treatment

In order to remove co-isolated RNA, pellets were dissolved into 200 µl of low salt $T_1E_{0.1}$ buffer and 3 µl of RNase A (stock 10 mg/µl). The solution was incubated at 37 °C for 30 min or overnight at room temperature.

3.5.1.7 Solvent extraction

After incubation, 200 µl of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added to each tube, mixed and centrifuged at 5000 rpm for 10 minutes. The aqueous phase in each tube was transferred to a fresh tube (Marsh Biomarket) and 200 µl of chloroform: isoamyl alcohol (24: 1) was added to each tube, mixed and centrifuged at 5000 rpm for 10 minutes. The aqueous layer was transferred to fresh tube.

3.5.1.8 DNA precipitation

To the aqueous layer, 15 µl (approximately $1/10^{th}$ volume) of 3 M sodium acetate (pH 5.2) and 300 µl (2 volumes) of absolute ethanol (kept at -20 °C) were added and the mixtures were subsequently incubated in a freezer (-20 °C) for 5 min and the tubes were centrifuged at 6200 rpm for 15 min.

3.5.1.9 Ethanol wash

After centrifugation, the supernatant was carefully decanted from each tube in order to ensure that the pellet remained inside the tube. Subsequently, 200 µl of 70% ethanol was added to each of the tubes, followed by centrifugation at 5000 rpm for 5 minutes.

3.5.1.10 Final re-suspension

The supernatant was carefully decanted and pellet allowed to air dry for one hour. Dried pellets were re-suspended in 100 µl of $T_{10}E_1$ buffer and kept overnight at room temperature to dissolve completely. The re-suspended DNA samples were stored at 4 °C.

3.5.2 DNA Quantification and Quality Check

The quality and quantity of DNA were checked by agarose gel electrophoresis as described below

3.5.2.1 Reagents preparation:

3.5.2.1.1TBE buffer (1X): For 10X TBE buffer, 109 g of Tris and 55 g of boric acid were dissolved one by one in 800 ml distilled water, then 40 ml of 0.5 M EDTA (pH 8.0) was added. The volume was made up to 1 litre with distilled water and sterilized by autoclaving. This was stored at 4 °C. To prepare working solution (1X), the stock solution was diluted 10 times.

3.5.2.1.2 Ethidium bromide (10 mg/ml): A quantity of 100 mg ethidium bromide was dissolved in 10 ml of distilled water. The vessel containing this solution was wrapped in aluminium foil and stored at 4 °C.

3.5.2.1.3 Orange loading dye: Mixed 10 ml of 0.5 M EDTA (pH 8.0), 1 ml of 5 M NaCl, 50 ml of glycerol and 39 ml of distilled water and orange dye powder (Orange G, GurrCertistain®) was added till the color became sufficiently dark.

3.5.2.1.4 Procedure: A quantity of 0.8 g of agarose was added to 100 ml of 1X TBE buffer and the slurry was heated using microwave oven until the agarose was completely dissolved. After cooling the solution to about 60 °C, 5µl of ethidium bromide solution was added and the resulting mixture was poured into the gel-casting tray for solidification. Before the gel solidified, an acrylic comb of desired well number was placed on the agarose solution to form wells for loading samples. Each well was loaded with 5 µl of sample aliquot having 3 µl distilled water, 1 µl Orange dye and 1 µl of DNA sample. The DNA samples of known concentration (lambda DNA of 5 and 10 ng/µl) were also loaded on to the gel to estimate the DNA concentration of the experimental samples. The gel was run at 70 V for 20 minutes. After completing the electrophoresis run, DNA on the gel was visualized under UV light and photographed. The DNA was normalized to 5 ng/µl concentration with visual comparison by loading DNA samples with the standard lambda DNA.

3.5.4 Selection of SSR Markers for Diversity Analysis

A total of 26 SSR markers previously reported by Winter *et al*. (1999) (TA-, TAA-, GA-, TR- and TS-series), Nayak *et al.* (2010) (ICCM-series), Thudi *et al.* (2011) (CaM-series) and NCPGR- series developed by Sethy *et al*. (2006a) and Gaur *et al.* (2011) were used in this study. The pre-determined SSR markers were selected based on their coverage and distribution on the linkage groups. The 26 SSR markers representing all the linkage groups (8 chromosomes) of the chickpea were selected for diversity analysis (Table 3.7).

3.5.5 Polymerase chain reaction (PCR) amplification

Components of PCR mixture were presented hereunder Table 3.8. PCR reactions were conducted in 384 well micro-titre plates in a GeneAmp PCR system 9700 Perkin Elmer (Applied Biosystem, USA) DNA thermocycler.

Table 3.8. Components of polymerase chain reaction (PCR) mixture

Component	Stock concentration	Volume (µl)
DNA	5 ng/µl	1.00
Primers	10 pm/µl	0.50
$MgCl_2$	25 mM	1.00
Buffer	10X	0.50
dNTPs	2 mM	0.25
Taq polymerase enzyme	0.5 U/µl	0.20
Millipore water		1.55
Total		5.00

Table 3.7. Details of SSR markers used to assess genetic diversity in wild relatives of chickpea

Marker	Linkage group	SSR motif	Forward primer 5'→ 3'	Reverse primer 5'→ 3'
H2E13	7	(GA)9	TGGGGGTATACATGAATTGAATAA	AATCCCATCAATGTTGTACTTTTC
CaM1515	3	(AG)5n(TA)18	GCAATGAGAAGGGAAGGAAA	GCGGAAAACCAATTTACCAA
CaM0958	7	(TTA)7	TCGTATATGAAGCCAATGTTGC	AAATTTTGTTGTCCTTTTCATCA
ICCM0249	4	(T)12n(TAA)29	TTTCTTCGCATGGGCTTAAC	GGAGATTTGTTGGGTAGGCTC
ICCM0120a	5	(TTA)12	TGTCTCGATAAGAGTTTGTTATTTTTC	CGTTTTGTTTCATATTCAAACTCG
TAA58	7	(AAT)41	CATTGCTTAAGAACCAAAATGG	CAATTTTACATCGA CGTGTGC
GA6	8	(GA)23	ATTTTTCTCCGGTGTTGCAC	AAACGACAGAGAGTGGCGAT
TA21	7	(TAA)51	GTACCTCGAAGATGTAGCCGATA	TTTTCCATTTAGAGTAGGATCTTCTTG
NCPGR21	4	(CT)15	TCTACCTCGTTTTTCGTGCC	TTGCTCCTTCAACAAAACCC
TA71	5	(ATT)32	CGATTTAACACAAAACACAA	CCTATCCATTGTCATCTCGT
TA200	2	(TTA)37	TTTCTCCTCTACTATTATGATCACCAG	TTGAGAGGGTTAGAACTCATTATGTTT
TA142	3	(TTA)15	TGTTAACATTCCCTAATATCAATAACTT	TTCCACAATGTTGTATGTTTTGTAAG
CaM0244	6	(TCTCT)6	TTTCCCCTTCTTTCTCAACA	TTCAGAGATTGGATGAGAAGGTT
GAA47	4	(GAA)11	CACTCCTCATGCCAACTCCT	AAAATGGAATAGTCGTATGGGG
CaM2064	6	(TA)8n(TA)5	AGATCACCAATTGGGAACAAA	CACCTTCTCGACTTTCCTATGTG
ICCM0130a	8	(ATT)22	GGATTTCGACTTTTATCCCTTTTT	CGGACTGGAATCAAAAGCTC
CaM0799	5	(T)10n(ATT)6	TGGAGCATTGTCACTTAAGCC	ACGGTGTCGAACACACCATA
CaM1451	1	(TTAT)7	AGACGTGGTCAACCACAAAA	CACAACTTAATTATGCCCCCA
TA116	5	(TAA)5TT(A)3(TAA)20	AATTCAATGACGAATTTTTATAAGGG	AAAAAGAAAAGGGAAAAGTAGGTTTTA
CaSTMS11	4	(GA)20	GTATCTACTTGTAATATTCTCTTCTCT	ATATCATAAACCCCCCAC
CaM2036	8	(GAGG)5	TGTGCGACCAATTTTGTTGT	CTGATAGGAACCCGGATTGA
NCPGR19	7	(GA)19	TCCATTGTAGCTTAGCTTAG	TCTTACTCTTAGCTTACCTCTT
TA59	2	(TAA)29	ATCTAAAGAGAAATCAAAATTGTCGAA	GCAAATGTGAAGCATGTATAGATAAAG
TR42	1	(TAA)57	TCTGTCATTCATAATGA TGTATTCT	CAACTCAACATGCTTTAATTGA T
GA16	2	(GA)22	CACCTCGTACCATGGTTTCTG	TAAATTTCATCCTCTCCGGC
TA30	1	(TAA)18TA(TAA)19	TCATTAAAATTCTATTGTCCTGTCCTT	ATCGTTTTTCTAAACTAAATTGTGCAT

For separation of amplicons using capillary electrophoresis M-13 tailed, and direct flourophore labelled primers were used. The M-13 tailed forward primer from each primer pair was labelled with different flourophores, FAM (Blue), VIC (Green), NED (Yellow) and PET (Red) (Applied Biosystems) before amplification. The reactions were performed in volumes of 5 µl.

3.5.6 Reaction Conditions for the PCR Program

A touch down PCR program was used to amplify the DNA fragments. Initial denaturation was done for 5 minutes at 94 °C (to activate the *Taq* DNA polymerase), subsequently 10 cycles of denaturation at 94 °C for 20 s, annealing at 60 °C for 30 s (temperature reduced by 1 °C each cycle) and extension at 72 °C for 30 s. This was followed by 40 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s with the final extension of 20 min at 72 °C to ensure amplification to equal lengths of both DNA strands.

3.5.7 Capillary Electrophoresis

3.5.7.1 Sample preparation

After confirming the PCR amplification on 1.2% agarose gel, the PCR products were separated by capillary electrophoresis using ABI prism 3730XL automatic DNA-sequencer (Applied Biosystems, USA). The capillary electrophoresis technique has a resolution of less than 2 bp and hence, can be used to clearly distinguish polymorphisms of less than 2 bp. Moreover, as this technique is a fluorescence based detection system, it dispenses with the need for radioactive or laborious manual polyacrylamide gel screening techniques. Prior to electrophoresis, multiplexing was done *i.e.,* the amplified products of primers labelled with different dyes or same flourophores labelled primers with non-overlapping amplicons (in terms of size) were pooled. For multiplexing, 1 µl of each of the amplified products were pooled and mixed with 0.25 µl of GeneScan LIZ 500 size standard (Applied Biosystems) and 7 µl of Hi-Di formamide (Applied Biosystems) and 2.8 µl of distilled water. This final product was then denatured for 5 minutes at 95 °C (Perkin Elmer 9700, Applied Biosystems) and cooled immediately and resolved in automated 96 capillary ABI 3730xl DNA analyser.

3.5.7.2 SSR fragment analysis

The electrophoregram containing trace files produced from ABI Prism 3730 xl DNA analyzer were analysed using GeneMapper version 4.0 (Applied Biosystems) to size the peak patterns in relation to the internal size standard GeneScan 500™ LIZ®. GeneMapper version 4.0 software automatically calculates the size of the unknown DNA sample fragments by generating a calibration sizing curve based upon the migration times of the known fragments in the standard. The peaks were displayed with base pair size and height (amplitude) values in a chromatogram and the allelic data were exported into excel spread sheet for further analysis.

3.5.7.3 Diversity analysis

Summary statistics for all the markers was derived using PowerMarker v 3.25 software (Liu and Muse, 2005). This software uses the following formulas to calculate different parameters:

3.5.7.3.1 Major allele frequency

Major allele frequency = Number of genotypes having major allele x 100

Total number of genotypes

3.5.7.3.2 Gene diversity: Gene diversity, often referred to as expected heterozygosity is defined as the probability that two randomly chosen alleles from the population are different. An unbiased estimator of gene diversity at the l^{th} locus is

$$He = (1 - \sum_{i=1}^{n} P_i^2)/(1 - \frac{1+f}{n})$$

Where Pi = i^{th} allele frequency, f = inbreeding coefficient, n = number of individuals

3.5.7.3.3 Heterozygosity: Heterozygosity is the proportion of heterozygous individuals in the population. At a single locus it was estimated as

$$Hi = 1 - \sum_{i=1}^{k} P_i$$

Where, P_i = i^{th} allele frequency

3.5.7.3.4 Polymorphism Information Content (PIC): As per Botstein *et al*. (1980) polymorphism information content (PIC) was estimated as

$$PIC = 1 - \left[\sum_{i=1} P_i^2\right] - \left[\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2\right]$$

Where, P_i and P_j are the frequencies of i^{th} and j^{th} alleles

3.5.7.3.5 Dissimilarity matrix: Dissimilarity matrix was calculated using PowerMarker v 3.25 software. Dissimilarity was calculated (Perrier *et al*., 2003) by pair-wise simple matching using the following formula as follows

$$d_{ij} = 1 - \frac{1}{L}\sum_{i=1}^{L} \frac{m_i}{\pi}$$

Where, d_{ij} = dissimilarity between units *i* and *j*, L = number of loci, π= ploidy, m_i = number of matching alleles for locus *i.*

3.5.7.3.6 Dendrogram/tree construction: Genetic dissimilarities among wild relatives of chickpea genotypes were calculated and dendrogram was constructed using un-weighted pair group method with arithmetic mean (UPGMA) as implemented in PowerMarker v 3.25 software.

Chapter ~ IV

Results & Discussion

Chapter IV

RESULTS AND DISCUSSION

The results of the present investigation "Biochemical and molecular mechanisms of resistance to *Helicoverpa armigera* (Hubner) in wild relatives of chickpea" are presented hereunder. The experiments were conducted in the field, glasshouse and laboratory conditions at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana State, India, during 2014-15 and 2015-16.

4.1 ABUNDANCE OF POD BORERS, *Helicoverpa armigera* AND *Spodoptera exigua* ON WILD RELATIVES OF CHICKPEA UNDER NATURAL INFESTATION

Under field conditions, observations were recorded on abundance of pod borers on wild relatives of chickpea at fortnight intervals during post-rainy season 2014-15 and 2015-16. The results are presented hereunder (Table 4.1 to 4.14).

4.1.1 Oviposition by the *H. armigera* on Different Genotypes of Wild Relatives of Chickpea

Oviposition by *H. armigera* was not significantly different among genotypes except at 75 days after emergence (DAE) during post-rainy season, 2014-15 (Table 4.5). Highest number of eggs per five plants were recorded on PI 599109 (9.00) followed by PI 599046 (3.50), IG 70018 (3.00), IG 70006 (2.50), ICC 3137 (2.00), IG 70022 (1.00) and PI 599077 (1.00) while no oviposition was observed on other genotypes. During post-rainy season, 2015-16 no significant differences were observed among genotypes in terms of number of eggs per five plants except at 15 DAE (Table 4.8). Highest number of eggs per five plants was observed on KAK 2 and IG 72933 (3.00), while no ovipostion was observed on IG 599076, IG 69979, IG 70006, IG 70022, PI 510663, PI 599046, PI 599077 and PI 599109. Oviposition was not observed on any genotype at 60, 90 and 105 DAE.

The peak oviposition activity was observed on all genotypes at 30 DAE *i.e.* last week of November with a range of 11.50 in IG 70006 and 0.50 in PI 510663 and again reduced at 45 and 60 DAE during post-rainy season, 2014-15. Suganthy *et al.* (2003) reported that number of eggs laid by *H. armigera* per 20 plants were high

(44.30) even at 15 days after sowing (DAS), while maximum number of eggs (67.30/ 20 plants) were recorded at 50 DAS and maximum egg laying was observed during second fortnignt of December. This relative preference for oviposition on different genotypes is thought to arise from the balance between attractants and deterrents from genotypes to which the insect respond (Renwick and Chew, 1994).

4.1.2 Abundance of *H. armigera* Larvae on Different Genotypes of Wild Relatives of Chickpea

Significant differences were exhibited among different genotypes of wild relatives of chickpea with respect to abundance of *H. armigera* larvae throughout cropping period during post-rainy season, 2014-15 except at 30 DAE. All genotypes of wild relatives recorded less number of larvae compared to cultivated chickpea. At 15 DAE, highest number of larvae were recorded on ICC 3137 and ICCL 86111 (14.50 larvae/5 plants) followed by KAK 2 (14.00 larvae/5 plants), while lowest number of larvae was recorded on IG 599076 and ICCW 17148 (3.00 larvae/5 plants) (Table 4.1). At 45 DAE, highest number of larvae observed was 13.00 larvae/5 plants in ICC 3137 followed by 12.00 larvae/5 plants (KAK 2) and lowest was 1.00 larvae/5 plants in IG 599076 (Table 4.3). Larval abundance was observed in a range of 2.00 larvae/5 plants (IG 599076) and 18.50 larvae/5 plants (IG 72933) at 60 DAE, except on ICC 3137 (32.50 larvae/5 plants) which was significantly highest compared to all other genotypes (Table 4.4). At 75 DAE, all genotypes recorded significantly less number of larvae compared to susceptible check, ICC 3137 (Table 4.5). All genotypes of wild relatives showed significantly less number of larvae compared to cultivated chickpea at 90 DAE, where highest larval count was recorded on ICC 3137 (33.00 larvae/5 plants) and lowest was recorded on PI 510663 (1.00 larvae/5 plants) followed by IG 69979 (2.00 larvae/5 plants) (Table 4.6). At 105 DAE, observations were recorded only on wild relatives of chickpea, as cultivated genotypes attained their physiological maturity (Table 4.7), among which lowest number of larvae were recorded on ICCW 17148 (20.50 larvae/5 plants) and highest was observed on IG 70022 (40.50 larvae/5 plants).

During post-rainy season, 2015-16 significant differences were exhibited among genotypes of wild relatives of chickpea with respect to larval abundance of *H. armigera* throughout cropping period except at 105 DAE. Low larval counts were recorded on all genotypes of wild relatives (except *C. reticulatum*) compared to

Table 4.1. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 15 DAE (Post-rainy season, 2014-15)

Species	Genotype	Numbers per 5 plants		DR
		***H. armigera* larvae**	***S. exigua* larvae**	
C. chrossanicum	IG 599076	3.00 (1.85) [a]	0.00 (0.71)	3.00 [abc]
C. cuneatum	IG 69979	4.00 (2.11) [ab]	0.00 (0.71)	2.50 [ab]
C. bijugum	IG 70006	7.00 (2.71) [abcd]	0.00 (0.71)	2.50 [ab]
C. bijugum	IG 70012	9.00 (3.08) [bcde]	1.00 (1.14)	3.50 [abc]
C. bijugum	IG 70018	7.50 (2.83) [abcde]	0.00 (0.71)	3.25 [abc]
C. bijugum	IG 70022	10.00 (3.23) [cde]	0.50 (0.97)	3.84 [bc]
C. reticulatum	IG 72933	6.00 (2.54) [abcd]	0.00 (0.71)	3.50 [abc]
C. reticulatum	IG 72953	6.00 (2.52) [abcd]	0.00 (0.71)	3.50 [abc]
C. pinnatifidum	PI 510663	7.00 (2.71) [abcd]	0.00 (0.71)	2.50 [ab]
C. judaicum	PI 568217	6.00 (2.54) [abcd]	0.50 (0.97)	2.50 [ab]
C. bijugum	PI 599046	9.00 (3.08) [bcde]	0.50 (0.97)	3.00 [abc]
C. bijugum	PI 599066	10.00 (3.23) [cde]	0.00 (0.71)	3.50 [abc]
C. judaicum	PI 599077	4.50 (2.23) [abc]	0.50 (0.97)	2.75 [ab]
C. pinnatifidum	PI 599109	9.00 (3.04) [bcde]	0.00 (0.71)	3.00 [abc]
C. microphyllum	ICCW 17148	3.00 (1.85) [a]	0.50 (0.97)	2.50 [ab]
C. arietinum	JG 11 (C)	12.50 (3.59) [de]	0.00 (0.71)	4.00 [bc]
C. arietinum	KAK 2 (S)	14.00 (3.81) [e]	0.50 (0.97)	4.50 [c]
C. arietinum	ICC 3137 (S)	14.50 (3.86) [e]	0.00 (0.71)	6.50 [d]
C. arietinum	ICCL 86111 (R)	14.50 (3.85) [e]	1.00 (1.14)	3.50 [abc]
C. arietinum	ICC 506 EB (R)	6.50 (2.60) [abcd]	0.00 (0.71)	2.00 [a]
	Fpr	**0.006**	**NS**	**0.01**
	Mean	**2.92**	**0.83**	**3.29**
	SE±	**0.31**	**0.21**	**0.48**
	LSD (P= 0.05)	**0.93**	**-**	**1.43**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$ (DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.2. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 30 DAE (Post-rainy season, 2014-15)

Species	Genotype	Numbers per 5 plants				DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* egg masses	*S. exigua* larvae	
C. chrossanicum	IG 599076	1.50 (1.35)	3.50 (1.94)	0.00 (0.72)	3.50 (2.02) ^{b}	3.80 ^{cd}
C. cuneatum	IG 69979	1.00 (1.14)	3.00 (1.78)	0.00 (0.71)	0.00 (0.71) ^{a}	3.00 ^{bcd}
C. bijugum	IG 70006	11.50 (3.20)	4.00 (2.07)	0.00 (0.71)	0.00 (0.71) ^{a}	2.50 ^{bc}
C. bijugum	IG 70012	2.50 (1.67)	5.50 (2.39)	0.00 (0.71)	0.50 (0.97) ^{a}	3.50 ^{cd}
C. bijugum	IG 70018	1.00 (1.14)	2.00 (1.58)	0.00 (0.71)	0.00 (0.71) ^{a}	2.00 ^{ab}
C. bijugum	IG 70022	5.00 (1.97)	4.50 (1.89)	0.00 (0.71)	1.00 (1.14) ^{a}	3.00 ^{bcd}
C. reticulatum	IG 72933	8.00 (2.77)	7.50 (2.82)	0.00 (0.71)	0.00 (0.71) ^{a}	2.50 ^{bc}
C. reticulatum	IG 72953	1.00 (1.14)	4.50 (2.16)	0.00 (0.71)	0.00 (0.71) ^{a}	2.50 ^{bc}
C. pinnatifidum	PI 510663	0.50 (0.97)	2.00 (1.55)	0.00 (0.71)	0.00 (0.71) ^{a}	6.00 ^{e}
C. judaicum	PI 568217	3.50 (2.00)	4.00 (1.81)	0.00 (0.71)	1.50 (1.29) ^{a}	4.00 ^{d}
C. bijugum	PI 599046	4.00 (1.81)	4.50 (2.21)	0.00 (0.71)	0.50 (0.97) ^{a}	2.00 ^{ab}
C. bijugum	PI 599066	4.00 (2.12)	5.00 (2.30)	0.00 (0.71)	0.50 (0.97) ^{a}	4.00 ^{d}
C. judaicum	PI 599077	1.50 (1.29)	1.50 (1.29)	0.00 (0.71)	0.00 (0.71) ^{a}	3.50 ^{cd}
C. pinnatifidum	PI 599109	6.50 (2.60)	5.00 (2.34)	0.50 (0.97)	0.00 (0.71) ^{a}	2.50 ^{bc}
C. microphyllum	ICCW 17148	1.50 (1.29)	2.50 (1.73)	0.00 (0.71)	2.00 (1.41) ^{ab}	3.00 ^{bcd}
C. arietinum	JG 11 (C)	4.50 (2.23)	4.50 (2.21)	0.00 (0.71)	0.00 (0.71) ^{a}	2.00 ^{ab}
C. arietinum	KAK 2 (S)	7.50 (2.63)	10.00 (3.24)	0.00 (0.71)	0.00 (0.71) ^{a}	3.00 ^{bcd}
C. arietinum	ICC 3137 (S)	5.00 (2.34)	8.00 (2.87)	0.00 (0.71)	0.50 (0.97) ^{a}	7.00 ^{e}
C. arietinum	ICCL 86111 (R)	8.00 (2.83)	9.50 (2.97)	0.00 (0.71)	0.00 (0.71) ^{a}	3.50 ^{cd}
C. arietinum	ICC 506 EB (R)	4.00 (1.81)	5.50 (2.43)	0.00 (0.71)	0.00 (0.71) ^{a}	1.00 ^{a}
	Fpr	**NS**	**NS**	**NS**	**0.041**	**<0.001**
	Mean	**1.92**	**2.18**	**0.72**	**0.91**	**3.22**
	SE±	**0.71**	**0.59**	**0.06**	**0.22**	**0.42**
	LSD (p=0.05)	**-**	**-**	**-**	**0.66**	**1.23**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.3. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 45 DAE (Post-rainy season, 2014-15)

Species	Genotype	Numbers per 5 plants					DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* egg masses	*S. exigua* larvae	*C. chlorideae* cocoons	
C. chrossanicum	IG 599076	0.00 (0.71)	1.00 (1.10) [a]	0.00 (0.71)	10.00 (3.24)	0.00 (0.71)	5.07 [ef]
C. cuneatum	IG 69979	0.50 (0.97)	5.00 (2.34) [a-f]	0.00 (0.71)	1.50 (1.40)	0.00 (0.71)	1.75 [ab]
C. bijugum	IG 70006	0.00 (0.71)	3.00 (1.78) [a-d]	0.00 (0.71)	9.50 (3.03)	0.00 (0.71)	1.75 [ab]
C. bijugum	IG 70012	0.00 (0.71)	2.50 (1.73) [abc]	0.00 (0.71)	7.00 (2.56)	0.50 (0.97)	3.00 [bcd]
C. bijugum	IG 70018	0.50 (0.97)	5.00 (2.35) [a-f]	0.50 (0.97)	3.00 (1.63)	0.00 (0.71)	2.75 [bcd]
C. bijugum	IG 70022	0.00 (0.71)	3.50 (1.96) [a-e]	0.00 (0.71)	7.00 (2.63)	0.00 (0.71)	3.75 [cde]
C. reticulatum	IG 72933	0.00 (0.71)	11.50 (3.43) [def]	0.00 (0.71)	8.50 (2.90)	2.50 (1.73)	3.50 [cd]
C. reticulatum	IG 72953	4.00 (1.81)	8.50 (2.97) [b-f]	0.00 (0.71)	5.50 (2.45)	1.00 (1.14)	3.00 [bcd]
C. pinnatifidum	PI 510663	0.50 (0.97)	3.00 (1.87) [a-e]	0.00 (0.71)	4.00 (1.81)	0.50 (0.97)	4.00 [def]
C. judaicum	PI 568217	2.00 (1.41)	5.50 (2.39) [a-f]	0.00 (0.71)	5.00 (2.25)	0.50 (0.97)	2.50 [abcd]
C. bijugum	PI 599046	0.00 (0.71)	5.50 (2.33) [a-f]	0.00 (0.71)	7.00 (2.63)	0.00 (0.71)	2.50 [abcd]
C. bijugum	PI 599066	0.00 (0.71)	5.00 (2.34) [a-f]	0.00 (0.71)	2.00 (1.55)	0.00 (0.71)	2.75 [bcd]
C. judaicum	PI 599077	0.00 (0.71)	3.50 (2.00) [a-f]	0.00 (0.71)	2.50 (1.73)	0.00 (0.71)	2.25 [abc]
C. pinnatifidum	PI 599109	0.00 (0.71)	2.00 (1.41) [ab]	0.00 (0.71)	7.50 (2.63)	0.00 (0.71)	2.50 [abcd]
C. microphyllum	ICCW 17148	0.00 (0.71)	4.50 (2.07) [a-f]	0.00 (0.71)	0.00 (0.71)	0.50 (0.97)	3.25 [bcd]
C. arietinum	JG 11 (C)	5.50 (2.39)	10.00 (3.24) [c-f]	0.00 (0.71)	8.00 (2.83)	1.00 (1.14)	1.75 [ab]
C. arietinum	KAK 2 (S)	3.00 (1.87)	12.00 (3.49) [ef]	0.00 (0.71)	3.50 (1.89)	0.50 (0.97)	3.00 [bcd]
C. arietinum	ICC 3137 (S)	5.50 (2.39)	13.00 (3.65) [f]	0.00 (0.71)	6.00 (2.55)	1.00 (1.14)	5.50 [f]
C. arietinum	ICCL 86111 (R)	7.00 (2.26)	11.00 (3.34) [c-f]	0.00 (0.71)	5.50 (2.39)	1.50 (1.29)	1.75 [ab]
C. arietinum	ICC 506 EB (R)	1.50 (1.90)	8.50 (2.90) [b-f]	0.00 (0.71)	5.00 (1.97)	0.50 (0.97)	1.00 [a]
	Fpr	**NS**	**0.045**	**NS**	**NS**	**NS**	**<.001**
	Mean	**1.17**	**2.43**	**0.72**	**2.24**	**0.93**	**2.87**
	SE±	**0.54**	**0.49**	**0.06**	**0.67**	**0.25**	**0.49**
	LSD (P=0.05)	**-**	**1.45**	**-**	**-**	**-**	**1.44**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.4. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 60 DAE (Post-rainy season, 2014-15)

Species	Genotype	Numbers per 5 plants					DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* egg masses	*S. exigua* larvae	*C. chlorideae* cocoons	
C. chrossanicum	IG 599076	1.50 (1.39)	2.00 (1.56) ^{a}	0.00 (0.71)	3.00 (1.85) ^{f}	0.00 (0.73)	3.84 ^{bc}
C. cuneatum	IG 69979	0.00 (0.71)	10.50 (3.27) $^{a-e}$	0.00 (0.71)	0.00 (0.71) ^{a}	0.00 (0.71)	2.50 ^{ab}
C. bijugum	IG 70006	3.00 (1.85)	8.00 (2.89) $^{a-e}$	0.00 (0.71)	1.00 (1.14) $^{a-e}$	0.50 (0.97)	2.50 ^{ab}
C. bijugum	IG 70012	5.00 (2.25)	5.50 (2.43) ^{abc}	0.00 (0.71)	0.00 (0.71) ^{a}	0.00 (0.71)	3.00 ^{abc}
C. bijugum	IG 70018	6.50 (2.38)	3.50 (2.00) ^{ab}	0.00 (0.71)	2.00 (1.55) ^{ef}	0.50 (0.97)	3.25 ^{abc}
C. bijugum	IG 70022	2.50 (1.53)	3.50 (2.00) ^{ab}	0.00 (0.71)	0.50 (0.97) $^{a-d}$	1.00 (1.22)	3.50 ^{abc}
C. reticulatum	IG 72933	5.50 (2.23)	18.50 (4.36) ^{ef}	0.00 (0.71)	0.00 (0.71) ^{a}	0.00 (0.71)	4.00 ^{bc}
C. reticulatum	IG 72953	4.00 (2.07)	11.00 (3.38) $^{b-e}$	0.00 (0.71)	0.00 (0.71) ^{a}	1.00 (1.22)	3.50 ^{abc}
C. pinnatifidum	PI 510663	1.00 (1.14)	4.00 (2.11) ^{ab}	0.00 (0.71)	0.00 (0.71) ^{a}	1.00 (1.22)	3.50 ^{abc}
C. judaicum	PI 568217	3.00 (1.85)	7.00 (2.68) $^{a-d}$	0.00 (0.71)	0.00 (0.71) ^{a}	0.50 (0.97)	2.50 ^{ab}
C. bijugum	PI 599046	3.50 (1.96)	4.00 (2.07) ^{ab}	0.00 (0.71)	1.00 (1.22) ^{ade}	0.00 (0.71)	3.00 ^{abc}
C. bijugum	PI 599066	0.50 (0.97)	3.50 (2.00) ^{ab}	0.00 (0.71)	0.00 (0.71) ^{a}	0.50 (0.97)	3.50 ^{abc}
C. judaicum	PI 599077	0.00 (0.71)	6.00 (2.41) ^{abc}	0.50 (0.97)	0.50 (0.97) $^{a-d}$	0.00 (0.71)	3.00 ^{abc}
C. pinnatifidum	PI 599109	1.50 (1.29)	3.00 (1.63) ^{a}	0.00 (0.71)	0.00 (0.71) ^{a}	0.00 (0.71)	2.75 ^{ab}
C. microphyllum	ICCW 17148	1.00 (1.14)	5.00 (2.34) ^{ab}	0.00 (0.71)	0.00 (0.71) ^{a}	0.50 (0.97)	2.50 ^{ab}
C. arietinum	JG 11 (C)	4.00 (1.81)	16.50 (4.09) ^{cde}	0.00 (0.71)	1.00 (1.22) $^{a-e}$	1.50 (1.40)	2.50 ^{ab}
C. arietinum	KAK 2 (S)	2.00 (1.58)	17.00 (4.18) ^{def}	0.00 (0.71)	0.00 (0.71) ^{ab}	1.00 (1.14)	4.50 ^{c}
C. arietinum	ICC 3137 (S)	2.00 (1.41)	32.50 (5.72) ^{f}	0.00 (0.71)	0.00 (0.71) ^{abc}	0.50 (0.97)	6.50 ^{d}
C. arietinum	ICCL 86111 (R)	0.00 (0.71)	6.50 (2.56) $^{a-d}$	0.00 (0.71)	0.00 (0.71) ^{abc}	0.50 (0.97)	3.50 ^{abc}
C. arietinum	ICC 506 EB (R)	1.00 (1.14)	5.00 (2.25) ^{ab}	0.00 (0.71)	0.00 (0.71) ^{abc}	0.00 (0.71)	2.00 ^{a}
	Fpr	**NS**	**<.001**	**NS**	**<.001**	**NS**	**0.01**
	Mean	**1.51**	**2.79**	**0.72**	**0.91**	**0.93**	**3.29**
	SE±	**0.59**	**0.50**	**0.06**	**0.15**	**0.20**	**0.48**
	LSD (P=0.05)	**-**	**1.47**	**-**	**0.45**	**-**	**1.43**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.5. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 75 DAE (Post-rainy season, 2014-15)

Species	Genotype	Numbers per 5 plants					DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* egg masses	*S. exigua* larvae	*C. chlorideae* cocoons	
C. chrossanicum	IG 599076	0.00 (0.73) [a]	3.00 (1.88) [a]	1.00 (1.20)	3.00 (1.87) [c]	0.00 (0.74)	2.84 [ab]
C. cuneatum	IG 69979	0.00 (0.71) [a]	3.00 (1.85) [a]	0.00 (0.71)	0.00 (0.71) [a]	1.00 (1.14)	2.50 [a]
C. bijugum	IG 70006	2.50 (1.67) [ab]	4.50 (2.21) [ab]	0.50 (0.97)	0.00 (0.71) [a]	0.00 (0.71)	2.50 [a]
C. bijugum	IG 70012	0.00 (0.71) [a]	4.50 (2.23) [ab]	0.00 (0.71)	0.00 (0.71) [a]	0.00 (0.71)	3.50 [ab]
C. bijugum	IG 70018	3.00 (1.85) [ab]	5.00 (2.35) [ab]	0.00 (0.71)	0.50 (0.97) [ab]	1.50 (1.29)	3.50 [ab]
C. bijugum	IG 70022	1.00 (1.14) [a]	3.50 (1.96) [a]	0.50 (0.97)	1.00 (1.22) [b]	1.00 (1.14)	3.50 [ab]
C. reticulatum	IG 72933	0.00 (0.71) [a]	5.00 (2.34) [ab]	0.50 (0.97)	0.00 (0.71) [a]	0.00 (0.71)	4.50 [bc]
C. reticulatum	IG 72953	0.00 (0.71) [a]	3.50 (2.00) [a]	0.00 (0.71)	0.00 (0.71) [a]	0.50 (0.97)	4.00 [ab]
C. pinnatifidum	PI 510663	0.00 (0.71) [a]	4.00 (1.98) [a]	0.00 (0.71)	0.00 (0.71) [a]	0.00 (0.71)	3.00 [ab]
C. judaicum	PI 568217	0.00 (0.71) [a]	2.00 (1.58) [a]	0.00 (0.71)	0.50 (0.97) [ab]	1.00 (1.14)	2.50 [a]
C. bijugum	PI 599046	3.50 (2.00) [ab]	3.00 (1.87) [a]	0.00 (0.71)	0.50 (0.97) [ab]	0.00 (0.71)	3.50 [ab]
C. bijugum	PI 599066	0.00 (0.71) [a]	4.00 (2.12) [ab]	0.50 (0.97)	0.50 (0.97) [ab]	1.00 (1.14)	4.00 [ab]
C. judaicum	PI 599077	1.00 (1.14) [a]	3.00 (1.87) [a]	0.00 (0.71)	0.00 (0.71) [a]	0.00 (0.71)	3.00 [ab]
C. pinnatifidum	PI 599109	9.00 (2.82) [b]	3.00 (1.85) [a]	0.00 (0.71)	0.00 (0.71) [a]	0.00 (0.71)	3.00 [ab]
C. microphyllum	ICCW 17148	0.00 (0.71) [a]	3.00 (1.85) [a]	0.00 (0.71)	0.00 (0.71) [a]	0.50 (0.97)	2.50 [a]
C. arietinum	JG 11 (C)	0.00 (0.71) [a]	9.00 (3.08) [b]	0.00 (0.71)	0.00 (0.71) [a]	0.50 (0.97)	3.50 [ab]
C. arietinum	KAK 2 (S)	0.00 (0.71) [a]	5.00 (2.35) [ab]	0.00 (0.71)	0.00 (0.71) [a]	0.00 (0.71)	6.00 [cd]
C. arietinum	ICC 3137 (S)	2.00 (1.41) [a]	15.50 (3.98) [c]	0.00 (0.71)	0.00 (0.71) [a]	0.00 (0.71)	7.00 [d]
C. arietinum	ICCL 86111 (R)	0.00 (0.71) [a]	3.50 (1.96) [a]	0.00 (0.71)	0.00 (0.71) [a]	0.00 (0.71)	3.50 [ab]
C. arietinum	ICC 506 EB (R)	0.00 (0.71) [a]	3.00 (1.85) [a]	0.00 (0.71)	0.00 (0.71) [a]	0.50 (0.97)	2.50 [a]
	Fpr	**0.03**	**0.01**	**NS**	**<.001**	**NS**	**0.01**
	Mean	**1.06**	**2.16**	**0.78**	**0.84**	**0.88**	**3.54**
	SE±	**0.39**	**0.30**	**0.12**	**0.12**	**0.23**	**0.55**
	LSD (P=0.05)	**1.15**	**0.88**	**-**	**0.36**	**-**	**1.65**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.6. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 90 DAE (Post-rainy season, 2014-15)

Species	Genotype	Numbers per 5 plants					DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* egg masses	*S. exigua* larvae	*C. chlorideae* cocoons	
C. chrossanicum	IG 599076	0.00 (0.71)	3.00 (1.86) [a]	0.50 (0.97)	4.50 (2.23)	1.00 (1.20)	4.13 [bcde]
C. cuneatum	IG 69979	0.00 (0.71)	2.00 (1.58) [ab]	0.00 (0.71)	0.00 (0.71)	1.00 (1.22)	2.00 [a]
C. bijugum	IG 70006	1.00 (1.14)	7.50 (2.76) [ab]	0.00 (0.71)	4.50 (2.07)	1.00 (1.22)	3.50 [abc]
C. bijugum	IG 70012	0.00 (0.71)	4.50 (2.21) [ab]	0.00 (0.71)	1.50 (1.40)	1.00 (1.22)	4.00 [bcd]
C. bijugum	IG 70018	0.00 (0.71)	2.50 (1.67) [ab]	0.00 (0.71)	1.50 (1.29)	1.00 (1.14)	6.00 [e]
C. bijugum	IG 70022	0.00 (0.71)	2.50 (1.73) [ab]	0.00 (0.71)	0.50 (0.97)	0.00 (0.71)	5.50 [de]
C. reticulatum	IG 72933	1.00 (1.14)	6.50 (2.64) [ab]	0.00 (0.71)	0.00 (0.71)	2.50 (1.67)	3.50 [abc]
C. reticulatum	IG 72953	1.00 (1.14)	9.00 (3.08) [b]	0.00 (0.71)	3.50 (1.72)	3.50 (2.00)	3.50 [abc]
C. pinnatifidum	PI 510663	0.00 (0.71)	1.00 (1.22) [ab]	0.00 (0.71)	4.50 (1.89)	1.00 (1.22)	4.00 [bcd]
C. judaicum	PI 568217	0.00 (0.71)	3.50 (1.96) [ab]	0.00 (0.71)	0.50 (0.97)	2.50 (1.73)	3.00 [ab]
C. bijugum	PI 599046	0.00 (0.71)	3.50 (2.00) [ab]	0.00 (0.71)	1.00 (1.22)	0.50 (0.97)	3.50 [abc]
C. bijugum	PI 599066	0.00 (0.71)	5.00 (2.34) [ab]	0.00 (0.71)	1.00 (1.14)	1.00 (1.14)	3.50 [abc]
C. judaicum	PI 599077	2.00 (1.41)	3.00 (1.87) [ab]	0.50 (0.97)	1.00 (1.14)	0.00 (0.71)	2.00 [a]
C. pinnatifidum	PI 599109	0.00 (0.71)	4.00 (2.11) [ab]	0.00 (0.71)	1.00 (1.14)	0.00 (0.71)	4.50 [bcde]
C. microphyllum	ICCW 17148	0.00 (0.71)	3.00 (1.85) [ab]	0.00 (0.71)	1.50 (1.29)	2.00 (1.55)	3.00 [ab]
C. arietinum	JG 11 (C)	0.00 (0.71)	7.00 (2.73) [ab]	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	5.00 [cde]
C. arietinum	KAK 2 (S)	-	-	-	-	-	-
C. arietinum	ICC 3137 (S)	0.00 (0.71)	33.00 (5.58) [c]	0.00 (0.71)	0.00 (0.71)	1.50 (1.29)	8.00 [f]
C. arietinum	ICCL 86111 (R)	0.00 (0.71)	8.00 (2.91) [b]	0.00 (0.71)	0.50 (1.06)	1.00 (1.18)	4.50 [bcde]
C. arietinum	ICC 506 EB (R)	-	-	-	-	-	-
	Fpr	**NS**	**0.005**	**NS**	**NS**	**NS**	**<.001**
	Mean	**0.81**	**2.34**	**0.73**	**1.24**	**1.20**	**4.08**
	SE±	**0.26**	**0.48**	**0.09**	**0.41**	**0.27**	**0.57**
	LSD (P=0.05)	**-**	**1.45**	**-**	**-**	**-**	**1.70**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at p ≤ 0.05(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.7. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 105 DAE (Post-rainy season, 2014-15)

Species	Genotype	Numbers per 5 plants		DR
		H. armigera larvae	*C. chlorideae* cocoons	
C. chrossanicum	IG 599076	-	-	-
C. cuneatum	IG 69979	22.00 (4.72) ^{ab}	4.50 (2.21) ^{ab}	3.00 ^{a}
C. bijugum	IG 70006	29.00 (5.43) ^{bcd}	8.00 (2.89) ^{ab}	3.75 ^{abc}
C. bijugum	IG 70012	29.00 (5.42) ^{bcd}	10.50 (3.24) ^{b}	4.00 ^{abc}
C. bijugum	IG 70018	35.00 (5.96) ^{def}	7.50 (2.79) ^{ab}	6.00 ^{d}
C. bijugum	IG 70022	40.50 (6.40) ^{f}	9.50 (3.15) ^{b}	5.50 ^{cd}
C. reticulatum	IG 72933	37.50 (6.16) ^{ef}	3.00 (1.87) ^{a}	6.50 ^{d}
C. reticulatum	IG 72953	30.50 (5.57) ^{cde}	5.00 (2.35) ^{ab}	6.25 ^{d}
C. pinnatifidum	PI 510663	28.00 (5.34) ^{bcd}	3.00 (1.85) ^{a}	4.00 ^{abc}
C. judaicum	PI 568217	28.00 (5.34) ^{bcd}	7.50 (2.82) ^{ab}	3.50 ^{ab}
C. bijugum	PI 599046	37.50 (6.16) ^{ef}	3.50 (1.96) ^{a}	5.00 ^{bcd}
C. bijugum	PI 599066	35.00 (5.96) ^{def}	7.00 (2.71) ^{ab}	5.00 ^{bcd}
C. judaicum	PI 599077	25.00 (5.05) ^{abc}	3.50 (2.00) ^{a}	3.50 ^{ab}
C. pinnatifidum	PI 599109	38.50 (6.24) ^{ef}	4.50 (2.21) ^{ab}	5.50 ^{cd}
C. microphyllum	ICCW 17148	20.50 (4.58) ^{a}	3.50 (2.00) ^{a}	3.00 ^{a}
C. arietinum	JG 11 (C)	-	-	-
C. arietinum	KAK 2 (S)	-	-	-
C. arietinum	ICC 3137 (S)	-	-	-
C. arietinum	ICCL 86111 (R)	-	-	-
C. arietinum	ICC 506 EB (R)	-	-	-
	Fpr	**<.001**	**0.047**	**0.004**
	Mean	**5.59**	**2.43**	**4.61**
	SE±	**0.21**	**0.30**	**0.55**
	LSD (P=0.05)	**0.64**	**0.92**	**1.69**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

cultivated chickpea throughout cropping period. At 15 DAE, highest number of larvae were recorded on IG 72953 (14.50 larvae/5 plants) followed by ICC 3137 (11.00 larvae/5 plants), while lowest was recorded on PI 599109 (1.00 larva/5 plants) (Table 4.8). At 30 DAE, highest number of larvae were recorded on IG 72933 (17.50 larvae/5 plants) followed by ICC 3137 (14.50 larvae/5 plants), while lowest was recorded on IG 599076 (1.50 larvae/5 plants) and IG 69979 (2.00 larvae/5 plants) (Table 4.9). At 45 DAE, larval count was observed in a range of 1.50 larvae/5 plants (PI 568217) to 12.50 larvae/5 plants (IG 72933) (Table 4.10). The genotype, ICC 3137 recorded highest number of larvae (11.50 larvae/5 plants) compared to all other genotypes, while genotypes IG 70012 and IG 70022 recorded lowest number of larvae (1.00 larva/5 plants) at 60 DAE (Table 4.11). Among all genotypes, lowest of 1.00 larva/5 plants was recorded on IG 599076, PI 510663, PI 568217 and PI 599109, whereas highest of 11.50 larvae/5 plants was recorded on ICC 3137 at 75 DAE (Table 4.12). All wild relatives of chickpea exhibited larval count in a range of 3.00 larvae/5 plants (IG 70018 and PI 568217) to 13.50 larvae/5 plants (IG 72933), whereas cultivated genotypes exhibited a range of 5.00 larvae/5 plants (ICC 506EB) to 25.00 larvae/5 plants (ICC 3137) at 90 DAE (Table 4.13).

During both the seasons the genotypes, IG 70012, IG 70018, IG 70022, PI 510663, PI 599109, PI 599077, ICCW 17148 and IG 69979 recorded significantly lower numbers of *H. armigera* larvae compared to cultivated chickpea. The larval abundance was observed throughout the crop growth period, while the peak larval abundance was recorded during 60 and 90 DAE during post-rainy season, 2014-15, whereas 90 DAE during post-rainy season, 2015-16. The present results are in agreement with Rao *et al*. (2001) who observed pod borer damage on chickpea at 38 days after sowing (DAS) whereas the peak incidence was recorded at 87 DAS. Suganthy *et al*. (2003) also revealed that pest incidence started at 15 DAS, then gradually increased until first peak at 29 DAS, second peak at 57 DAS and the third peak at 85 DAS. Yadav and Jat (2009) reported that, the infestation of *H. armigera* on chickpea started in the second fortnight of November and reached its peak by the end of February. The larval population of the pest occurred throughout the crop growth period and maximum population was recorded at pod formation and grain developmental stages. Similar results were also observed by Altaf *et al*. (2008) and Yadav *et al*. (2016).

4.1.3 Oviposition by *S. exigua* on Different Genotypes of Wild Relatives of Chickpea

There were no significant differences in oviposition by *S. exigua* (number of eggmass per five plants) among all the genotypes throughout crop growth period during post-rainy seasons, 2014-15 and 2015-16.

4.1.4 Abundance of *S. exigua* Larvae on Different Genotypes of Wild Relatives of Chickpea

Though there were significant differences in abundance of *S. exigua* larvae on different genotypes of wild relatives of chickpea at 30, 60 and 75 DAE during post-rainy season, 2014-15, the number of larvae were very less to the time for assessing levels of resistance (Table 4.2, 4.4 and 4.5, respectively). During post-rainy season, 2015-16 significant differences were observed in abundance of *S. exigua* larvae on different genotypes of wild relatives of chickpea only at 45, 90 and 105 DAE. At 45 DAE, highest larval count was observed on JG 11 (6.50 larvae/5 plants) followed by IG 599076 and ICCL 86111 (4.00 larvae/5 plants) and lowest (0.50 larva/5 plants) was observed on IG 70018, IG 72933, PI 568217 and PI 599066 (Table 4.10). Highest larval count was recorded on IG 70006 (9.00 larvae/5 plants), while lowest was observed on PI 568217, PI 599077 and ICC 506EB (0.50 larva/5 plants) at 90 DAE (Table 4.13). Highest larval count of 7.00 larvae/5 plants (PI 599066) was recorded at 105 DAE among the observed genotypes, while no incidence was observed on IG 69979 and ICCW 17148 (Table 4.14).

Among all the genotypes, IG 599076 was highly suffered against *S. exigua* larvae throughout cropping period. During this post-rainy season, 2014-15, highest abundance was observed at 45 DAS but there were no significant differences between genotypes with respect to larval count, while no larvae was recorded on any genotype at 105 DAE. The present findings are in agreement with findings of Shankar (2013) who reported that *S. exigua* population was high during early stage of the crop than the later stages. On contrary, larval population was observed during later stages i.e. at 90 and 105 DAE during post-rainy season, 2015-16.

4.1.5 Parasitisation of *H. armigera* by Larval Parasitoid, *Campoletis chlorideae*

During post-rainy season, 2014-15 parasitisation of *H. armigera* by *C. chlorideae* was first observed at 45 DAE, but significant differences among

Table 4.8. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 15 DAE (Post-rainy season, 2015-16)

Species	Genotype	Numbers per 5 plants				DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* egg masses	*S. exigua* larvae	
C. chrossanicum	IG 599076	0.00 (0.71) ^{a}	4.00 (2.15) ^{abcd}	0.00 (0.71)	0.00 (0.71)	2.50 ^{abc}
C. cuneatum	IG 69979	0.00 (0.71) ^{a}	2.00 (1.55) ^{abc}	0.00 (0.71)	0.00 (0.71)	3.50 ^{bcd}
C. bijugum	IG 70006	0.00 (0.71) ^{a}	3.00 (1.78) ^{abcd}	0.00 (0.71)	0.00 (0.71)	1.00 ^{a}
C. bijugum	IG 70012	1.00 (1.14) ^{abcd}	2.00 (1.58) ^{abc}	0.50 (0.97)	0.50 (0.97)	1.00 ^{a}
C. bijugum	IG 70018	0.50 (0.97) ^{abc}	1.50 (1.40) ^{ab}	0.00 (0.71)	1.00 (1.22)	1.00 ^{a}
C. bijugum	IG 70022	0.00 (0.71) ^{a}	3.50 (1.96) ^{abcd}	0.00 (0.71)	9.50 (2.76)	1.00 ^{a}
C. reticulatum	IG 72933	3.00 (1.78)^{cd}	9.00 (3.04) ^{cde}	0.00 (0.71)	5.00 (1.97)	3.00^{bc}
C. reticulatum	IG 72953	1.50 (1.40)^{abcd}	14.50 (3.78)^{e}	0.00 (0.71)	1.50 (1.29)	5.00^{d}
C. pinnatifidum	PI 510663	0.00 (0.71)^{a}	2.50 (1.67)^{abcd}	0.00 (0.71)	1.50 (1.29)	2.50^{abc}
C. judaicum	PI 568217	0.50 (0.97)^{abc}	2.50 (1.73)^{abcd}	0.00 (0.71)	3.00 (1.63)	2.00^{ab}
C. bijugum	PI 599046	0.00 (0.71)^{a}	1.50 (1.40)^{ab}	0.50 (0.97)	0.00 (0.71)	1.00^{a}
C. bijugum	PI 599066	2.00 (1.58)^{acd}	5.50 (2.45)^{abcde}	0.50 (0.97)	0.50 (0.97)	1.00^{a}
C. judaicum	PI 599077	0.00 (0.71)^{a}	2.00 (1.55)^{abc}	0.00 (0.71)	0.00 (0.71)	3.50^{bcd}
C. pinnatifidum	PI 599109	0.00 (0.71)^{ab}	1.00 (1.22)^{a}	1.00 (1.14)	0.50 (0.97)	2.00^{ab}
C. microphyllum	ICCW 17148	0.50 (0.97)^{abc}	3.50 (1.96)^{abcd}	0.00 (0.71)	0.00 (0.71)	4.00^{cd}
C. arietinum	JG 11 (C)	1.50 (1.40)^{abcd}	9.00 (3.01)^{cde}	0.00 (0.71)	10.00 (2.94)	2.75^{bc}
C. arietinum	KAK 2 (S)	3.00 (1.85)^{d}	9.00 (2.90)^{bcde}	0.50 (0.97)	4.00 (2.07)	4.00^{cd}
C. arietinum	ICC 3137 (S)	1.50 (1.29)^{abcd}	11.00 (3.14)^{de}	0.00 (0.71)	0.50 (0.97)	3.50^{bcd}
C. arietinum	ICCL 86111 (R)	2.00 (1.58)^{abcd}	5.50 (2.43)^{abcde}	0.00 (0.71)	0.00 (0.71)	3.00^{bc}
C. arietinum	ICC 506 EB (R)	0.50 (0.97)^{abc}	4.50 (2.23)^{abcd}	0.00 (0.71)	0.50 (0.97)	3.00^{bc}
	Fpr	**0.03**	**0.02**	**NS**	**NS**	**<.001**
	Mean	**1.08**	**2.15**	**0.78**	**1.25**	**2.51**
	SE±	**0.26**	**0.44**	**0.15**	**0.61**	**0.50**
	LSD (P= 0.05)	**0.76**	**1.30**	**-**	**-**	**1.49**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.9. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 30 DAE (Post-rainy season, 2015-16)

Species	Genotype	Numbers per 5 plants			DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* larvae	
C. chrossanicum	IG 599076	0.00 (0.71)	1.50 (1.40)^{a}	7.50 (2.52)	2.80^{bcdef}
C. cuneatum	IG 69979	0.00 (0.71)	2.00 (1.58)^{a}	0.50 (0.97)	1.75^{abc}
C. bijugum	IG 70006	0.00 (0.71)	12.50 (3.60)^{cde}	2.50 (1.67)	1.50^{ab}
C. bijugum	IG 70012	0.00 (0.71)	9.00 (3.08)^{bcd}	1.00 (1.14)	1.50^{ab}
C. bijugum	IG 70018	0.00 (0.71)	9.00 (3.06)^{bcd}	2.00 (1.58)	1.50^{ab}
C. bijugum	IG 70022	0.00 (0.71)	5.50 (2.39)^{ab}	1.00 (1.22)	2.25^{abcd}
C. reticulatum	IG 72933	1.00 (1.14)	17.50 (4.24)^{e}	1.50 (1.29)	3.25^{cdef}
C. reticulatum	IG 72953	0.00 (0.71)	10.50 (3.27)^{bcde}	3.50 (2.00)	3.75^{dfg}
C. pinnatifidum	PI 510663	0.00 (0.71)	2.50 (1.73)^{a}	1.00 (1.14)	2.00^{abc}
C. judaicum	PI 568217	0.50 (0.97)	2.50 (1.73)^{a}	1.00 (1.22)	5.50^{h}
C. bijugum	PI 599046	0.00 (0.71)	10.50 (3.29)^{bcde}	0.00 (0.71)	1.00^{a}
C. bijugum	PI 599066	0.00 (0.71)	9.50 (3.16)^{bcd}	1.50 (1.29)	2.00^{abc}
C. judaicum	PI 599077	0.00 (0.71)	5.00 (2.30)^{ab}	0.50 (0.97)	5.00^{gh}
C. pinnatifidum	PI 599109	0.00 (0.71)	3.00 (1.85)^{a}	8.50 (2.90)	2.25^{abcde}
C. microphyllum	ICCW 17148	0.00 (0.71)	2.50 (1.73)^{a}	0.50 (0.97)	4.00^{fg}
C. arietinum	JG 11 (C)	0.00 (0.71)	11.50 (3.46)^{cde}	7.00 (2.63)	4.00^{fg}
C. arietinum	KAK 2 (S)	0.00 (0.71)	13.00 (3.65)^{cde}	4.00 (2.11)	4.00^{fg}
C. arietinum	ICC 3137 (S)	0.00 (0.71)	14.50 (3.86)^{de}	3.00 (1.85)	3.75^{defg}
C. arietinum	ICCL 86111 (R)	0.00 (0.71)	12.00 (3.51)^{cde}	5.00 (1.97)	3.25^{cdef}
C. arietinum	ICC 506 EB (R)	0.00 (0.71)	7.50 (2.82)^{bc}	3.00 (1.78)	1.00^{a}
	Fpr	**NS**	**<.001**	**NS**	**<.001**
	Mean	**0.74**	**2.79**	**1.60**	**2.80**
	SE±	**0.11**	**0.30**	**0.56**	**0.46**
	LSD (P= 0.05)	**-**	**0.88**	**-**	**1.36**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at p ≤ 0.05(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.10. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 45 DAE (Post-rainy season, 2015-16)

Species	Genotype	Numbers per 5 plants				DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* egg masses	*S. exigua* larvae	
C. chrossanicum	IG 599076	0.00 (0.71)	2.00 (1.58)^{ab}	0.00 (0.71)	4.00 (2.11)^{bc}	2.00^{bcdefg}
C. cuneatum	IG 69979	0.00 (0.71)	2.00 (1.55)^{ab}	0.00 (0.71)	1.00 (1.14)^{ab}	2.00^{abcdefg}
C. bijugum	IG 70006	0.00 (0.71)	6.50 (2.60)^{cde}	0.00 (0.71)	2.50 (1.73)^{abc}	1.50^{abc}
C. bijugum	IG 70012	0.00 (0.71)	3.50 (2.00)^{abc}	0.00 (0.71)	1.50 (1.40)^{ab}	1.50^{abcd}
C. bijugum	IG 70018	0.00 (0.71)	6.00 (2.54)^{cde}	0.00 (0.71)	0.50 (0.97)^{a}	1.50^{abcde}
C. bijugum	IG 70022	0.50 (0.97)	3.00 (1.85)^{abc}	0.00 (0.71)	2.00 (1.55)^{ab}	1.25^{ab}
C. reticulatum	IG 72933	0.00 (0.71)	12.50 (3.57)^{f}	0.00 (0.71)	0.50 (0.97)^{a}	3.50^{h}
C. reticulatum	IG 72953	0.00 (0.71)	8.50 (3.00)^{def}	0.00 (0.71)	3.50 (1.96)^{bc}	2.75^{gh}
C. pinnatifidum	PI 510663	0.00 (0.71)	2.50 (1.73)^{abc}	0.50 (0.97)	1.50 (1.29)^{ab}	1.50^{abcde}
C. judaicum	PI 568217	0.00 (0.71)	1.50 (1.40)^{a}	0.00 (0.71)	0.50 (0.97)^{a}	2.50^{fg}
C. bijugum	PI 599046	0.00 (0.71)	4.00 (2.12)^{abcd}	0.00 (0.71)	1.00 (1.14)^{ab}	1.00^{a}
C. bijugum	PI 599066	0.00 (0.71)	5.00 (2.34)^{bcd}	0.00 (0.71)	0.50 (0.97)^{a}	1.50^{abcde}
C. judaicum	PI 599077	0.50 (0.97)	3.50 (2.00)^{abc}	0.00 (0.71)	1.00 (1.14)^{ab}	2.50^{cfg}
C. pinnatifidum	PI 599109	0.00 (0.71)	2.50 (1.73)^{abc}	0.00 (0.71)	2.50 (1.73)^{abc}	1.75^{abcdef}
C. microphyllum	ICCW 17148	0.00 (0.71)	2.00 (1.58)^{ab}	0.00 (0.71)	0.50 (0.97)^{a}	2.50^{cdfg}
C. arietinum	JG 11 (C)	0.00 (0.71)	8.00 (2.91)^{def}	0.00 (0.71)	6.50 (2.63)^{c}	2.75^{gh}
C. arietinum	KAK 2 (S)	0.00 (0.71)	10.50 (3.32)^{ef}	0.50 (0.97)	2.50 (1.67)^{abc}	3.50^{h}
C. arietinum	ICC 3137 (S)	0.00 (0.71)	11.50 (3.40)^{ef}	0.00 (0.71)	3.50 (2.00)^{bc}	3.50^{h}
C. arietinum	ICCL 86111 (R)	0.00 (0.71)	10.00 (3.24)^{ef}	0.00 (0.71)	4.00 (2.11)^{bc}	1.75^{abcdef}
C. arietinum	ICC 506 EB (R)	0.00 (0.71)	4.50 (2.23)^{abcd}	0.00 (0.71)	1.50 (1.40)^{ab}	1.25^{ab}
	Fpr	**NS**	**<.001**	**NS**	**0.01**	**<.001**
	Mean	**0.73**	**2.33**	**0.73**	**1.49**	**2.10**
	SE±	**0.08**	**0.26**	**0.08**	**0.29**	**0.29**
	LSD (P=0.05)	**-**	**0.78**	**-**	**0.85**	**0.87**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.11. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 60 DAE (Post-rainy season, 2015-16)

Species	Genotype	Numbers per 5 plants		DR
		***H. armigera* larvae**	***S. exigua* larvae**	
C. chrossanicum	IG 599076	2.00 (1.58) ^{ab}	3.00 (1.85)	2.00^{abcde}
C. cuneatum	IG 69979	3.00 (1.78) ^{abcde}	0.00 (0.71)	2.50^{ce}
C. bijugum	IG 70006	2.50 (1.67) ^{abc}	3.50 (1.96)	1.25^{ab}
C. bijugum	IG 70012	1.00 (1.22)^{a}	1.50 (1.40)	1.50^{abc}
C. bijugum	IG 70018	4.00 (2.12) ^{abcde}	0.50 (0.97)	1.25^{ab}
C. bijugum	IG 70022	1.00 (1.22)^{a}	3.00 (1.85)	1.50^{abc}
C. reticulatum	IG 72933	8.50 (2.90) ^{cef}	0.00 (0.71)	3.50^{fg}
C. reticulatum	IG 72953	6.50 (2.60) ^{bcdef}	5.00 (2.30)	2.75^{ef}
C. pinnatifidum	PI 510663	1.50 (1.40)^{ab}	2.00 (1.41)	1.00^{a}
C. judaicum	PI 568217	2.00 (1.58) ^{ab}	0.00 (0.71)	1.50^{abc}
C. bijugum	PI 599046	2.00 (1.55) ^{ab}	1.00 (1.14)	1.25^{ab}
C. bijugum	PI 599066	3.00 (1.78) ^{abcde}	1.00 (1.14)	2.50^{cde}
C. judaicum	PI 599077	2.50 (1.67) ^{abcd}	0.00 (0.71)	1.75^{abcde}
C. pinnatifidum	PI 599109	1.50 (1.40)^{ab}	2.00 (1.41)	1.00^{a}
C. microphyllum	ICCW 17148	2.00 (1.55)^{ab}	0.50 (0.97)	2.00^{abcde}
C. arietinum	JG 11 (C)	3.50 (2.00) ^{abcde}	8.50 (2.64)	2.25^{bcde}
C. arietinum	KAK 2 (S)	8.00 (2.89) ^{ef}	1.00 (1.14)	3.50^{fg}
C. arietinum	ICC 3137 (S)	11.50 (3.46) ^{f}	2.00 (1.41)	3.75^{g}
C. arietinum	ICCL 86111 (R)	8.50 (2.97) ^{ef}	5.00 (2.30)	1.50^{abc}
C. arietinum	ICC 506 EB (R)	2.00 (1.58) ^{ab}	0.00 (0.71)	1.50^{abcd}
	Fpr	**0.01**	**NS**	**<.001**
	Mean	**1.95**	**1.37**	**1.98**
	SE±	**0.36**	**0.49**	**0.30**
	LSD (P= 0.05)	**1.07**	**-**	**0.89**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.12. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 75 DAE (Post-rainy season, 2015-16)

Species	Genotype	Numbers per 5 plants			DR
		H. armigera eggs	*H. armigera* larvae	*S. exigua* larvae	
C. chrossanicum	IG 599076	0.00 (0.71)	1.00 (1.22)^{a}	1.50 (1.40)	1.25 ^{ab}
C. cuneatum	IG 69979	0.00 (0.71)	1.50 (1.40)^{ab}	0.00 (0.71)	1.25 ^{ab}
C. bijugum	IG 70006	0.00 (0.71)	2.00 (1.58)^{ab}	2.00 (1.55)	1.25 ^{ab}
C. bijugum	IG 70012	0.00 (0.71)	3.50 (2.00)^{bcd}	1.50 (1.29)	1.00 ^{a}
C. bijugum	IG 70018	0.00 (0.71)	2.50 (1.73)^{abc}	3.00 (1.85)	1.00 ^{a}
C. bijugum	IG 70022	0.00 (0.71)	2.00 (1.55)^{ab}	1.00 (1.14)	1.50 ^{ab}
C. reticulatum	IG 72933	1.00 (1.14)	8.50 (2.97)^{fg}	0.50 (0.97)	2.75 ^{c}
C. reticulatum	IG 72953	0.00 (0.71)	5.00 (2.34)^{cdef}	1.00 (1.14)	2.50 ^{c}
C. pinnatifidum	PI 510663	0.00 (0.71)	1.00 (1.22)^{a}	0.50 (0.97)	1.00 ^{a}
C. judaicum	PI 568217	0.00 (0.71)	1.00 (1.22)^{a}	0.00 (0.71)	1.75 ^{b}
C. bijugum	PI 599046	0.00 (0.71)	2.50 (1.67)^{ab}	0.00 (0.71)	1.25 ^{ab}
C. bijugum	PI 599066	0.50 (0.97)	2.50 (1.73)^{abc}	0.00 (0.71)	1.50 ^{ab}
C. judaicum	PI 599077	1.50 (1.29)	3.50 (2.00)^{bcd}	0.00 (0.71)	1.00 ^{a}
C. pinnatifidum	PI 599109	0.00 (0.71)	1.00 (1.22)^{a}	1.50 (1.29)	1.25 ^{ab}
C. microphyllum	ICCW 17148	0.00 (0.71)	2.00 (1.55)^{ab}	0.00 (0.71)	1.75 ^{b}
C. arietinum	JG 11 (C)	0.00 (0.71)	6.50 (2.63)^{df}	0.00 (0.71)	1.75 ^{b}
C. arietinum	KAK 2 (S)	0.00 (0.71)	7.50 (2.83)^{f}	0.50 (0.97)	2.75 ^{c}
C. arietinum	ICC 3137 (S)	1.50 (1.29)	11.50 (3.46)^{g}	1.50 (1.29)	3.00 ^{c}
C. arietinum	ICCL 86111 (R)	0.00 (0.71)	5.50 (2.45)^{def}	1.00 (1.14)	1.50 ^{ab}
C. arietinum	ICC 506 EB (R)	0.00 (0.71)	3.50 (2.00)^{bcde}	0.00 (0.71)	1.50 ^{ab}
	Fpr	**NS**	**<.001**	**NS**	**<.001**
	Mean	**0.80**	**1.94**	**1.03**	**1.63**
	SE±	**0.20**	**0.20**	**0.31**	**0.21**
	LSD (P= 0.05)	**-**	**0.59**	**-**	**0.64**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.13. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 90 DAE (Post-rainy season, 2015-16)

Species	Genotype	Numbers per 5 plant		DR
		H. armigera larvae	*S. exigua* larvae	
C. chrossanicum	IG 599076	4.50 (2.27) abc	1.50 (1.29) abc	1.00 a
C. cuneatum	IG 69979	8.00 (2.87)cde	1.00 (1.14) ab	1.25 ab
C. bijugum	IG 70006	5.50 (2.45) abc	9.00 (3.04) d	1.25 ab
C. bijugum	IG 70012	5.50 (2.45) abc	2.50 (1.73) abc	1.25 ab
C. bijugum	IG 70018	3.00 (1.87) a	5.00 (2.34) bcd	1.50 ab
C. bijugum	IG 70022	5.00 (2.34) abc	5.50 (2.43) cd	1.25 ab
C. reticulatum	IG 72933	13.50 (3.73)fgh	3.50 (2.00) abcd	3.00 ef
C. reticulatum	IG 72953	11.50 (3.45)efg	4.00 (2.12) abcd	2.75 def
C. pinnatifidum	PI 510663	6.50 (2.63)abcd	5.50 (2.45) cd	1.25 ab
C. judaicum	PI 568217	3.00 (1.87) a	0.50 (0.97) a	2.00 bcd
C. bijugum	PI 599046	7.00 (2.73)bcde	3.00 (1.78) abc	1.25 ab
C. bijugum	PI 599066	8.00 (2.89)cde	2.00 (1.58) abc	1.75 abc
C. judaicum	PI 599077	4.50 (2.23) abc	0.50 (0.97) a	1.00 a
C. pinnatifidum	PI 599109	3.50 (2.00) ab	2.50 (1.53) abc	1.75 abc
C. microphyllum	ICCW 17148	4.50 (2.23) abc	1.50 (1.40) abc	2.50 cde
C. arietinum	JG 11 (C)	17.50 (4.24)h	2.00 (1.58) abc	3.50f
C. arietinum	KAK 2 (S)	17.00 (4.18)gh	1.00 (1.14) ab	4.75g
C. arietinum	ICC 3137 (S)	25.00 (5.04)i	4.50 (2.23) bcd	5.75h
C. arietinum	ICCL 86111 (R)	11.00 (3.39)def	3.00 (1.85) abcd	3.25 ef
C. arietinum	ICC 506 EB (R)	5.00 (2.34) abc	0.50 (0.97) a	2.00 bcd
	Fpr	**<.001**	**0.02**	**<.001**
	Mean	**2.86**	**1.73**	**2.20**
	SE±	**0.24**	**0.36**	**0.27**
	LSD (P= 0.05)	**0.70**	**1.05**	**0.80**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

Table 4.14. Abundance of pod borers (*H. armigera* and *S. exigua*) on different genotypes of wild relatives of chickpea at 105 DAE (Post-rainy season, 2015-16)

Species	Genotype	Numbers per 5 plant		DR
		***H. armigera* larvae**	***S. exigua* larvae**	
C. chrossanicum	IG 599076	-	-	-
C. cuneatum	IG 69979	0.00 (0.71)	0.00 (0.71)[a]	2.50[bc]
C. bijugum	IG 70006	0.00 (0.71)	1.00 (1.14) [ab]	2.00 [ab]
C. bijugum	IG 70012	0.00 (0.71)	1.00 (1.14) [ab]	2.00 [ab]
C. bijugum	IG 70018	0.00 (0.71)	1.00 (1.14) [ab]	2.75 [bc]
C. bijugum	IG 70022	0.00 (0.71)	4.50 (2.21) [abc]	2.00 [ab]
C. reticulatum	IG 72933	0.00 (0.71)	1.50 (1.29) [ab]	4.00[d]
C. reticulatum	IG 72953	0.00 (0.71)	3.50 (2.00) [abc]	3.50[cd]
C. pinnatifidum	PI 510663	0.00 (0.71)	4.50 (2.23) [bc]	3.00 [bc]
C. judaicum	PI 568217	0.00 (0.71)	1.00 (1.22) [ab]	3.00 [bc]
C. bijugum	PI 599046	0.00 (0.71)	2.50 (1.53) [abc]	1.50 [a]
C. bijugum	PI 599066	0.00 (0.71)	7.00 (2.56) [bc]	2.25 [ab]
C. judaicum	PI 599077	0.50 (0.97)	1.00 (1.22) [ab]	2.50 [abc]
C. pinnatifidum	PI 599109	0.50 (0.97)	7.50 (2.83)[c]	2.50 [abc]
C. microphyllum	ICCW 17148	0.00 (0.71)	0.00 (0.71)[a]	3.00[bc]
C. arietinum	JG 11 (C)	-	-	-
C. arietinum	KAK 2 (S)	-	-	-
C. arietinum	ICC 3137 (S)	-	-	-
C. arietinum	ICCL 86111 (R)	-	-	-
C. arietinum	ICC 506 EB (R)	-	-	-
	Fpr	**NS**	**0.04**	**0.003**
	Mean	**0.74**	**1.64**	**2.63**
	SE±	**0.10**	**0.44**	**0.30**
	LSD (P= 0.05)	**-**	**1.32**	**0.90**

Figures in parentheses are square root (√x+0.5) transformed values; DAE- Days after emergence
The values followed by same alphabets did not differ significantly at $p \leq 0.05$(DMRT)
C- Commercial cultivar, S- Susceptible check, R- Resistance check
DR (Damage rating) = 1, <10% leaf area damaged and 9= >80% leaf area damaged

genotypes was exhibited only at 105 DAE. Highest number of cocoons at 105 DAE was observed in IG 70012 (10.50 cocoons/ five plants), while lowest was observed in PI 510663 and IG 72933 (3.00 cocoons per fie plants) (Table 4.7). During post-rainy season, 2015-16 parasitisation of *H. armigera* by *C. chlorideae* was not observed on any genotype throughout crop growth period. These variations could be due to differences in weather parameters in both the seasons. Larval parasitoid was first observed in 3rd standard week (SW) and attained peak population in 7th and 8th SW, respectively (Pillai *et al*., 2016). Similar kind of study was made by Bohria and Shukla (2006) who reported that peak parasitization of *H. armigera* by *C. chlorideae* was in the second week of January.

4.1.6 Damage Rating on Different Genotypes of Wild Relatives of Chickpea

All genotypes showed less damage rating compared to susceptible checks, ICC 3137 and KAK 2 during post-rainy season, 2014-15. At 15 DAE, lowest damage rating was observed on resistant check, ICC 506EB (2.00), whereas highest was observed on ICC 3137 (6.50) (Table 4.1). Among all the genotypes ICC 3137 (7.00) recorded highest damage rating followed by PI 510663 (6.00) at 30 DAE and lowest (2.00) was recorded on IG 70018, PI 599046 and JG 11 (Table 4.2). Damage rating was recorded in a range from 1.00 (ICC 506EB) to 5.50 (ICC 3137) at 45 DAE (Table 4.3). Among all the genotypes ICC 506EB (2.00) recorded lowest damage rating and highest (6.50) was recorded on ICC 3137 followed by KAK 2 (4.50) at 60 DAE (Table 4.4). All genotypes were on par with respect to damage rating except ICC 3137 (7.00) and KAK 2 (6.00) which showed highest damage rating compared to all other genotypes at 75 DAE (Table 4.5). At 90 DAE, damage rating was recorded as 8.00 on ICC 3137 which was highest compared to all other genotypes followed by IG 70018 (6.00) and damage rating recorded on IG 69979 and PI 599077 was 2.00, which was lowest among all genotypes (Table 4.6). At 105 DAE, damage rating was observed in a range of 3.00 (IG 69979 and ICCW 17148) to 6.50 (IG 72933) among all genotypes (Table 4.7).

During post-rainy season, 2015-16 significant differences were exhibited in damage rating among different genotypes of wild relatives of chickpea. At 15 DAE, lowest damage rating of 1.00 was recorded on IG 70006, IG 70012, IG 70018, IG 70022, PI 599046 and PI 599066, whereas highest of 4.00 was recorded on KAK 2 (Table 4.8). Damage rating was observed in a range of 1.00 (PI 599046 and ICC

506EB) to 5.50 (PI 568217) at 30 DAE among all the genotypes (Table 4.9). At 45 DAE lowest damage rating was observed on PI 599046 (1.00) and highest was observed on ICC 3137, KAK 2 and IG 72933 (3.50) (Table 4.10). Damage rating showed significant differences among all genotypes with a range of 1.00 (PI 510663 and PI 599109) to 3.75 (ICC 3137) at 60 DAE (Table 4.11). At 75 DAE, all genotypes showed significantly less damage rating compared to susceptible check, ICC 3137 (3.00) (Table 4.12). At 90 DAE, highest damage rating was observed on ICC 3137 (5.75) followed by KAK 2 (4.75), while lowest was observed in IG 599076 and PI 599077 (1.00) (Table 4.13). Among genotypes of wild relatives of chickpea lowest damage rating was observed on PI 599046 (1.50) and highest was observed on IG 72933 (4.00) followed by IG 72953 (3.50) at 105 DAE (Table 4.14). Sharma *et al*. (2006) also observed that under natural infestation, accessions of *C. microphyllum*, and *C. canariense* suffered a damage rating of less than 2.0 compared to 4.0 in *C. judaicum* accessions and 8.5 to 9.0 in the cultivated chickpeas.

4.1.7 Association of Abiotic Factors with Abundance of Pod Borers in Wild Relatives of Chickpea

Correlation studies (Table 4.15) revealed that, among the weather parameters evaporation, maximum temperature, wind velocity and solar radiation showed significant negative correlation with egg load of *H. armigera*, while significant positive correlation was showed with relative humidity1. Larval count of *H. armigera* was in significant positive association with evaporation, maximum temperature, wind velocity, solar radiation and sunshine hours, whereas significant negative association was observed with relative humidity. Oviposition by *S. exigua* has not shown significant association with any of the weather parameters. Larvae of *S. exigua* showed significant negative association with rainfall, evaporation, solar radiation and sunshine hours, while significant positive association showed with minimum temperature, relative humidity1 and wind velocity. *C. chlorideae* cocoons exhibited significant positive association with minimum temperature, evaporation, wind velocity, solar radiation and sunshine hours, whereas significant negative association has shown with rainfall and relative humidity.

Similar results were obtained by Reddy *et al*. (2009) and Yadav *et al*. (2016) who reported positive association of *H. armigera* larval abundance with temperature and negative association with relative humidity. On contrary, Zafar *et al*. (2013)

Table 4.15. Correlation of abiotic factors with abundance of *H. armigera*, *S. exigua* and coccons of *C. chlorideae* in wild relatives of chickpea during post-rainy seasons, 2014-15 and 2015-16

	***H. armigera* eggs**	***H. armigera* larvae**	***S. exigua* eggs**	***S. exigua* larvae**	***C. chlorideae* cocoons**	**Damage rating**
***H. armigera* larvae**	0.07					
***S. exigua* eggs**	-0.11	0.01				
***S. exigua* larvae**	0.11	0.07	0.03			
***C. chlorideae* cocoons**	-0.24	0.57**	0.14	0.01		
Damage rating	-0.01	0.58**	-0.09	0.01	0.25**	
Rainfall (mm)	-0.05	-0.07	0.05	-0.19*	-0.27**	-0.13
Evaporation (mm)	-0.32**	0.52**	0.12	-0.18*	0.79**	0.30**
Maximum temperature (ºC)	-0.30**	0.58**	0.10	0.11	0.78**	0.29**
Minimum temperature (ºC)	-0.03	0.17	0.05	0.34**	0.09	-0.03
Relative humidity 1	0.31**	-0.44**	-0.12	0.20*	-0.74**	-0.27**
Relative humidity 2	0.16	-0.35**	-0.04	0.15	-0.60**	-0.29**
Wind velocity (kmph)	-0.30**	0.37**	0.14	0.23*	0.64**	0.16
Solar radiation	-0.22*	0.37**	0.08	-0.27**	0.58**	0.29**
Bright sunshine (hours)	-0.10	0.24*	0.03	-0.24**	0.45**	0.26**

*,** Correlation co-efficient significant at P ≤ 0.05 and 0.01, respectively

observed that maximum temperature showed significant positive correlation with the *H. armigera* egg counts, whereas relative humidity and rainfall had significant negative correlation. Rohit *et al*. (2016) observed that rainfall showed significant negative correlation with cocoons of *C. chlorideae*, but on contrary they observed that maximum and minimum temperature had a highly negative significant correlation and relative humidity had a significant positive correlation with *C. chlorideae* cocoons. Pillai *et al*. (2016) also observed that larval parasitoid showed a significant negative correlation with maximum and minimum temperature, whereas significant positive correlation with maximum and minimum relative humidity. Dhillon and Sharma (2008) reported that fluctuations in temperature have a significant influence on parasitoid development.

4.1.8 Pod Damage Inflicted by *H. armigera* in Wild Relatives of Chickpea

Under multi-choice field conditions, significant differences were exhibited in per cent pod damage by *H. armigera* in different genotypes of wild relatives of chickpea (Table 4.16). During post-rainy season, 2014-15 the highest pod damage was recorded on ICC 3137 (36.30%) followed by KAK 2 (32.12%), while lowest pod damage was recorded on IG 69979 (15.52%). Pod damage on all other genotypes ranged from 18.23% on ICC 506EB to 31.57% on IG 72953. Similar trend was observed during post-rainy season, 2015-16, where lowest pod damage was recorded on IG 69979 (9.55%) and highest was recorded on KAK 2 (30.50%) followed by ICC 3137 (28.88%).

Based on observations on both the seasons, it was observed that wild relatives of chickpea were encountered with less damage compared to susceptible checks and damage ranged from 10.0 to 37.0% pod damage. The present results are in agreement with findings of Wakil *et al*. (2005), Hossain *et al*. (2007) and Cheema *et al*. (2010) who observed that pod damage by *H. armigera* was in a range of 10 to 38%. The genotypes with resistance to pod borer with less larval abundance and low per cent pod damage might have various morphological and biochemical factors contributing to resistance and they can be used sources for resistance.

4.2 ANTIXENOSIS MECHANISM OF RESISTANCE TO *H. armigera* IN WILD RELATIVES OF CHICKPEA

The oviposition preference of *H. armigera* adults on wild relatives of chickpea was studied under no-choice, dual-choice and multl-choice conditions.

Table 4.16. Per cent pod damage inflicted by pod borer, *H. armigera* in different genotypes of wild relatives of chickpea under field conditions

Species	Genotype	Post rainy season 2014-15	Post rainy season 2015-16
C. chrossanicum	IG 599076	20.80 (27.04) ^{abc}	16.52 (23.87) ^{abcd}
C. cuneatum	IG 69979	15.52 (23.20) ^{a}	9.55 (17.99) ^{a}
C. bijugum	IG 70006	28.22 (32.08) ^{cdef}	10.32 (18.71) ^{ab}
C. bijugum	IG 70012	24.70 (29.74) ^{bcde}	14.97 (22.76) ^{abcd}
C. bijugum	IG 70018	26.04 (30.65) ^{bcdef}	15.16 (22.80) ^{abcd}
C. bijugum	IG 70022	22.36 (28.22) ^{abcde}	12.48 (20.66) ^{abc}
C. reticulatum	IG 72933	29.66 (32.99) ^{cdef}	18.38 (25.30) ^{cd}
C. reticulatum	IG 72953	31.57 (34.17) ^{def}	21.84 (27.82) ^{de}
C. pinnatifidum	PI 510663	27.58 (31.67) ^{bcdef}	12.70 (20.84) ^{abc}
C. judaicum	PI 568217	28.23 (32.07) ^{cdef}	15.04 (22.81) ^{abcd}
C. bijugum	PI 599046	26.40 (30.91) ^{bcdef}	16.84 (24.21) ^{abcd}
C. bijugum	PI 599066	21.69 (27.76) ^{abcd}	17.93 (24.96) ^{bcd}
C. judaicum	PI 599077	26.09 (30.58) ^{bcdef}	16.73 (24.14) ^{abcd}
C. pinnatifidum	PI 599109	28.63 (32.32) ^{cdef}	18.23 (25.23) ^{cd}
C. microphyllum	ICCW 17148	24.58 (29.50) ^{abcde}	15.98 (23.48) ^{abcd}
C. arietinum	JG 11 (C)	29.17 (32.67) ^{cdef}	16.79 (24.19) ^{abcd}
C. arietinum	KAK 2 (S)	32.12 (34.51) ^{ef}	30.50 (33.52) ^{f}
C. arietinum	ICC 3137 (S)	36.30 (36.97) ^{f}	28.88 (32.51) ^{ef}
C. arietinum	ICCL 86111 (R)	24.02 (29.34) ^{abcde}	17.90 (24.87) ^{bcd}
C. arietinum	ICC 506 EB (R)	18.23 (25.19) ^{ab}	14.71 (22.38) ^{abcd}
	Fpr	**0.012**	**0.001**
	Mean	**30.58**	**24.15**
	SE±	**1.90**	**1.81**
	LSD (P= 0.05)	**5.67**	**5.36**

Figures in parentheses are angular transformed values

The values followed by same alphabets did not differ significantly at $p \leq 0.05$ (DMRT)

C- Commercial cultivar, S- Susceptible check, R- Resistant check

Under multi-choice cage condition (Figure 4.1.) when 50 pairs of *H. armigera* adults were provided with twigs of all genotypes for oviposition in a cage, lowest number of eggs were laid on IG 70012 (555.00 eggs) which was on par with PI 599046 (643.50 eggs), while highest number of eggs were laid on ICCW 17148 (1207.00 eggs). The genotypes IG 70012, PI 599046, IG 70022, PI 599066, IG 70006, IG 70018 (*C. bijugum*), ICC 506EB, ICCL 86111 (resistant checks), IG 72933, IG 72953 (*C. reticulatum*) IG 69979 (*C. cuneatum*) and IG 599076 (*C. chrossanicum*) showed significantly lowest preference for oviposition (555.0 to 814.00 eggs/genotype) compared to susceptible checks, ICC 3137 (1070.50 eggs) and KAK 2 (1041.00 eggs).

Under no-choice conditions (Figure 4.2.), significant differences were observed in ovipositon preference of *H. armigera* among wild relatives of chickpea. Highest oviposition was observed on PI 599077 (1516.33 eggs/five females) which was on par with ICCW 17148 (1508.33 eggs/five females), PI 568217 (1488.67 eggs/five females), IG 70022 (1462.67 eggs/ five females) and IG 70012 (1416.33 eggs/five females) and lowest was observed on IG 72933 (785.00 eggs/five females) and was on par with ICC 506EB (806.33 eggs/five females) and ICCL 86111 (840 eggs/five females). However, moderate levels of oviposition was recorded on genotypes, IG 599076, IG 72953, PI 599066, PI 599046, JG 11, PI 599046 and PI 599109, these genotypes showed <15.32% to 23.87% less oviposition compared to susceptible check.

Under dual-choice conditions (Figure 4.3.), when five pairs of *H. armigera* adults released for oviposition in a cage with choice of test genotype and the susceptible check (ICC 3137), significantly less preference for oviposition (128 to 636 eggs/genotype) was observed on IG 70022, PI 599066, IG 70012, ICC 506EB, PI 599046, PI 510663, IG 70018, PI 599109, IG 70006, IG 69979, ICCL 86111 and IG 599076 compared to susceptible check, ICC 3137 (413 to 854 eggs). Genotypes such as, PI 568217 (733 eggs), PI 599077 (736 eggs) and ICCW 17148 (897 eggs) showed more preference for oviposition compared to the susceptible check, ICC 3137 (391 to 802 eggs).

The genotypes showing resistance to *H. armigera* under field conditions also exhibited oviposition non-preference under laboratory conditions, suggesting that laboratory tests can be used to assess antixenosis for oviposition to *H. armigera*

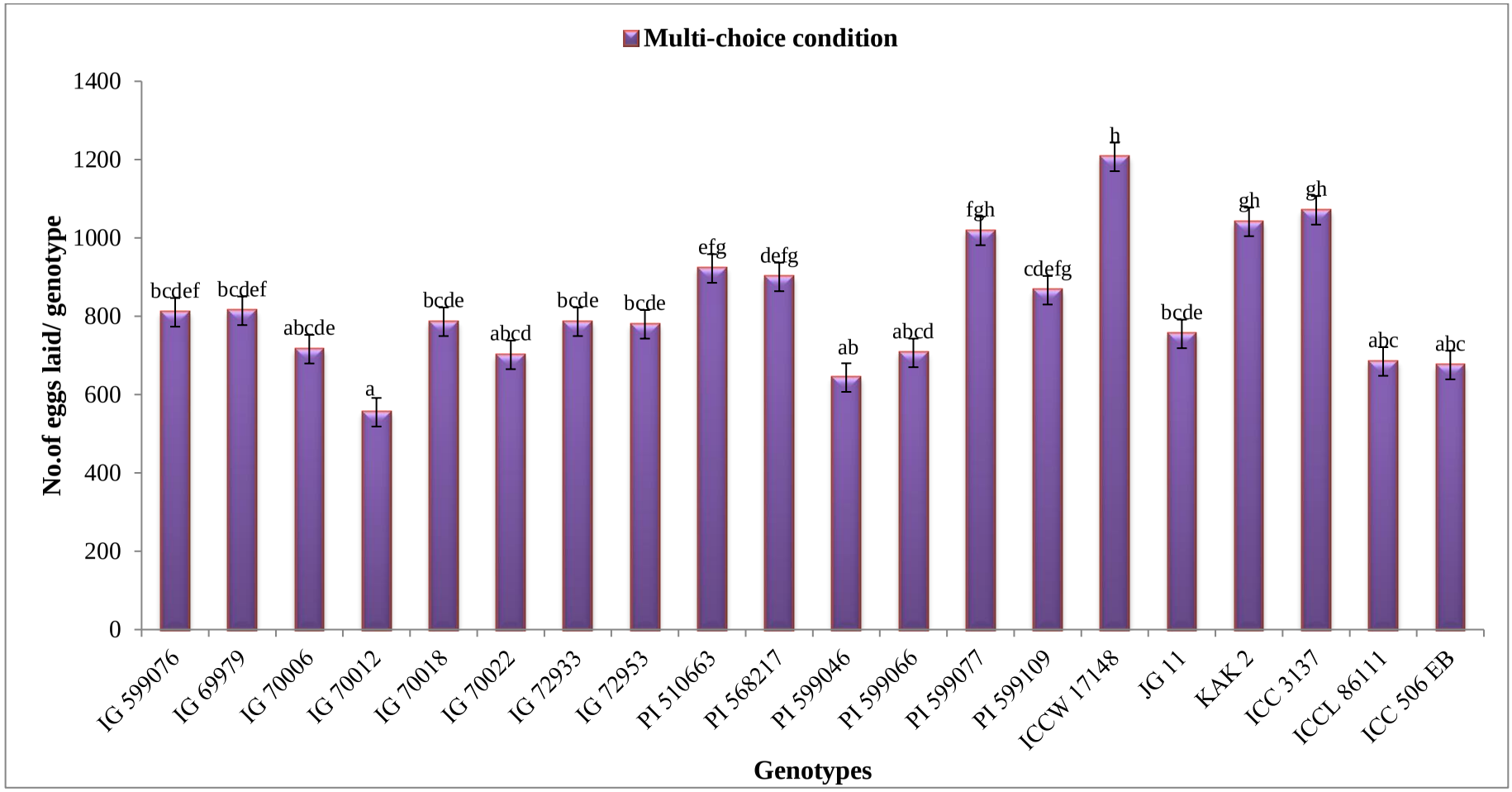


Figure 4.1. Oviposition preference by *H. armigera* females towards wild relatives of chickpea under multi-choice condition.

For experimentation 50 females were released in a cage per each replication

The means followed by the same alphabet did not differ significantly at LSD, $P \leq 0.05$

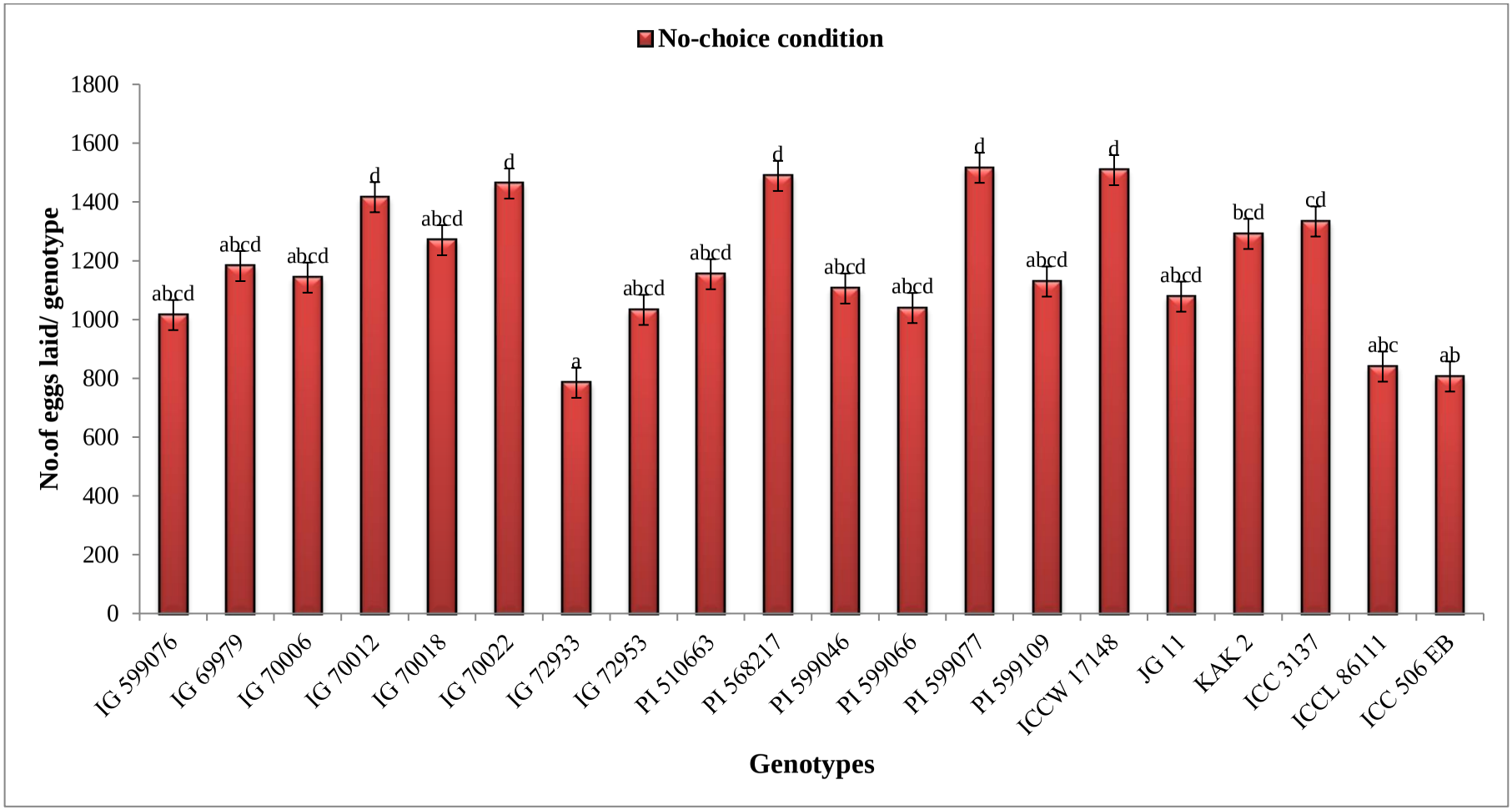


Figure 4.2. Oviposition preference by *H. armigera* females towards wild relatives of chickpea under no-choice condition.

For experimentation five females were released in a cage per each replication

The means followed by the same alphabet did not differ significantly at LSD, $P \leq 0.05$

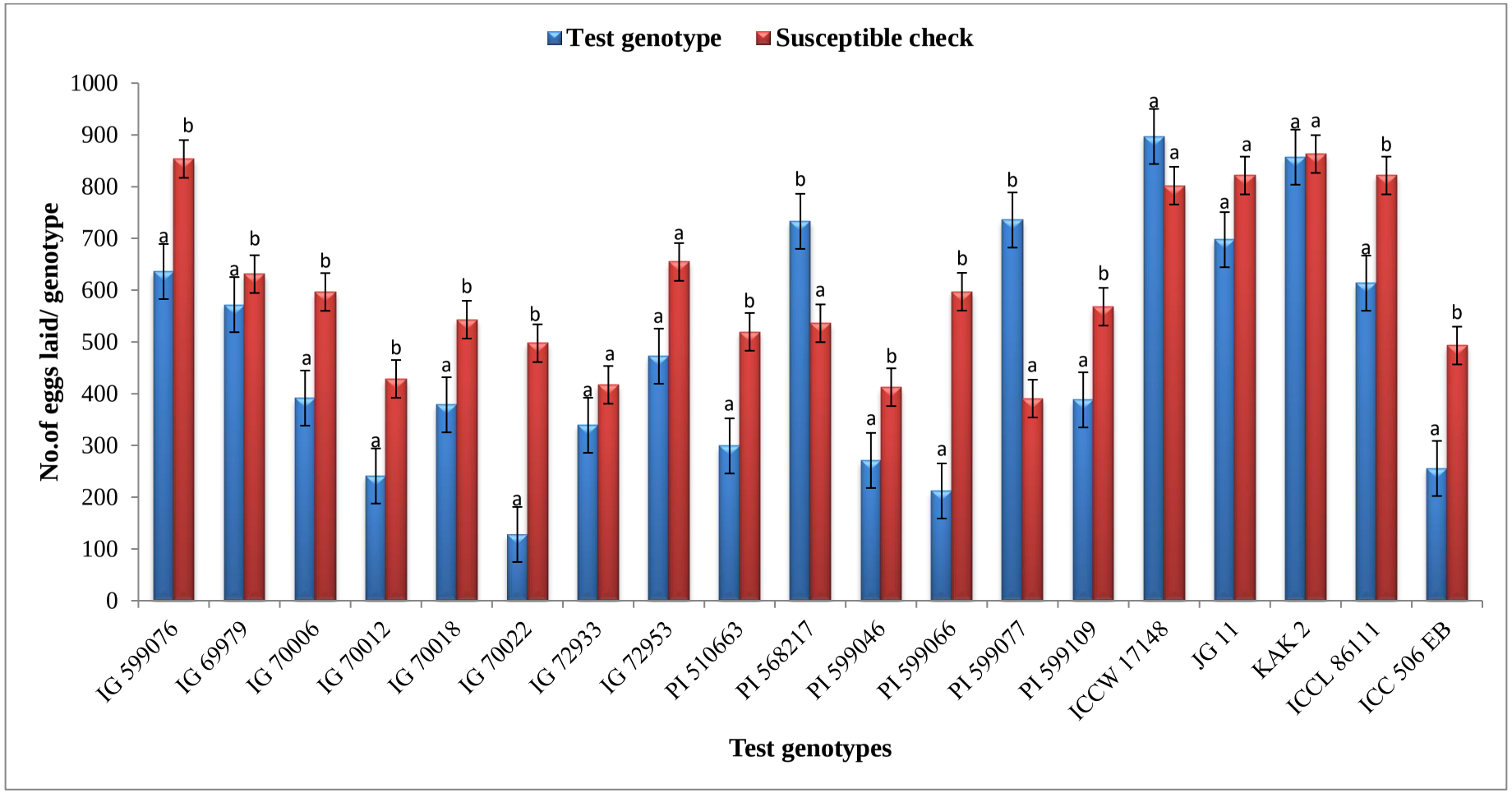


Figure 4.3. Oviposition preference by *H. armigera* females towards wild relatives of chickpea under dual-choice condition.

For experimentation five females were released in a cage per each replication

The genotypes with same alphabet with in a pair did not differ significantly from susceptible check, ICC 3137 at LSD, $P \leq 0.05$

(Kumari *et al*., 2006). The no-choice, dual-choice and multi-choice cage tests conducted in this study to assess the level of antixenosis to *H. armigera* revealed significant differences in number of eggs laid on different species and within different genotypes of the same species. All the genotypes of wild relatives of chickpea showed antixenosis for oviposition under multi-choice (except in *C. microphyllum*) and dualchoice (except in *C. microphyllum* and *C. judaicum*) conditions, of which accessions belonging to *C. bijugum* (PI 599066, PI 599046 and IG 70006), *C. reticulatum*, *C. chrossanicum*, *C. pinnatifidum*, *C. cuneatum* and resistant checks (ICC 506EB and ICCL 86111) also showed antixenosis for oviposition under no-choice conditions compared to susceptible checks (ICC 3137 and KAK 2).

The choice of oviposition may depend on the morphological characteristics (trichome density) and chemicals from the surfaces of various plant tissues (Navasero and Ramaswamy, 1991 and Udayagiri and Mason, 1995). Sarwar *et al*. (2009) reported that the preference or non-preference for oviposition on chickpea by female moth may be due to its varying behavioural response possibly due to different canopy structure of the plants. The variation in number of eggs laid on different genotypes in the present study could be due to variability in chickpea foliar secretions containing high concentrations of malic acid (Rembold, 1981). Yoshida *et al*. (1997) observed differences in oviposition preferences in relation with varying concentrations of acid exudates such as malic acid organic acids. Contributory effect of leaf surface chemicals on oviposition preference of *H. armigera* had also been reported by Sharma *et al*. (2001) and Green *et al*. (2003) who observed that methanol extracts of pigeonpea pods had a significant positive stimulant effect on oviposition by *H. armigera*, whereas methanol extracts from wild relatives of pigeonpea *C. scarabaeoides* pods showed no such effects.

4.3. ANTIBIOSIS MECHANISM OF RESISTANCE TO *H. armigera* IN WILD RELATIVES OF CHICKPEA

Results pertaining to different experiments *viz.* detached leaf assay, detached pod assay and diet incorporation assay to evaluate antibiosis mechanism of resistance to pod borer, *H. armigera* in wild relatives of chickpea were presented here under.

4.3.1 Detached Leaf Assay for Evaluation of Resistance to *H. armigera* in Wild Relatives of Chickpea

There were significant differences in leaf damage by neonates of *H. armigera* among different accessions of wild relatives of chickpea during post rainy season, 2014-15 (Table 4.17). The lowest damage rating was observed in IG 70012 (1.00) and resistant check, ICC 506EB (1.00) and which were on par with IG 70022 (1.33), whereas highest damage rating was recorded on susceptible check, KAK 2 (5.33) and it was on par with IG 599076 (4.67) and ICC 3137 (4.50). Damage rating of all other genotypes ranged between 2.00 (IG 70006, IG 70018, PI 568217 and ICCL 86111) to 4.33 (IG 72953). There were no significant differences in larval survival among different genotypes. Larval weights were lowest on all wild relatives of chickpea genotypes compared to cultivated chickpea and were in a range of 2.26 mg in ICCL 86111 to 2.79 mg in JG 11.

During post-rainy season 2015-16 (Table 4.18), significantly lowest damage rating was observed on genotypes, *C. cuneatum*, IG 69979 (1.33) and was on par with *C. bijugum*, IG 70022 (1.67) and PI 599046 (1.83) compared to resistant check, ICC 506EB (2.00), while highest damage rating was observed on susceptible check, ICC 3137 (5.33). Larval survival was lowest in IG 69979 (43.30%) and was on par with PI 599109 (53.30%), resistant check, ICC 506EB (53.30%), PI 599046 (56.70%) and IG 72953 (56.70%), whereas highest was observed in PI 599066 (96.70%) followed by IG 70012 (90.00%), IG 70018 (90.00%) and susceptible check, ICC 3137 (86.70%). Larval weight was ranged between 0.34 mg (IG 69979) and 2.10 mg (IG 599076 and KAK 2). Larval weights were significantly lower on IG 69979, IG 70022, PI 568217, PI 599077 and ICCW 17148 compared to that of the larvae reared on the resistant check, ICC 506EB (1.22 mg).

Wild relatives of chickpea genotypes grown under glasshouse conditions exhibited significant differences with respect to damage rating, larval survival percentage and larval weight (Table 4.19). All genotypes of wild relatives of chickpea showed less damage rating compared to susceptible checks KAK 2 (8.00) and ICC 3137 (6.67), whereas the lowest (1.33) was recorded on genotypes, IG 70022 and PI 599066. Larval survival ranged between 30.00% on resistant check, ICC 506EB and 96.67% on IG 70006 (*C. bijugum*). Wild relatives of chickpea genotypes IG 70006 (96.67%) and IG 70018 (90%) showed significantly higher

Table 4.17. Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea grown under field condition using detached leaf assay (Post-rainy season, 2014-15)

Species	Genotype	Damage rating (DR)[1]	Larval survival (%)	Mean larval weight (mg)
C. chrossanicum	IG 599076	4.67 [de]	20.00 (26.07)	2.04[abcd]
C. cuneatum	IG 69979	2.67 [abcd]	13.33 (21.14)	1.40 [abcd]
C. bijugum	IG 70006	2.00[ab]	23.33 (28.08)	1.81 [abcd]
C. bijugum	IG 70012	1.00[a]	30.00 (33.00)	0.99 [ab]
C. bijugum	IG 70018	2.00[a]	53.33 (46.92)	1.86 [abcd]
C. bijugum	IG 70022	1.33[a]	36.67 (37.22)	0.52 [a]
C. reticulatum	IG 72933	3.33 [abcde]	30.00 (32.30)	2.60 [bcd]
C. reticulatum	IG 72953	4.33 [bcde]	40.00 (38.86)	2.35 [bcd]
C. pinnatifidum	PI 510663	3.00 [abcde]	46.67 (43.08)	1.16 [abc]
C. judaicum	PI 568217	2.00[ab]	43.33 (41.07)	1.15 [abc]
C. bijugum	PI 599046	3.33 [abcde]	43.33 (41.07)	1.35 [abcd]
C. bijugum	PI 599066	3.33 [abcde]	40.00 (38.86)	0.98 [ab]
C. judaicum	PI 599077	2.67[abcd]	30.00 (33.00)	2.32 [bcd]
C. pinnatifidum	PI 599109	2.67[abcd]	43.33 (40.78)	1.14 [abc]
C. microphyllum	ICCW 17148	3.00[abcde]	36.67 (37.22)	1.11 [abc]
C. arietinum	JG 11 (C)	4.00 [bcde]	33.33 (34.93)	2.79[d]
C. arietinum	KAK 2 (S)	5.33[e]	56.67 (49.14)	2.72 [cd]
C. arietinum	ICC 3137(S)	4.50 [bde]	43.33 940.78)	2.69 [cd]
C. arietinum	ICCL 86111 (R)	2.00[abc]	26.67 (30.29)	2.26 [bcd]
C. arietinum	ICC 506EB (R)	1.00[a]	23.33 (28.78)	2.27 [bcd]
	Fpr	**0.004**	**NS**	**0.02**
	Mean	**2.91**	**35.49**	**1.78**
	SE±	**0.74**	**6.25**	**0.47**
	LSD (P= 0.05)	**2.11**	**17.91**	**1.35**

(DR)[1]= 1,<10% leaf area damaged and 9 = >80% leaf area damaged
Figures in the parentheses are angular transformed values
The values followed by same alphabet did not differ significantly at $p \leq 0.05$ (DMRT)
C-Commercial cultivar, S- Susceptible check, R- Resistant check

Table 4.18. Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea grown under field condition using detached leaf assay (Post-rainy season, 2015-16)

Species	Genotype	Damage rating (DR)[1]	Larval survival (%)	Mean larval weight (mg)
C. chrossanicum	IG 599076	4.67 de	86.70 (72.78) def	2.10 e
C. cuneatum	IG 69979	1.33a	43.30 (40.78)a	0.34 a
C. bijugum	IG 70006	3.67 cd	80.00 (63.93) bcde	0.85 ab
C. bijugum	IG 70012	2.67 abc	90.00 (78.93) ef	0.87 ab
C. bijugum	IG 70018	2.33 abc	90.00 (75.00) ef	0.82 ab
C. bijugum	IG 70022	1.67a	76.70 (61.92) bcde	0.60 a
C. reticulatum	IG 72933	3.33 bcd	76.70 (61.22) bcde	1.89 de
C. reticulatum	IG 72953	3.67 cd	56.70 (48.85)ab	1.90 de
C. pinnatifidum	PI 510663	3.33 bcd	73.30 (60.00) bcde	1.50 cd
C. judaicum	PI 568217	2.67 abc	66.70 (55.07)abcd	0.64 ab
C. bijugum	PI 599046	1.83a	56.70 (48.85)ab	0.73 ab
C. bijugum	PI 599066	3.50 cd	96.70 (83.86) f	0.84 ab
C. judaicum	PI 599077	2.00 ab	76.70 (61.92) bcde	0.67 ab
C. pinnatifidum	PI 599109	3.33 bcd	53.30 (47.01)ab	0.85 ab
C. microphyllum	ICCW 17148	2.33 abc	86.70 (68.86) cdef	0.71 ab
C. arietinum	JG 11 (C)	3.33 bcd	63.30 (53.07)abc	1.52 cde
C. arietinum	KAK 2 (S)	4.67 de	76.70 (61.22) bcde	2.10 e
C. arietinum	ICC 3137(S)	5.33e	86.70 (68.86) cdef	2.03 de
C. arietinum	ICCL 86111 (R)	3.33 bcd	76.70 (61.71) bcde	1.72 cde
C. arietinum	ICC 506EB (R)	2.00 ab	53.30 (46.92)ab	1.22 bc
	Fpr	**<.001**	**<.001**	**<.001**
	Mean	**3.05**	**61.04**	**1.20**
	SE±	**0.43**	**5.63**	**0.18**
	LSD (p=0.05)	**1.24**	**16.10**	**0.51**

(DR)[1]= 1,<10% leaf area damaged and 9 = >80% leaf area damaged
Figures in the parentheses are angular transformed values
The values followed by same alphabet did not differ significantly at $p \leq 0.05$ (DMRT)
C-Commercial cultivar, S- Susceptible check, R- Resistant check

Table 4.19. Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea grown under glasshouse condition using detached leaf assay

Species	Genotype	Damage rating (DR)[1]	Larval survival (%)	Mean larval weight (mg)
C. chrossanicum	IG 599076	4.67 ^{abcd}	76.67 (60.07) ^{bc}	1.80 ^{abcde}
C. cuneatum	IG 69979	3.33 ^{abc}	70.00 (57.00) ^{bc}	0.71 ^{a}
C. bijugum	IG 70006	3.83 ^{abcd}	96.67 (83.86) ^{d}	1.00 ^{ab}
C. bijugum	IG 70012	3.50 ^{abc}	86.67 (72.78) ^{cd}	1.42 ^{abcd}
C. bijugum	IG 70018	3.67 ^{abc}	90.00 (75.00) ^{cd}	1.38 ^{abcd}
C. bijugum	IG 70022	1.33 ^{a}	86.67 (68.86) ^{bcd}	1.22 ^{abc}
C. reticulatum	IG 72933	5.33 ^{abcd}	83.33 (70.07) ^{bcd}	2.75 ^{de}
C. reticulatum	IG 72953	5.33 ^{abcd}	73.33 (59.21) ^{bc}	3.20 ^{ef}
C. pinnatifidum	PI 510663	5.00 ^{abcd}	86.67 (72.78) ^{cd}	2.54 ^{cde}
C. judaicum	PI 568217	4.33 ^{abcd}	86.67 (72.78) ^{cd}	1.35 ^{abcd}
C. bijugum	PI 599046	2.00 ^{ab}	86.67 (68.86) ^{bcd}	1.27 ^{abc}
C. bijugum	PI 599066	1.33 ^{a}	70.00 (57.70) ^{bc}	1.09 ^{abc}
C. judaicum	PI 599077	4.67 ^{abcd}	83.33 (66.14) ^{bcd}	1.77 ^{abcde}
C. pinnatifidum	PI 599109	4.67 ^{abcd}	76.67 (61.71) ^{bc}	2.20 ^{abcde}
C. microphyllum	ICCW 17148	3.67 ^{abc}	73.33 (59.71) ^{bc}	1.20 ^{abc}
C. arietinum	JG 11 (C)	6.00 ^{bcd}	70.00 (57.00) ^{bc}	4.43 ^{fg}
C. arietinum	KAK 2 (S)	8.00 ^{d}	66.67 (54.78) ^{bc}	5.10 ^{fg}
C. arietinum	ICC 3137(S)	6.67 ^{cd}	76.67 (61.22) ^{bc}	4.40 ^{fg}
C. arietinum	ICCL 86111 (R)	4.67 ^{abcd}	60.00 (50.85) ^{b}	4.24 ^{fg}
C. arietinum	ICC 506EB (R)	4.67 ^{abcd}	30.00 (33.21) ^{a}	2.29 ^{bcde}
	Fpr	**0.05**	**0.001**	**<.001**
	Mean	**4.35**	**63.18**	**2.27**
	SE±	**1.22**	**6.06**	**0.44**
	LSD (p=0.05)	**3.50**	**17.38**	**1.25**

(DR)[1]= 1,<10% leaf area damaged and 9 = >80% leaf area damaged
Figures in the parentheses are angular transformed values
The values followed by same alphabet did not differ significantly at $p \leq 0.05$ (DMRT)
C-Commercial cultivar, S- Susceptible check, R- Resistant check

larval survival compared to susceptible check, ICC 3137 (76.67%). All wild relatives of chickpea genotypes had significantly lower larval weight ranged between 0.71 mg on IG 69979 (*C. cuneatum*) and 3.20 mg on IG 72953 (*C. reticulatum*) compared to the larvae weighed on the susceptible check, KAK 2 (5.10 mg), while larval weight reared on resistant check, ICC 506EB was 2.29 mg.

There were significant differences in leaf feeding, larval survival, and larval weight when the neonate larvae of *H. armigera* were released on the detached leaves from the wild relatives of chickpea genotypes across different seasons. There was a significant reduction in leaf feeding and larval weights when neonates were fed on the leaves of IG 70012, IG 70022, IG 70018, IG 70006, PI 599046, PI 599066(*C. bijugum*), IG 69979 (*C. cuneatum*), PI 568217, PI 599077 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*). The earlier studies also revealed that low larval survival and larval weights were recorded when larvae of *H. armigera* reared on wild relatives of chickpea, *C. bijugum*, *C. judaicum*, *C.cuneatum*, *C. pinnatificum*, *C. reticulatum* and *C. microphyllum* (Sharma *et al*., 2005a,b and 2006).

Though, larval survival was greater on the wild relatives than on the cultivated chickpea, the damage rating and larval weights were less, this could be due to presence of some anti-feedant or antibiosis mechanism in wild relatives of chickpea for resistance to *H. armigera* larvae. Sharma *et al*. (2004) observed that leaf feeding and larval survival were greater, while the larval weights on many wild relatives were much lower than those on the cultivated chickpea, indicating existence of antibiosis effect on *H. armigera* in wild relatives of chickpea. Acid exudates such as malic acid and oxalic acid on the leaves of chickpea are the principle component of resistance to *H. armigera* (Cowgill and Lateef, 1996). Green *et al*. (2002) reported the compounds present on the plant surface would play a critical role in determining food selection and initiation of feeding and also trichomes present on plant surface may act as barrier against feeding by neonates of *H. armigera*.

4.3.2 Detached Pod Assay for Evaluation of Resistance to *H. armigera* in Wild Relatives of Chickpea

There were significant differences in damage rating, pod damage percentage and weight gained by larvae when they were fed on pods of wild relatives of chickpea (Table 4.20). All wild relatives of chickpea genotypes exhibited low

Table 4.20. Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea using detached pod assay (Post-rainy season, 2015-16)

Species	Genotype	Damage rating (DR)	Weight gained by larvae (%)	Pod damage (%)
C. chrossanicum	IG 599076	5.6^{bcdefg}	62.7^{ab}	64.0^{bcde}
C. cuneatum	IG 69979	3.4^{ab}	28.8 ^{a}	30.0^{a}
C. bijugum	IG 70006	4.4^{abc}	94.8 ^{abcd}	48.0^{abcd}
C. bijugum	IG 70012	4.8 ^{abcdef}	97.7 ^{abcd}	51.0 ^{abcde}
C. bijugum	IG 70018	3.0 ^{a}	56.0 ^{ab}	41.0 ^{abc}
C. bijugum	IG 70022	4.7 ^{abcdef}	63.5 ^{ab}	52.0^{abcde}
C. reticulatum	IG 72933	4.6 ^{abcd}	74.6 ^{ab}	34.0^{ab}
C. reticulatum	IG 72953	3.6 ^{ab}	95.7 ^{abcd}	41.0^{abc}
C. pinnatifidum	PI 510663	5.8^{bcdefgh}	119.2 ^{bcd}	61.0 ^{abcde}
C. judaicum	PI 568217	5.0 ^{abcdefg}	103.3 ^{abcd}	56.0 ^{abcde}
C. bijugum	PI 599046	4.8 ^{abcdef}	92.6 ^{abcd}	53.0 ^{abcde}
C. bijugum	PI 599066	4.6 ^{abcde}	32.9 ^{ab}	52.0 ^{abcde}
C. judaicum	PI 599077	5.2 ^{abcdefg}	107.8 ^{abcd}	58.0 ^{abcde}
C. pinnatifidum	PI 599109	5.4 ^{bcdefg}	113.6 ^{abcd}	65.3 ^{bcde}
C. microphyllum	ICCW 17148	5.0^{abcdefg}	100.1 ^{abcd}	54.0 ^{abcde}
C. arietinum	JG 11 (C)	7.0^{defgh}	221.5^{e}	84.0^{e}
C. arietinum	KAK 2 (S)	8.0^{h}	174.6^{de}	66.7^{bcde}
C. arietinum	ICC 3137 (S)	7.2^{gh}	210.2^{e}	76.0^{de}
C. arietinum	ICCL 86111 (R)	6.4^{cdefgh}	163.8^{cde}	72.7 ^{cde}
C. arietinum	ICC 506EB (R)	6.2 ^{cdefgh}	170.0^{de}	72.7 ^{cde}
	Fpr	**<.001**	**<.001**	**0.01**
	Mean	**5.3**	**109.8**	**56.7**
	SE±	**0.7**	**26.0**	**9.8**
	LSD (*P*= 0.05)	**1.9**	**73.4**	**27.5**

DR= 1, <10% pod area damaged, and 9= >80% pod area damaged
C- Commercial cultivar, S- Susceptible check, R- Resistant check
The values followed by same alphabet did not differ significantly at $p \leq 0.05$ (DMRT)

damage rating and per cent pod damage when compared to cultivated chickpea. Percentage of weight gained by larvae was more when fed on cultivated chickpea than on wild relatives. Least damage rating (< 4.8) was exhibited in wild relatives of chickpea genotypes, IG 69979 (*C. cuneatum*), IG 72933, IG 72953 (*C. reticulatum*) and PI 5990066, IG 70006, IG 70012, IG 70018 (*C. bijugum*) and showed high levels of resistance compared to susceptible checks, 8.0 in KAK 2 and 7.2 in ICC 3137 and resistant checks, 6.2 in ICC 506EB and 6.4 in ICCL 86111. Based on per cent pod damage wild relatives of chickpea genotypes IG 69979 (*C. cuneatum*), IG 70006 and IG 70018 (*C. bijugum*) and IG 72933, IG 72953 (*C. reticulatum*) showed high levels of resistance with lowest per cent pod damage (< 48%) compared to the cultivated chickpea (84% in JG 11 and 76% in ICC 3137). Based on percentage of weight gained by larvae, accessions IG 69979 (*C. cuneatum*), PI 5990066, IG 70006, IG 70018, IG 70012, IG 70022, PI 599046 (*C. bijugum*), IG 599076 (*C. chrossanicum*) and IG 72933, IG 72953 (*C. reticulatum*) recorded lowest weight gained by larvae (< 97.7%) compared to resistant check, ICCL 86111 (163.8%), commercial cultivar, JG 11 (221.5%) and susceptible check, ICC 3137 (210.2%).

In support of the present investigations, Sharma *et al*. (2005a) also observed low per cent pod damage and weight gained by third instar larvae of *H. armigera* when fed on wild relatives of chickpea compared to cultivated species. It indicates that wild relatives of chickpea seem to have different mechanisms of resistance to *H. armigera* than in cultivated chickpea. Wild chickpea have shown significant variation in trypsin inhibitors for the *H. armigera* gut proteinases (Patankar *et al*., 1999). Giri *et al*. (1998) reported that there was a progressive increase in protease inhibitors throughout seed development in chickpea. Hence, interactions of these protease inhibitors with gut proteases of *H. armigera* might be one of the main factors for resistance in wild relatives of chickpea.

4.3.3 Diet Incorporation Assay for Evaluation of Resistance to *H. armigera* in Wild Relatives of Chickpea

4.3.3.1 Post-rainy Season, 2014-15

During post-rainy season, 2014-15, survival and development of *H. armigera* varied significantly when reared on artificial diet impregnated with lyophilized leaf powder of different genotypes of wild relatives of chickpea (Table 4.21). Larval survival on l0th day after release of the larvae was lowest on resistant check, ICC

506EB (58.33%) followed by C. *chrossanicum*, IG 599076 (60.42%), ICCL 86111 (60.42%), *C. bijugum*, PI 599066 (60.42%), IG 70012 (62.50%) and PI 599046 (62.50%), while highest larval survival (87.50%) was recorded on susceptible check, ICC 3137 and IG 72933.

Weight of 10 days old larvae reared on all wild relatives of chickpea were in a range of 2.55 mg in IG 69979 (*C. cuneatum*) and 10.31 mg in IG 72933 (*C. reticulatum*) and significantly lowest compared to commercial cultivar, JG 11 (19.94 mg) and susceptible checks, KAK 2 (17.46 mg) and ICC 3137 (16.03 mg). Larval period was significantly longer on all wild relatives of chickpea (> 25 days) compared to cultivated chickpea with a range of 24.45 days in KAK2 to 23.52 days in ICC 3137. Pupation was lowest (27.08%) when larvae reared on *C. bijugum* genotypes, IG 70012, IG 70018 and PI 599046. Pupation in all other genotypes was in a range of 31.25% in ICCW 17148 (*C. microphyllum*), IG 599076 (*C. chrossanicum*), IG 70022 and PI 599066 (*C. bijugum*) to 43.75% in PI 599077 (*C. judaicum*) and ICCL 86111 and significantly low compared to susceptible check, KAK 2 (52.08%) and commercial cultivar, JG 11 (54.17%). Weight of one day old pupa varied significantly among different genotypes of wild relatives of chickpea. Lowest pupal weight was recorded on *C. microphyllum*, ICCW 17148 (326.81 mg) and *C. bijugum*, IG 70018 (328.60 mg) and highest was observed on susceptible check, ICC 3137(417.27 mg), JG 11 (413.76 mg) and KAK 2 (403.73 mg). In all other genotypes pupal weight was varied in a range of 336.74 mg (IG 70012) to 382.23 mg (IG 72953).

Longest pupal period was observed on *C. bijugum* genotypes, IG 70018 (15.82 days) followed by IG 70022 (15.41 days), PI 599066 (15.39 days) and *C. pinnatifidum*, PI 510663 (15.35 days) compared to susceptible checks, KAK 2 (12.17 days) and ICC 3137 (12.43 days). Adult emergence in all wild species of chickpea was observed in a range of 16.67% (IG 70018 and PI 599046) and 33.33% (IG 72953, PI 510663, PI 568217 and PI 599077) and significantly lowest compared to susceptible checks, KAK 2 (47.92%) and ICC 3137 (45.83%). Lowest fecundity was observed when reared on PI 599066 (214.42), PI 568217 (215.50) and PI 599046 (216.00) whereas highest was observed on JG 11 (389.42). Fecundity was significantly lowest when reared on all wild relatives of chickpea genotypes compared to susceptible checks, ICC 3137 (349.25) and KAK 2 (340.17) except on

Table 4.21. Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea grown under field condition using diet incorporation assay (Post-rainy season, 2014-15)

Species	Genotype	Larval survival on 10 DAE (%) #	Mean larval weight on 10 DAE (mg)	Larval period (days)	Pupation (%) #	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) #	Fecundity ##
C. chrossanicum	IG 599076	60.42 (51.02) ^{ab}	4.34 ^{ab}	26.13 ^{cde}	31.25 (33.98) ^{a}	354.11 ^{ab}	14.30 ^{bcde}	29.17 (32.63) ^{abcde}	273.92 (16.56) ^{bcdef}
C. cuneatum	IG 69979	83.33 (67.77) ^{cd}	2.55 ^{a}	25.46 ^{abcde}	33.33 (35.22) ^{ab}	356.76 ^{ab}	14.02 ^{abcde}	31.25 (33.98) ^{abcdef}	233.25 (15.29)^{ab}
C. bijugum	IG 70006	77.08 (62.02) ^{abcd}	4.40 ^{ab}	25.34 ^{abcde}	35.42 (36.51)^{abc}	355.05 ^{ab}	14.44 ^{cde}	18.75 (25.35) ^{ab}	252.00 (15.89) ^{abcd}
C. bijugum	IG 70012	62.50 (52.27) ^{abc}	2.90 ^{a}	25.70 ^{cde}	27.08 (31.34) ^{a}	336.74^{a}	14.49 ^{cde}	18.75 (25.63) ^{abc}	226.42 (15.06) ^{a}
C. bijugum	IG 70018	70.83 (57.54) ^{abcd}	3.91 ^{ab}	26.66^{e}	27.08 (31.34) ^{a}	328.60^{a}	15.82^{e}	16.67 (23.93) ^{a}	222.42 (14.92) ^{a}
C. bijugum	IG 70022	77.08 (61.42) ^{abcd}	6.77 ^{abc}	26.69^{e}	31.25 (33.98) ^{a}	351.03^{ab}	15.41 ^{de}	22.92 (28.58) ^{abcd}	275.17 (16.60) ^{bcdef}
C. reticulatum	IG 72933	87.50 (70.53) ^{d}	10.31 ^{cd}	25.37 ^{abcde}	35.42 (36.51)^{abc}	372.91^{abc}	13.26 ^{abc}	25.00 (29.92) ^{abcd}	326.25 (18.07) ^{ghi}
C. reticulatum	IG 72953	77.08 (61.42) ^{abcd}	7.60 ^{bc}	25.26 ^{abcde}	39.58 (38.94) ^{abcd}	382.23^{abc}	13.50 ^{abcd}	33.33 (35.22) ^{bcdef}	343.25 (18.54) ^{ij}
C. pinnatifidum	PI 510663	72.92 (58.79) ^{abcd}	3.58 ^{ab}	26.12 ^{cde}	41.67 (40.13) ^{abcd}	354.28^{ab}	15.35 ^{de}	33.33 (35.26) ^{bcdef}	285.75 (16.92) ^{cdefg}
C. judaicum	PI 568217	85.42 (68.03) ^{cd}	5.69 ^{ab}	26.13 ^{cde}	39.58 (38.98) ^{abcd}	363.73^{abc}	13.35 ^{abc}	33.33 (35.22) ^{bcdef}	215.50 (14.69) ^{a}
C. bijugum	PI 599046	62.50 (52.35) ^{abc}	3.36 ^{ab}	25.73 ^{cde}	27.08 (31.34) ^{a}	341.76^{a}	14.22 ^{bcde}	16.67 (23.93) ^{a}	216.00 (14.71) ^{a}
C. bijugum	PI 599066	60.42 (51.02) ^{ab}	3.19 ^{a}	25.67 ^{bcde}	31.25 (33.98) ^{a}	344.54^{a}	15.39 ^{de}	22.92 (28.58) ^{abcd}	214.42 (14.66) ^{a}

Table 4.21 (cont.).

Species	Genotype	Larval survival on 10 DAE (%) #	Mean larval weight on 10 DAE (mg)	Larval period (days)	Pupation (%) #	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) #	Fecundity ##
C. judaicum	PI 599077	75.00 (60.00) abcd	4.17 ab	26.37 de	43.75 (41.38) abcd	352.38ab	14.59 cde	33.33 (35.22) bcdef	240.25 (15.51) abc
C. pinnatifidum	PI 599109	75.00 (60.00) abcd	4.43 ab	25.00 abcde	39.58 (38.94) abcd	353.35ab	14.05 abcde	27.08 (31.21) abcd	256.92 (16.04) abcde
C. microphyllum	ICCW 17148	83.33 (65.91) bcd	4.57 ab	25.84 cde	31.25 (33.68) a	326.81 a	14.71 cde	25.00 (29.23) abcd	305.67 (17.50) fghi
C. arietinum	JG 11 (C)	85.42 (67.60) cd	19.94 e	23.65 ab	54.17 (47.42) d	413.76c	12.28 a	45.83 (42.58) ef	389.42 (19.75) j
C. arietinum	KAK 2 (S)	75.00 (60.00) abcd	17.46 e	24.45 abcd	52.08 (46.22) cd	403.73bc	12.17 a	47.92 (43.80) f	340.17 (18.45) hij
C. arietinum	ICC 3137 (S)	87.50 (69.56) d	16.03 e	23.52 a	50.00 (45.00) bcd	417.27c	12.43 ab	45.83 (42.58) ef	349.25 (18.68) ij
C. arietinum	ICCL 86111 (R)	60.42 (51.02) ab	10.73 cd	24.16 abc	43.75 (41.38) abcd	379.09abc	13.06 abc	35.42 (36.51) cdef	306.75 (17.50) efghi
C. arietinum	ICC 506EB (R)	58.33 (49.96) a	11.93 d	24.30 abc	41.67 (40.19) abcd	381.13abc	13.06 abc	37.50 (37.730 def	291.00 (17.07) defgh
	Fpr	**0.044**	**<.001**	**0.027**	**0.02**	**0.024**	**0.003**	**0.004**	**<.001**
	Mean	**59.91**	**7.39**	**25.38**	**37.81**	**363.46**	**13.99**	**32.86**	**16.62**
	SE±	**4.60**	**1.26**	**0.59**	**3.01**	**16.26**	**0.56**	**3.21**	**0.44**
	LSD (p=0.05)	**13.61**	**3.73**	**1.75**	**8.92**	**48.12**	**1.66**	**9.50**	**1.30**

#Figures in the parentheses are angular transformed values; DAE- days after initiation of experiment

Figures in the parentheses are square root (√x+0.5) transformed values

The values followed by same alphabet did not differ significantly at $p \leq 0.05$ (DMRT)

C-Commercial cultivar, S- Susceptible check, R- Resistant check

C. microphyllum, ICCW 17148 (305.67), *C. reticulatum*, IG 72933 (326.25) and IG 72953 (343.25) which were low but non-significant.

4.3.3.2 Post-rainy Season, 2015-16

Survival and development of *H. armigera* on artificial diet impregnated with lyophilized leaf powders of wild relatives of chickpea varied significantly during post-rainy season, 2015-16 (Table 4.22). Larval survival on genotypes of *C. bijugum*, IG 70006, IG 7012, IG 70018, IG 70022, PI 599046 and PI 599066, *C. judaicum*, PI 568217, *C. pinnatifidum*, PI 510663 and PI 599109, *C. chrossanicum*, IG 599076, *C. reticulatum*, IG 72933 and IG 72953 and ICCL 86111 (resistant check) were significantly lower (50.00 to 75.00%) after 10 days compared to susceptible checks, KAK 2 (91.67%) and ICC 3137 (87.50%). Significant lower larval weights were recorded on all genotypes of wild relatives of chickpea with a range of 3.61 mg (IG 70018) and 11.24 mg (IG 72953) compared to cultivated chickpea genotypes.

Larval period was delayed for two to three days when reared on wild relatives of chickpea genotypes compared to susceptible check, ICC 3137 (22.35 days), whereas longest larval period was observed on *C. microphyllum*, ICCW 17148 (26.94 days) and *C. bijugum*, IG 70018 (26.77 days). Pupation percentage was lowest on PI 599066 (31.25%), IG 70012 (33.33%), compared to susceptible checks, KAK 2 (72.92%) and ICC 3137 (70.83%) and resistant checks, ICC 506EB (62.50%) and ICCL 86111 (62.50%). Mean pupal weights were lowest on all genotypes of wild relatives of chickpea with a range of 321.68 mg (IG 70012) and 410.63 mg (IG 72953) compared to susceptible check, ICC 3137 (446.31 mg), while pupal weight were 395.94 mg and 398.65 mg on resistant checks, ICCL 86111 and ICC 506EB, respectively.

Pupal period was significantly longest on all wild relatives of chickpea genotypes compared to susceptible check, ICC 3137 (11.77 days), except on PI 568217 (12.19 days) and IG 72933 (12.95 days) where the difference was non-significant. Pupal period was 12.46 days in resistant check, ICC 506EB. Adult emergence was observed in a range of 12.50% (IG 70006) and 56.25% (JG 11). Adult emergence on wild relatives of chickpea genotypes (12.50 to 39.58%) was significantly lower compared to susceptible checks, ICC 3137 (54.17%) and KAK 2 (50.00%), while on resistant check, ICC 506EB it was 43.75%. Lowest fecundity

Table 4.22. Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea grown under field condition using diet incorporation assay (Post-rainy season, 2015-16)

Species	Genotype	Larval survival on 10 DAE (%)[#]	Mean larval weight on 10 DAE (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) [#]	Fecundity [##]
C. chrossanicum	IG 599076	75.00 (60.08) [bcde]	4.26 [ab]	26.43 [ghij]	39.58 [ab]	346.02 [abc]	13.82 [cdefghi]	27.08 (31.21) [de]	265.33 (16.29) [bcdef]
C. cuneatum	IG 69979	79.17 (73.22) [cde]	4.43 [ab]	25.52 [efghi]	43.75 [abcd]	360.63 [abcde]	14.14 [defghij]	35.42 (36.45) [fg]	230.00 (15.17) [abcd]
C. bijugum	IG 70006	50.00 (71.26)[a]	5.81 [abcde]	24.19 [bcde]	41.67 [abc]	353.91 [abcde]	14.15 [defghij]	12.50 (20.70)[a]	255.00 (15.93) [abcde]
C. bijugum	IG 70012	58.33 (64.37) [ab]	8.15 [abcdefg]	25.04 [defg]	33.33 [a]	321.68[a]	13.47 [abcdefg]	18.75 (25.63) [b]	211.33 (14.52) [a]
C. bijugum	IG 70018	62.50 (52.27) [abc]	3.61 [a]	26.77 [ij]	35.42 [a]	328.21[ab]	15.73 [ij]	20.83 (27.05) [bc]	207.33 (14.40) [a]
C. bijugum	IG 70022	62.50 (52.35) [abc]	5.17 [abcd]	26.14 [fghij]	37.50 [ab]	358.28 [abcde]	15.81[j]	25.00 (30.00) [cd]	287.33 (16.95) [ef]
C. reticulatum	IG 72933	58.33 (49.87) [ab]	9.94 [efgh]	25.08 [defg]	41.67 [abc]	391.62 [cdefg]	12.95 [abcdef]	22.92 (28.58) [bcd]	349.00 (18.68) [ghi]
C. reticulatum	IG 72953	70.83 (57.31) [bcd]	11.24 [fgh]	24.57 [cde]	52.08 [abcde]	410.63 [fgh]	13.67 [bcdefgh]	37.50 (37.73) [fgh]	344.00 (18.54) [ghi]
C. pinnatifidum	PI 510663	70.83 (57.37) [bcd]	9.29 [cdefgh]	26.74 [hij]	52.08 [abcde]	351.44 [abcd]	15.62 [hij]	33.33 (35.22) [fg]	277.00 (16.66) [def]
C. judaicum	PI 568217	62.50 (52.27) [abc]	9.58 [defgh]	26.27 [fghij]	43.75 [abcd]	369.78 [bcdef]	12.19 [abcd]	31.25 (33.98) [ef]	223.00 (14.93) [ab]
C. bijugum	PI 599046	64.58 (69.30) [abc]	4.74 [abc]	25.36 [efgh]	43.75 [abcd]	341.07 [ab]	14.23 [efghij]	22.92 (28.58) [bcd]	227.00 (15.06) [abc]
C. bijugum	PI 599066	62.50 (62.95) [abc]	3.82 [ab]	25.50 [efghi]	31.25 [a]	339.16 [ab]	14.64 [fghij]	22.92 (28.58) [bcd]	216.33 (14.72) [a]

Table 4.22 (cont.).

Species	Genotype	Larval survival on 10 DAE (%)[#]	Mean larval weight on 10 DAE (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) [#]	Fecundity [##]
C. judaicum	PI 599077	87.50 (69.30) ^{de}	8.49 ^{bcdefg}	24.88 ^{cdef}	64.58 ^{de}	345.14 ^{ab}	15.32 ^{ghij}	39.58 (38.98) ^{gh}	228.00 (15.11) ^{abc}
C. pinnatifidum	PI 599109	75.00 (60.08) ^{bcde}	7.24 ^{abcdef}	23.63^{abc}	58.33 ^{bcde}	347.50 ^{abc}	13.49 ^{abcdefg}	35.42 (36.45) ^{fg}	272.33 (16.47) ^{cdef}
C. microphyllum	ICCW 17148	79.17 (62.95) ^{cde}	4.32 ^{ab}	26.94 ^{j}	52.08 ^{abcde}	340.08 ^{ab}	13.56 ^{abcdefg}	39.58 (38.98) ^{gh}	295.33 (17.19) ^{efg}
C. arietinum	JG 11 (C)	89.58 (45.00) ^{de}	17.12 ^{j}	22.93^{ab}	72.92 ^{e}	464.73 ^{i}	11.63 ^{a}	56.25 (48.59) ^{i}	382.33 (19.50) ^{i}
C. arietinum	KAK 2 (S)	91.67 (52.27) ^{e}	16.19 ^{ij}	22.93^{ab}	72.92^{e}	427.42 ^{ghi}	12.11 ^{abc}	50.00 (45.00) ^{i}	351.33 (18.72) ^{hi}
C. arietinum	ICC 3137 (S)	87.50 (53.92) ^{de}	13.28 ^{hij}	22.35^{a}	70.83 ^{e}	446.31 ^{hi}	11.77 ^{ab}	54.17 (47.40) ^{i}	382.00 (19.51) ^{i}
C. arietinum	ICCL 86111 (R)	75.00 (60.32) ^{bcde}	12.34 ^{ghi}	23.88^{bcd}	62.50 ^{cde}	395.94 ^{defg}	12.35 ^{abcde}	39.58 (38.94) ^{gh}	341.00 (18.48) ^{ghi}
C. arietinum	ICC 506EB (R)	81.25 (49.87) ^{cde}	13.38 ^{hij}	23.81^{bcd}	62.50 ^{cde}	398.65 ^{efg}	12.46 ^{abcde}	43.75 (41.41) ^{h}	307.00 (17.52) ^{fgh}
	Fpr	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**	**<.001**
	Mean	**58.82**	**8.62**	**24.95**	**50.62**	**371.91**	**13.65**	**34.97**	**16.72**
	SE±	**3.69**	**1.39**	**0.42**	**6.27**	**13.59**	**0.58**	**1.20**	**0.47**
	LSD (p=0.05)	**10.922**	**4.10**	**1.23**	**18.54**	**40.22**	**1.71**	**3.54**	**1.35**

[#]Figures in the parentheses are angular transformed values; DAE- days after initiation of experiment

[##] Figures in the parentheses are square root (√x+0.5) transformed values

The values followed by same alphabet did not differ significantly at $p \leq 0.05$ (DMRT)

C-Commercial cultivar, S- Susceptible check, R- Resistant check

was observed when the insects reared on IG 70018 (207.33), IG 70012 (211.33) and PI 599066 (216.33) among all the genotypes tested, while fecundity was highest reared on JG 11 (382.33) and ICC 3137 (382.00).

4.3.3.3 Glasshouse Condition

Survival and development of *H. armigera* larvae reared on artificial diet with lyophilized leaf powders of wild relatives of chickpea genotypes grown under glasshouse condition varied significantly (Table 4.23). The larval survival and larval weight on 10^{th} day, larval and pupal periods, pupation, pupal weight, adult emergence and fecundity were observed in a range of 47.92 to 81.25%, 1.69 to 14.63 mg, 24.38 to 27.18 days, 12.22 to 16.14 days, 22.92 to 50.00%, 313.54 to 388.23 mg, 10.42 to 45.83% and 205.00 to 396.50, respectively. Lowest larval survival was observed on IG 70018 (47.92%) followed by ICCW 17148 (52.08%) and IG 70012 (52.08%), while the highest was observed on susceptible check, ICC 3137 (81.25%).

Larval survival in all other genotypes was ranged from 58.33% (IG 70006, IG 70022 and PI 599046) to 79.17% (JG 11). Lowest larval weights were observed on ICCW 17148 (1.69 mg), PI 599046 (2.02 mg), IG 70018 (3.42 mg) and IG 70022 (3.94 mg) compared to all other genotypes, whereas highest larval weight was observed on susceptible checks, KAK 2 (14.63 mg) and ICC 3137 (14.34 mg). The larval period was prolonged by one to three days on IG 72933, PI 599077, IG 599076, PI 599066, IG 72953, PI 568217, PI 599046, IG 70012, PI 599109, IG 70006, IG 70022, ICCW 17148 and IG 700018 (25.67 to 27.18 days) as compared to that on the susceptible check, ICC 3137 (24.70 days). Pupation was significantly lower on *C. microphyllum*, ICCW 17148 and *C. bijugum*, PI 599046, IG 70018, IG 70012 and IG 70022 (20.83 to 35.42%) compared to susceptible check, KAK 2 (50.00%). Lowest pupal weight was observed on all genotypes of wild relatives of chickpea (313.54 to 362.20 mg) compared to susceptible checks, ICC 3137 (388.23 mg) and KAK 2 (380.03 mg).

Pupal period was delayed when reared on all genotypes of wild relatives of chickpea with a range of 13.33 days (IG 72953) to 16.14 days (PI 599066) compared to susceptible check, KAK 2 (12.22 days) and ICC 3137 (12.50 days), while it was 13.66 days in ICC 506EB (resistant check). Lowest adult emergence was observed on ICCW 17148 (10.42%) followed by IG 70018 (14.58%) and PI 599046

Table 4.23. Expression of antibiosis mechanism of resistance to *H. armigera* in wild relatives of chickpea grown under glasshouse condition using diet incorporation assay

Species	Genotype	Larval survival on 10 DAE (%)	Mean larval weight on 10 DAE (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) #	Fecundity ##
C. chrossanicum	IG 599076	60.42 abcd	9.15 bcdef	25.83 abcdef	41.67 cd	362.20 cde	14.78 bcdef	35.42 (36.51) fghi	282.50 (16.82) abc
C. cuneatum	IG 69979	60.42 abcd	8.65 abcdef	25.41 abcde	37.50 bcd	352.88 bcd	13.89 abcdef	29.17 (32.46) cdef	236.50 (15.34) ab
C. bijugum	IG 70006	58.33 abc	4.97 abc	26.50 def	43.75 cd	356.19 bcde	14.73 abcdef	25.00 (29.92) bcd	249.00 (15.79) ab
C. bijugum	IG 70012	52.08 ab	6.52 abcd	26.37 cdef	35.42 abcd	351.79 bcd	15.50 def	22.92 (28.58) bc	241.50 (15.51) ab
C. bijugum	IG 70018	47.92 a	3.42 ab	27.18 f	33.33 abc	328.98 ab	15.90 ef	14.58 (22.40) a	237.50 (15.38) ab
C. bijugum	IG 70022	58.33 abc	3.94 ab	26.61 def	35.42 abcd	343.77 bc	15.00 bcdef	22.92 (28.39) bc	263.00 (16.16) ab
C. reticulatum	IG 72933	70.83 bcde	10.12 bcdef	25.67 abcdef	41.67 cd	354.20 bcd	13.57 abcde	31.25 (33.98) defg	303.50 (17.38) abc
C. reticulatum	IG 72953	68.75 bcde	7.61 abcde	25.96 abcdef	41.67 cd	353.83 bcd	13.33 abcd	29.17 (32.63) cdef	342.50 (18.51) bc
C. pinnatifidum	PI 510663	64.58 abcde	6.91 abcd	25.50 abcde	41.67 cd	357.12 bcde	15.07 cdef	33.33 (35.26) efghi	294.50 (17.15) abc
C. judaicum	PI 568217	66.67 abcde	8.23 abcdef	26.00 abcdef	47.92 cd	357.68 bcde	14.50 abcdef	37.50 (37.73) ghi	208.00 (14.43) a
C. bijugum	PI 599046	58.33 abc	2.02 a	26.10 bcdef	22.92 ab	342.45 abc	14.21 abcdef	14.58 (22.40) a	205.00 (14.32) a
C. bijugum	PI 599066	60.42 abcd	6.07 abcd	25.84 abcdef	43.75 cd	349.91 bcd	16.14 f	27.08 (31.21) bcde	212.50 (14.59) a

Table 4.23. (cont.).

Species	Genotype	Larval survival on 10 DAE (%)	Mean larval weight on 10 DAE (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%) #	Fecundity ##
C. judaicum	PI 599077	60.42 ^{abcd}	7.07 ^{abcd}	25.80 ^{abcdef}	45.83 ^{cd}	359.62 ^{bcde}	13.86 ^{abcdef}	31.25 (33.98)^{defgh}	252.50 (15.90) ^{ab}
C. pinnatifidum	PI 599109	62.50 ^{abcde}	5.74 ^{abcd}	26.37 ^{cdef}	39.58^{cd}	359.20 ^{bcde}	14.60 ^{abcdef}	20.83 (27.05)^{b}	241.50 (15.50) ^{ab}
C. microphyllum	ICCW 17148	52.08^{ab}	1.69^{a}	26.80^{ef}	20.83^{a}	313.54^{a}	15.85 ^{def}	10.42 (18.74)^{a}	316.00 (17.78) ^{bc}
C. arietinum	JG 11 (C)	79.17^{de}	11.16 ^{cdef}	24.38^{a}	47.92 ^{cd}	362.79^{cde}	12.92^{abc}	39.58 (38.94)^{gij}	396.50 (19.91)^{c}
C. arietinum	KAK 2 (S)	75.00 ^{cde}	14.63^{f}	24.80^{abc}	50.00^{d}	380.03^{de}	12.22^{a}	45.83 (42.60)^{j}	329.00 (18.14) ^{ab}
C. arietinum	ICC 3137 (S)	81.25^{e}	14.34^{ef}	24.70^{ab}	47.92 ^{cd}	388.23^{e}	12.50^{ab}	37.50 (37.65)^{ghi}	316.50 (17.80) ^{bc}
C. arietinum	ICCL 86111 (R)	70.83 ^{bcde}	11.29 ^{cdef}	25.39 ^{abcde}	45.83^{cd}	362.23 ^{cde}	14.35 ^{abcdef}	33.33 (35.26)^{efghi}	272.50 (16.50) ^{ab}
C. arietinum	ICC 506EB (R)	68.75 ^{bcde}	12.35^{def}	25.02^{abcd}	45.83^{cd}	363.61 ^{cde}	13.66 ^{abcdef}	33.33 (35.22)^{efghi}	275.00 (16.56) ^{ab}
	Fpr	**0.039**	**<.001**	**0.035**	**0.01**	**0.015**	**0.04**	**<.001**	**0.02**
	Mean	**63.85**	**7.79**	**25.81**	**40.52**	**355.01**	**14.33**	**32.05**	**16.47**
	SE±	**5.83**	**2.09**	**0.48**	**4.66**	**9.43**	**0.73**	**1.47**	**0.92**
	LSD (p=0.05)	**17.27**	**5.86**	**1.41**	**13.79**	**27.92**	**2.16**	**4.34**	**2.73**

#Figures in the parentheses are angular transformed values; DAE- days after initiation of experiment

Figures in the parentheses are square root ($\sqrt{x+0.5}$) transformed values

The values followed by same alphabet did not differ significantly at $p \leq 0.05$ (DMRT)

C-Commercial cultivar, S- Susceptible check, R- Resistant check

(14.58%), while highest was observed on KAK 2 (45.83%). Fecundity was significantly lower when reared on PI 599046 (205.00), PI 568217 (208.00), PI 599066 (212.50), IG 69979 (236.50) and IG 70018 (237.50) compared to susceptible check, KAK 2 (329.00).

The above results based on the survival and development of *H. armigera* on artificial diet impregnated with lyophilized leaf powders of different genotypes of wild relatives of chickpea across seasons revealed that antibiosis to *H. armigera* in wild relatives of chickpea was expressed in terms of lower larval survival, pupation percentage and adult emergence, decreased larval and pupal weight, prolonged larval and pupal developmental periods and reduced fecundity. Higher levels of antibiosis against *H. armigera* in wild relatives compared to cultigens in terms of reduced survival and delayed developmental periods had also been studied in chickpea (Sharma *et al*., 2005a, 2006) and pigeonpea (Sujana *et al*., 2008 and Shanower *et al*., 1997). However, antibiosis seems to be the major component of resistance in the wild relatives of chickpea, which may be due to secondary plant substances such as flavonoids, protease inhibitors and lectins. Simmonds and Stevenson (2001) reported that isoflavonoids, judaicin 7-o-glucoside, 2-methoxy judaicin, judaicin and maakiain present in wild relatives of chickpea had shown antifeedant activity and reduction in weight towards the larvae of *H. armigera*. Shukla *et al*. (2005) reported that chickpea and snowdrop lectins had shown marked antibiosis effects on *H. armigera*. Antibiosis effect of chickpea trypsin inhibitor on *H. armigera* had been reported by Kansal *et al*. (2008). Narayanamma *et al*. (2008) also reported that, F_1 hybrids based on resistant genotypes of chickpea were recorded lower larval survival, pupation, pupal weight compared to susceptible check suggested transfer of antibiosis mechanism of resistance to progeny from resistant parents. Slower larval growth, which resulted in prolonged development, may also increase the probability of predation, parasitism and infection by pathogens, resulting in reduced survival of *H. armigera*. Hence, these wild relatives of chickpea with higher levels of antibiosis mechanism of resistance could be used as sources for development of cultivars resistance to *H. armigera*.

4.4 Morphological Characterization of Wild Relatives of Chickpea in Relation with Expression of Resistance to *H. armigera*

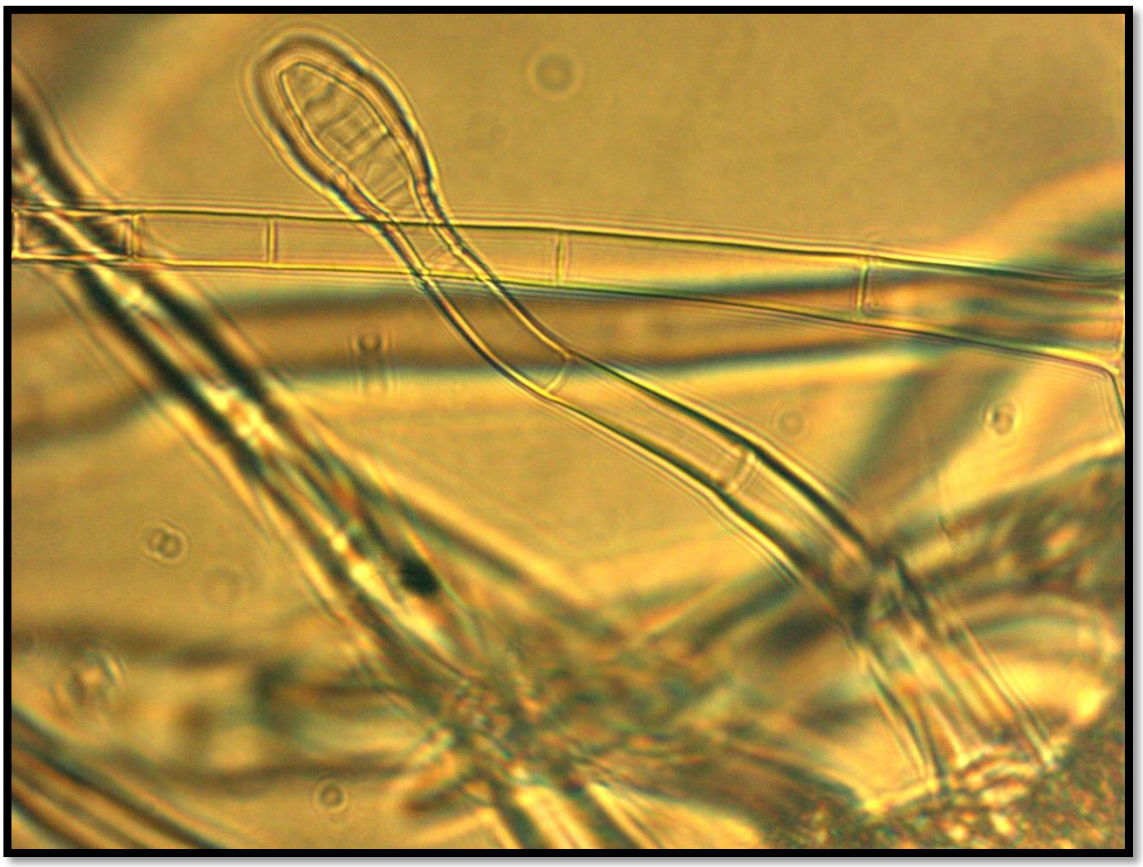
Results on morphological characters *viz*., trichome density and pod wall thickness in different wild relatives of chickpea are presented hereunder.

4.4.1 Trichome Density

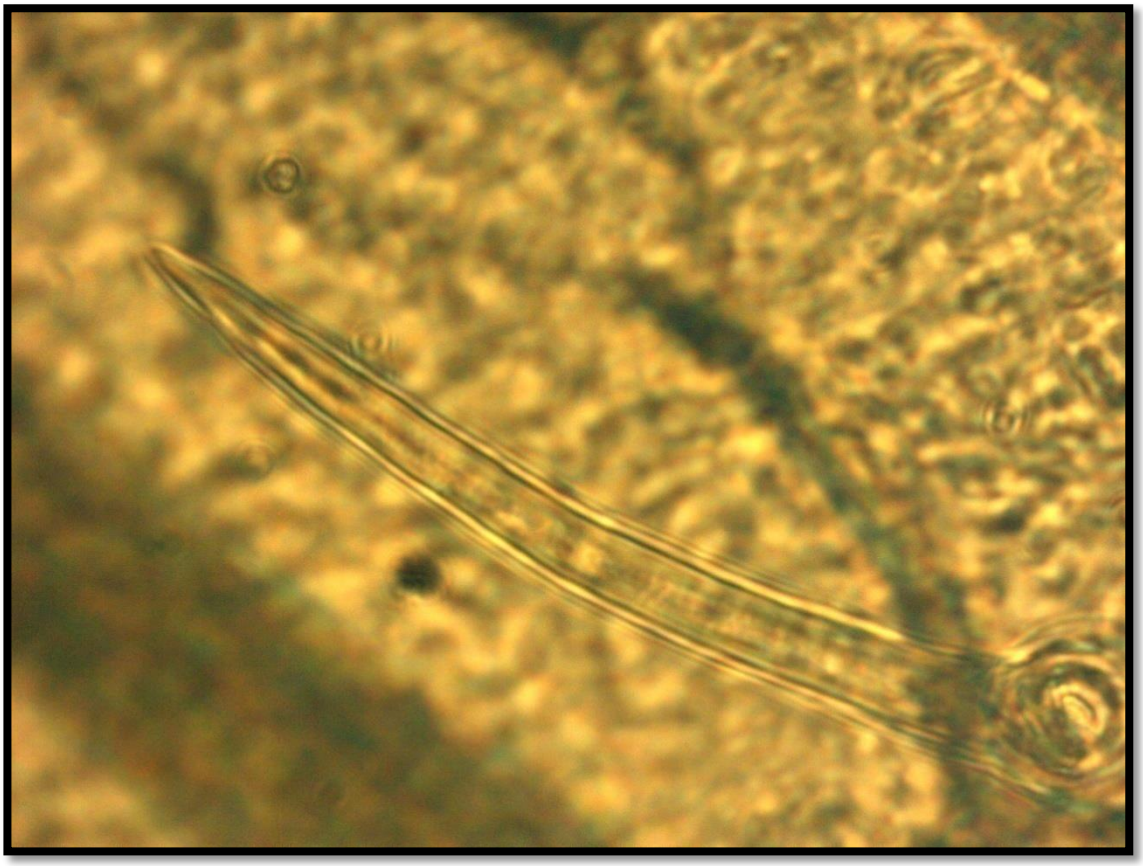
In the present investigation, two different types of trichomes *viz.,* glandular and non-glandular trichomes were observed on different wild relatives of chickpea (Table 4.24) (Plate 9). The glandular trichomes had a basal cell, long vacuolate stalk cells and a terminal cluster of dense secretary head cells (Schnepf, 1965 and Lazzaro and Thomson 1989), whereas non-glandular trichomes were unsegmented and long. Significant differences were observed in both glandular and non-glandular trichomes with respect to their density (number of trichomes per 10X microscopic field) among genotypes. Highest numbers of glandular trichomes were observed on *C. bijugum* genotypes, PI 599046, IG 70012, IG 70018, IG 70006, PI 599066 and IG 70022 (15.90 to 14.20) and lowest was observed on *C. chrossanicum*, IG 599076 (4.50). In cultivated chickpea genotypes glandular trichome density was less in susceptible check, KAK 2 (6.50) and ICC 3137 (7.70), while more was observed in resistant checks, ICCL 86111 (12.30) and ICC 506EB (11.40).

Among genotypes, lowest non-glandular trichome density was observed in PI 599077 (0.90) and ICCW 17148 (0.90), while highest trichome density was observed in IG 72933 (42.20) followed by JG 11 (39.00) and resistant check, ICC 506EB (37.00). Non-glandular trichomes were completely absent in *C. pinnatifidum* genotypes (PI 510663 and PI 599109).

The correlation studies (Table 4.25) revealed that, glandular and non-glandular trichomes showed negative association with oviposition preference under multi-choice (r= -0.75) and no-choice conditions (r= -0.63), respectively. Correlation of trichome density with detached leaf assay revealed that, glandular trichomes had significant negative association with damage rating (r= -0.58), whereas non-glandular trichomes had significant positive association with damage rating and larval weight (r= 0.55 and 0.68, respectively) but negative (r= -0.53) with larval survival percentage.



9a. Glandular trichome



9b. Non-glandular trichome

Plate 9. Different types of trichomes in wild relatives of chickpea

Table 4.24. Morphological characterization of wild relatives of chickpea exhibiting resistance or susceptibility to *H. armigera*

Species	Genotype	Trichome density on leaves (Number/10X microscopic field)		Pod wall thickness (mm)
		Glandular trichomes	Non glandular trichomes	
C. chrossanicum	IG 599076	4.50	12.60	0.18
C. cuneatum	IG 69979	8.80	4.00	0.30
C. bijugum	IG 70006	14.60	4.40	0.40
C. bijugum	IG 70012	15.40	4.00	0.40
C. bijugum	IG 70018	14.70	2.50	0.36
C. bijugum	IG 70022	14.20	3.60	0.37
C. reticulatum	IG 72933	11.30	42.20	0.39
C. reticulatum	IG 72953	8.00	31.90	0.41
C. pinnatifidum	PI 510663	5.10	0.00	0.23
C. judaicum	PI 568217	5.10	1.10	0.25
C. bijugum	PI 599046	15.90	3.30	0.38
C. bijugum	PI 599066	14.50	3.50	0.32
C. judaicum	PI 599077	5.70	0.90	0.24
C. pinnatifidum	PI 599109	5.70	0.00	0.26
C. microphyllum	ICCW 17148	6.10	0.90	0.24
C. arietinum	JG 11 (C)	10.40	39.00	0.26
C. arietinum	KAK 2 (S)	6.50	17.30	0.25
C. arietinum	ICC 3137 (S)	7.70	29.30	0.22
C. arietinum	ICCL 86111 (R)	12.30	25.90	0.28
C. arietinum	ICC 506 EB (R)	11.40	37.00	0.27
	Fpr	**<.001**	**<.001**	**<.001**
	Mean	**9.89**	**13.17**	**0.313**
	SE±	**0.87**	**1.74**	**0.015**
	LSD (p=0.05)	**2.43**	**4.85**	**0.043**

C- Commercial cultivar, S- Susceptible check, R- Resistance check

Table 4.25. Association of trichome density with oviposition preference and detached leaf assay for resistance to *H. armigera* in wild relatives of chickpea

	Oviposition preference		Detached leaf assay		
	Multi-choice condition	**No-choice condition**	**Damage rating**	**Larval survival (%)**	**Mean larval weight (mg)**
Glandular trichomes	-0.75**	-0.21	-0.58**	0.11	-0.26
Non-glandular trichomes	-0.13	-0.63**	0.55*	-0.53*	0.68**

*,** Correlation coefficients significant at P ≤ 0.05 and 0.01, respectively

Presence of trichomes is an important insect resistance mechanism in a number of crops, and wild relatives have often been exploited as a source for trichomes (Peter *et al*., 1995). Chemicals produced by glandular trichomes in chickpea had antixenosis and antibiosis effects on *H. armigera* (Yoshida *et al*., 1995). Peter and Shanower (1998) documented that dense mat of non-glandular trichomes in chickpea prevents the small larvae from feeding on the plant. Shahzad *et al*. (2005) reported that larval survival decreased with increase in trichome density in chickpea. Negative effects of trichomes on *H. armigera* in chickpea have been documented by several authors (Girija *et al*., 2008., Hossain *et al*., 2008b and Shabbir *et al*., 2014). Green *et al*. (2002) reported that first and second instars of *H. armigera* preferred pods of *Cajanus scarabaeoides* with trichomes removed to pods with trichomes present, which indicates the trichomes might be reason for non-preference for larval feeding. Presence of non-glandular trichomes in wild relatives of pigeonpea might be one of the reasons for oviposition non-preference (Peter *et al*., 1995 and Romeis *et al*., 1999).

4.4.2 Pod Wall Thickness

There were significant differences in pod wall thickness of different accessions of wild relatives of chickpea (Table 4.24). Lowest pod wall thickness was recorded in IG 599076 (0.18 mm), whereas highest was recorded in IG 72953 (0.41 mm) followed by IG 70006 (0.40 mm) and IG 70012 (0.40 mm). Pod wall thickness of other genotypes was in a range of 0.22mm in ICC 3137 to 0.39 mm in IG 72933.

4.5 HPLC FINGER PRINTS OF LEAF ORGANIC ACIDS IN DIFFERENT GENOTYPES OF WILD RELATIVES OF CHICKPEA

Variations in leaf organic acid exudates were identified and quantified through HPLC fingerprints based on their retention time (RT) and peak area in different wild relatives of chickpea and represented in mg/g fresh weight of sample.

4.5.1 Oxalic Acid Content

Oxalic acid identified at RT of 4.04 to 4.16 min in different genotypes. Significant differences were exhibited in oxalic acid concentrations among different genotypes (Table 4.26 and Figure 4.4). During post-rainy season, 2014-15 all wild relatives of chickpea genotypes recorded low amounts of oxalic acid compared to cultivated chickpea genotypes except in IG 72933 (2.36 mg/g) which was significantly higher compared to susceptible check, ICC 3137 (1.43 mg/g) and significantly lower compared to resistant checks, ICCL 86111 (3.00 mg/g) and ICC 506EB (3.13 mg/g). During post-rainy season, 2015-16 lowest amount of oxalic acid was observed in PI 599046 (0.44 mg/g), while highest amount of oxalic acid was recorded in IG 69979 (2.92 mg/g). Oxalic acid content in cultivated chickpea ranged from 1.84 mg/g (ICC 3137) to 2.45 mg/g (ICC 506EB) which was comparatively higher with wild relatives of chickpea genotypes. Similar trend was observed in glass house grown condition, where significantly higher amounts of oxalic acid were observed in all cultivated chickpea genotypes than wild relatives of chickpea genotypes. Oxalic acid content in wild relatives ranged from 0.16 mg/g (PI 568217) to 1.35 mg/g (IG 72953), while it was ranged from 1.21 mg/g (ICC 3137, susceptible check) to 4.27 mg/g (ICC 506EB, resistant check) in cultivated chickpea.

4.5.2 Malic Acid Content

Malic acid identified at RT of 5.24 to 5.29 min in different genotypes. Significant differences were exhibited in malic acid concentrations among different genotypes (Table 4.26 and Figure 4.4). During post-rainy season, 2014-15 lowest amount of malic acid was recorded in *C. reticulatum*, IG 72933 (1.94 mg/g) and IG 72953 (2.09 mg/g), while highest was recorded in *C. judaicum*, PI 599077 (10.46 mg/g) and PI 568217 (7.93 mg/g) followed by *C. microphyllum*, ICCW 17148 (7.46 mg/g). During post-rainy season, 2015-16 no traces of malic acid was recorded in

Table 4.26. Expression of leaf organic acids in wild relatives of chickpea exhibiting different levels of resistance to *H. armigera*

Species	Genotype	Post-rainy season, 2014-15		Post-rainy season, 2015-16		Glass house condition	
		Oxalic acid mg/g fresh weight	Malic acid mg/g fresh weight	Oxalic acid mg/g fresh weight	Malic acid mg/g fresh weight	Oxalic acid mg/g fresh weight	Malic acid mg/g fresh weight
C. chrossanicum	IG 599076	1.08	3.04	0.78	4.26	0.34	1.78
C. cuneatum	IG 69979	0.85	4.86	2.92	6.51	0.18	8.28
C. bijugum	IG 70006	0.37	5.28	0.80	1.41	0.22	1.36
C. bijugum	IG 70012	0.44	4.49	0.47	0.28	0.33	1.48
C. bijugum	IG 70018	0.46	3.30	0.63	1.24	0.18	1.49
C. bijugum	IG 70022	0.69	2.97	0.48	2.81	0.26	1.91
C. reticulatum	IG 72933	2.36	1.94	1.31	4.62	0.76	0.61
C. reticulatum	IG 72953	1.07	2.09	1.10	2.78	1.35	0.56
C. pinnatifidum	PI 510663	0.41	6.11	0.77	0.88	0.27	6.07
C. judaicum	PI 568217	0.63	7.93	1.57	4.50	0.16	5.26
C. bijugum	PI 599046	0.50	6.52	0.44	2.06	0.18	3.53
C. bijugum	PI 599066	0.41	4.01	0.72	0.00	0.17	3.58
C. judaicum	PI 599077	0.61	10.46	0.68	7.94	0.24	11.52
C. pinnatifidum	PI 599109	0.57	2.91	1.75	4.00	0.27	3.46
C. microphyllum	ICCW 17148	0.49	7.46	1.23	5.53	0.26	8.29
C. arietinum	JG 11	1.59	2.65	2.36	4.90	1.27	0.94
C. arietinum	KAK 2	1.19	6.08	2.03	1.98	1.80	1.56
C. arietinum	ICC 3137	1.43	5.99	1.84	4.86	1.21	2.14
C. arietinum	ICCL 86111	3.00	3.60	2.21	4.87	3.06	3.25
C. arietinum	ICC 506 EB	3.13	7.42	2.45	4.70	4.27	4.02
	Fpr	**<.001**	**0.02**	**<.001**	**<.001**	**<.001**	**<.001**
	Mean	**1.062**	**4.96**	**1.33**	**3.51**	**0.84**	**3.55**
	SE±	**0.1933**	**1.397**	**0.25**	**0.63**	**0.16**	**0.38**
	LSD (p=0.05)	**0.5723**	**4.135**	**0.71**	**1.80**	**0.45**	**1.10**

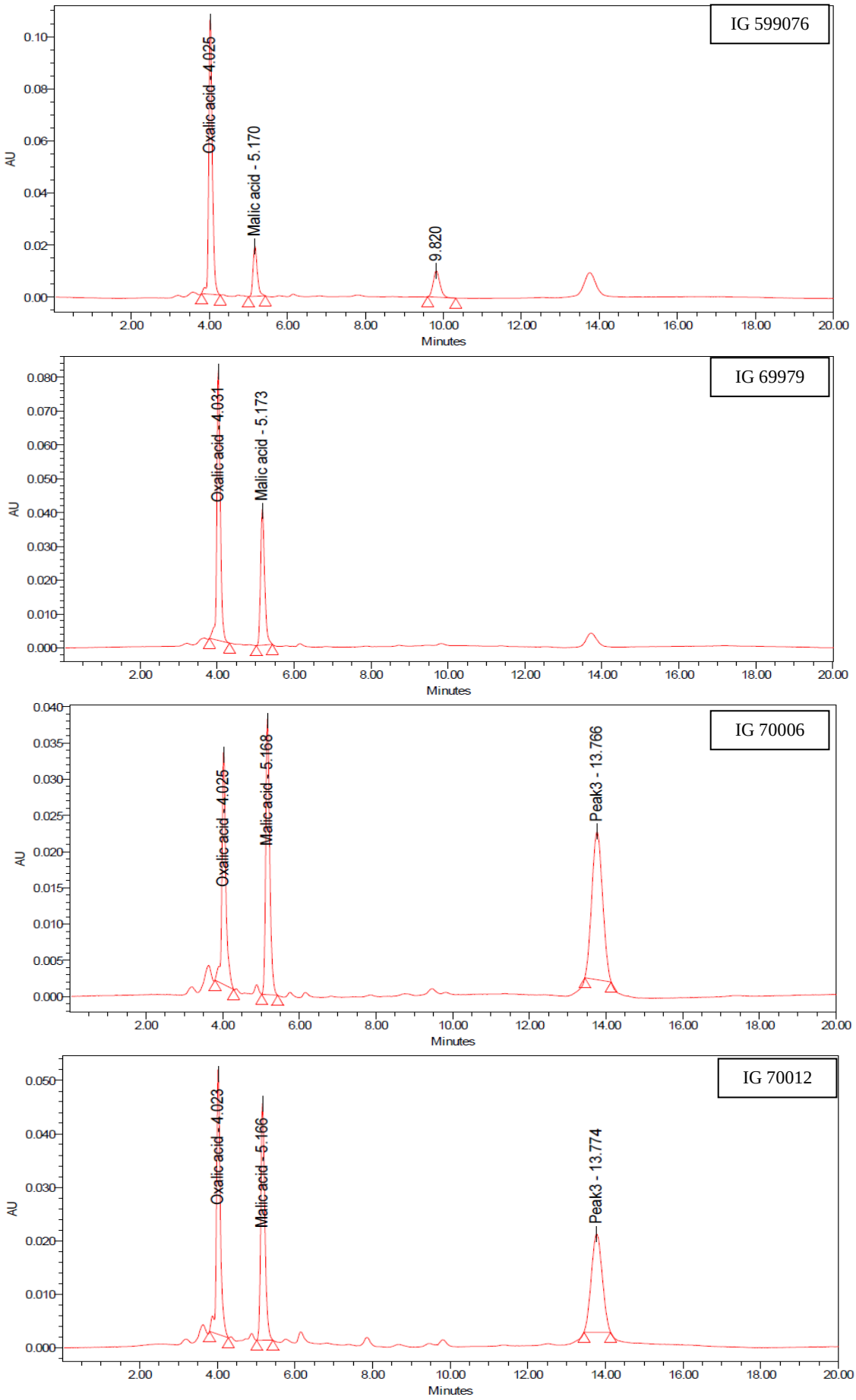


Figure 4.4. HPLC finger prints of leaf organic acids in wild relatives of chickpea.

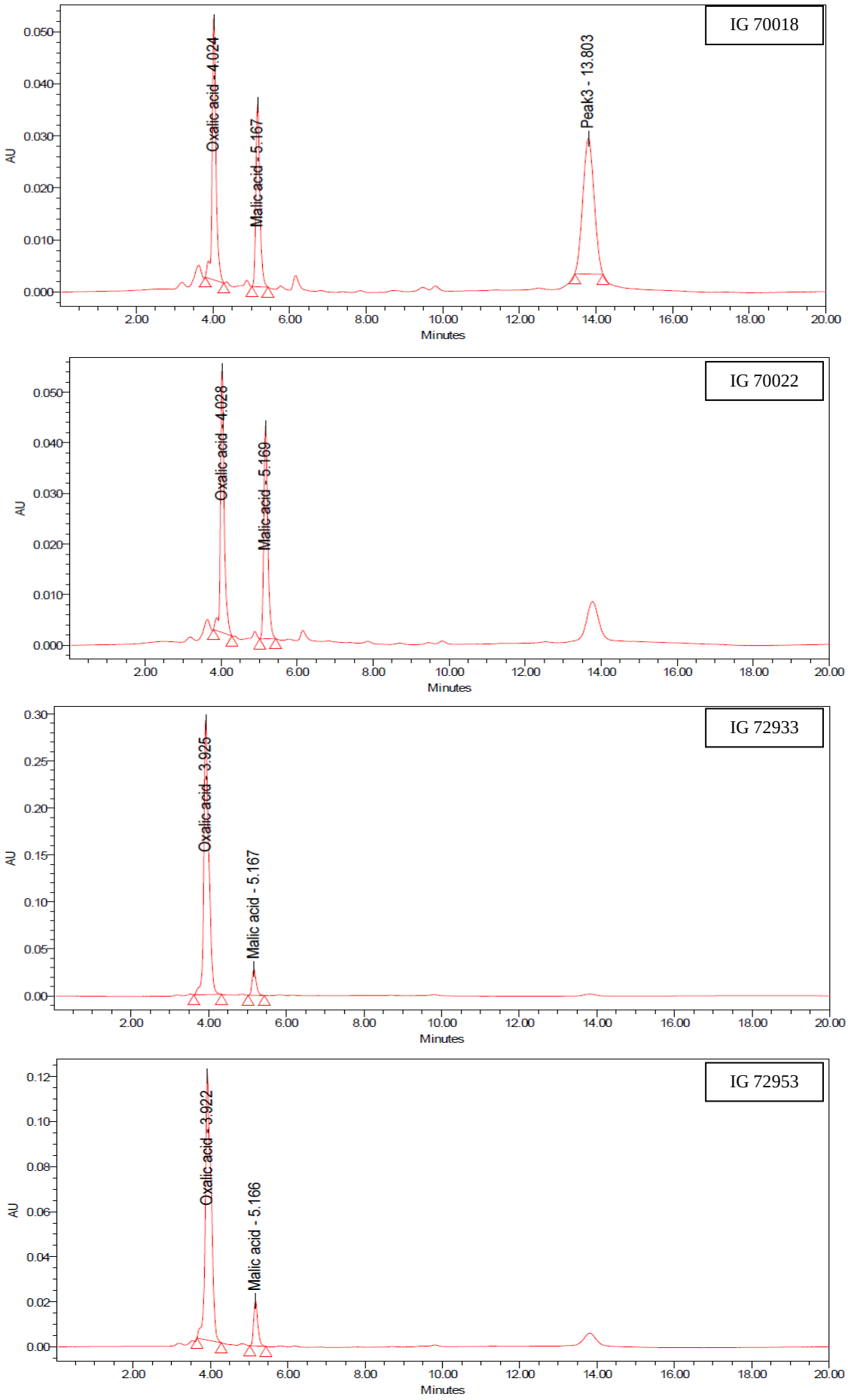


Figure 4.4. (Cont.)..

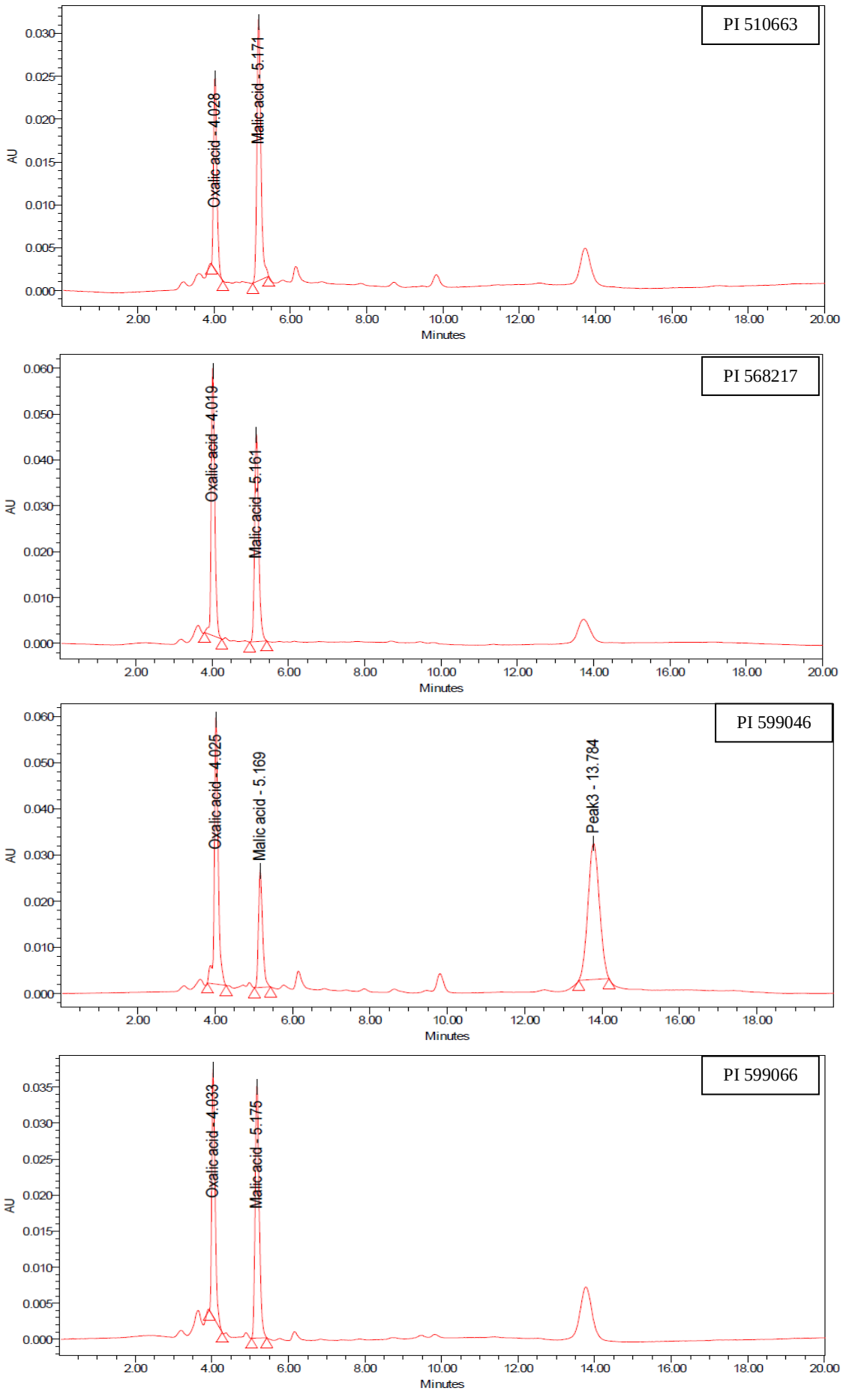


Figure 4.4. (Cont.)..

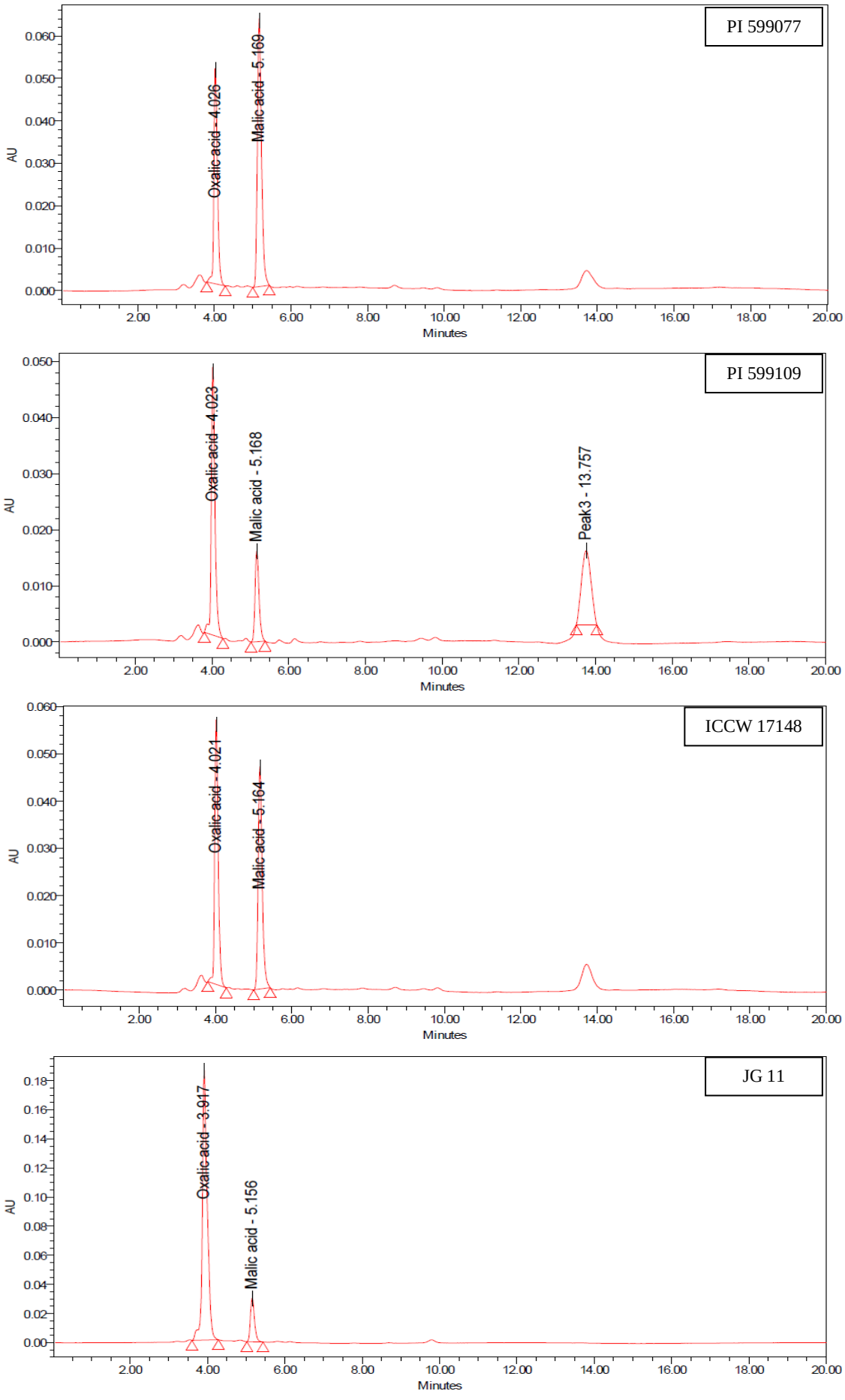


Figure 4.4. (Cont.)..

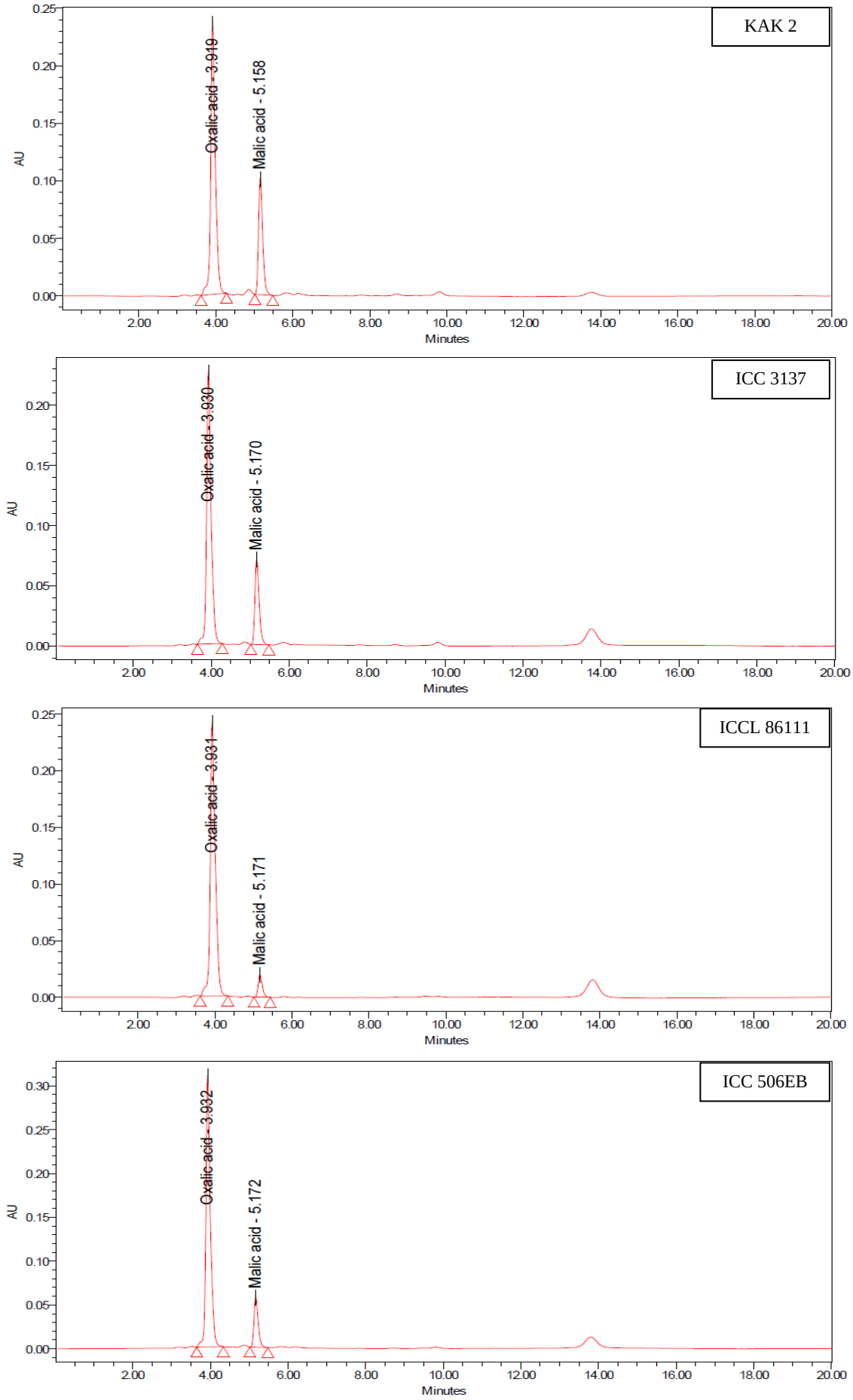


Figure 4.4. (Cont.)...

PI 599066, among other genotypes, IG 70012, IG 70018, IG 70006 (0.28 to 1.41 mg/g) observed less amount of malic acid and genotypes, PI 599077, IG 69979 and ICCW 17148 (7.94 to 5.53 mg/g) showed highest amount of malic acid. Under glasshouse condition, genotypes of *C. reticulatum*, IG 72953 (0.56 mg/g) and IG 72933 (0.61 mg/g) had less amount of malic acid, while PI 599077 (11.52 mg/g), ICCW 17148 (8.29 mg/g) and IG 69979 (8.28 mg/g) had highest amount of malic acid. Malic acid content in cultivated chickpea ranged from 0.94 mg/g in commercial cultivar, JG 11 to 4.02 mg/g in resistant check, ICC 506EB.

4.5.3 Association of Leaf Organic Acids with Oviposition Preference and Detached Leaf Assay for Resistance to *H. armigera* in Wild Relatives of Chickpea

Oxalic acid showed negative association with ovipoition preference, where the relation was significant under no-choice condition (r= -0.55) and non-significant under no-choice condition. Malic acid showed positive and significant (r= 0.48) association with oviposition preference under multi-choice condition (Table 4.27).

Association of organic acids with detached leaf assay revealed that, oxalic acid and malic acid had significant and negative correlation with larval survival (r= -0.35 and -0.29, respectively), which indicates that presence of higher amounts of these acids resulted in reduced larval survival in cultivated chickpea compared to wild relatives in detached leaf assay. Oxalic acid showed positive correlation (r= 0.36) with mean larval weight. This positive correlation might be due to nutrition conditions of cultivated chickpea favouring for establishment of larvae after survival against higher amounts of leaf organic acids.

Table 4.27. Association of leaf organic acids with oviposition preference and detached leaf assay for resistance to *H. armigera* in wild relatives of chickpea

	Oviposition preference		Detached leaf assay		
	Multi-choice condition	**No-choice condition**	**Damage rating**	**Larval survival (%)**	**Mean larval weight (mg)**
Oxalic acid (mg g^{-1})	-0.16	-0.55*	0.10	-0.35**	0.36**
Malic acid (mg g^{-1})	0.48*	0.41	-0.21	-0.29*	-0.18

*,** Correlation coefficients significant at P ≤ 0.05 and 0.01, respectively

The present results are in agreement with Yoshida *et al*. (1997) who reported that malic acid on the leaves stimulated oviposition during the vegetative and flowering stages. Acid exudates from leaf hairs contribute to plant resistance to *H. armigera* in chickpea (Yoshida *et al*., 1995). High amounts of malic and oxalic acids in leaves affected the survival of *H. amigera* and might result in resistance to pod borer (Simmonds and Stevenson, 2001). Malic acid and oxalic acid on the leaves are responsible for chickpea resistance to pod borer (Cowgill and Lateef, 1996). According to Yoshida *et al*., (1995), the concentration of oxalic acid is higher on the leaf surface of resistant genotypes than on susceptible genotypes, and this acid retards the growth of *H. armigera* larvae. Patnaik and Senapati (1995) reported that egg and larval counts of pod borer, *H. armigera* were negatively correlated with increasing concentration of acid exudates of chickpea. According to Baghwat *et al.* (1995) highest amount of malic acid was observed in ICC 506EB at 60 days after sowing, which harboured lowest numbers of *H. armigera* larvae. The amounts of malic acid were negatively correlated with leaf feeding by *H. armigera* larvae at flowering and maturity and with pod damage, whereas oxalic acid showed a negative association with leaf damage Narayanamma *et al*. (2013). Hence, oxalic acid and malic acid levels could be used as marker for resistance to *H. armigera*.

4.6 HPLC FINGER PRINTS OF FLAVONOIDS IN DIFFERENT GENOTYPES OF WILD RELATIVES OF CHICKPEA

The HPLC finger prints of 20 genotypes (both wild relatives and cultivated chickpea) had altogether 39 peaks with varying retention times (RT) from 2.15 to 25.70 min (Table 4.28 and Figure 4.5). To identify and quantify the flavonoid compounds present in the different wild relatives of chickpea genotypes 19 standards were run under similar conditions, of which the RT of nine compounds matched with the HPLC profiles of genotypes and their amounts were quantified. Among cultivated chickpea genotypes least number of peaks were observed in resistant check, ICC 506EB (11) followed by JG 11 (18), while another resistant check, ICCL 86111 (20) and susceptible checks KAK 2 (20) and ICC 3137 (20) recorded same number of peaks. The common peaks with varying peak areas in all the genotypes were observed at RT of 2.77 min, 3.43 min and 20.39 min (Genestein).

Table 4.28. Flavonoid profiles (areas) of wild relatives of chickpea estimated through HPLC fingerprinting

Compound (Peak areas)	Unknown	Unknown	Unknown	Unknown	Chlorogenic acid [#]	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
RT (min)	**2.15**	**2.49**	**2.77**	**3.43**	**8.11**	**9.12**	**10.09**	**10.73**	**11.18**	**11.45**	**11.86**
IG 599076	969902		1802778	1710895					2286316		
IG 69979			1206115	1191634				940561		4249093	
IG 70006	1448405		737066	1373379					8640061		122662
IG 70012	1628748		839827	1578497	2.74		2191886	1075853	4585993		144877
IG 70018	2061653		974199	1739637					2411904	887465	
IG 70022		1646662	790364	2555451				4419424	5082852		134643
IG 72933	1452726		995014	2595811			1433362	243911	1602852		
IG 72953	435619	1471498	1005947	3060061			469387		1604032		
PI 510663			1552907	1036095		3640032			5582455	330974	
PI 568217	1150850		869545	1796539		891798	863752		2478675		
PI 599046	2016696		925569	2363828		1997999		1796201	2820051	1292462	
PI 599066		1423691	807466	2787366	1.86			3282853	2417957	1813983	
PI 599077			1657445	1273153		1470383	2161142		5412314		
PI 599109			1653195	1730254					1023328		224424
ICCW 17148	536268		1307816	1820730		825133			2510414		
JG 11	1696415	73192	1501893	1995918			385753		2176041		
KAK 2	1207498		2949791	2944487	1.39		551415		2405930		
ICC 3137	763254		2344334	2979677	0.38	548399	302638		1056796		
ICCL 86111	1956036		1461059	2426156			462404		1478802		
ICC 506 EB	1380812		3149400	1925343							

[#] Concentrations of identified compounds were estimated by comparing the mean peak area of standards at known concentration and represented as mg g^{-1} of sample

Table 4.28 (cont.).

Compound (Peak areas)	Unknown	Unknown	Unknown	Unknown	Ferulic acid [#]	Naringin [#]	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
RT (min)	**11.18**	**11.45**	**11.86**	**12.17**	**12.68**	**13.01**	**13.37**	**13.54**	**13.92**	**14.14**	**14.40**	**14.51**
IG 599076	2286316			5070733		3.16		7311230				1473720
IG 69979		4249093		2048639		4.90	6281801			775894		457607
IG 70006	8640061		122662	9583305		3.93	10308405		6069797			
IG 70012	4585993		144877	11862585		4.85	12802164		8030990			
IG 70018	2411904	887465		8968185	0.61		11953023		4799323			
IG 70022	5082852		134643	9647142	0.92	4.14	9410064		7014720			
IG 72933	1602852			6434608				12519827		1397951		
IG 72953	1604032			6966271				11411405		907177		
PI 510663	5582455	330974		9985816	0.19		13986282		4793796		2667032	875428
PI 568217	2478675			8514091			8208389				1472919	26909
PI 599046	2820051	1292462		9625405	1.03	4.61	16096370			2237553		
PI 599066	2417957	1813983		9489135		4.28	10213479		6044029			
PI 599077	5412314			13696061		4.37	14107987				2214000	207265
PI 599109	1023328		224424		0.76		6449854		7525456			
ICCW 17148	2510414			8360959			8744219				1409738	49342
JG 11	2176041			7686120			11256992					
KAK 2	2405930			6747535			11820012					
ICC 3137	1056796			3458268			5132322					
ICCL 86111	1478802			4102233			8032041				175546	
ICC 506 EB				1996752			2145966					

[#] Concentrations of identified compounds were estimated by comparing the mean peak area of standards at known concentration and represented as mg g^{-1} of sample

Table 4.28 (cont.).

Compound (Peak areas)	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	3,4-Dihydroxy flavones [#]	Quercetin [#]	Unknown
RT (min)	**14.76**	**14.92**	**15.14**	**15.45**	**16.00**	**16.38**	**16.56**	**16.84**	**17.43**	**17.79**	**18.23**
IG 599076			2476796	2232396			954877			3.52	
IG 69979	4363294		6933762		4490626				0.74	0.63	
IG 70006	3008030		10118261	5335263	7955569		251675	1377264	1.61	10.56	2885606
IG 70012	2775211		12851666	6599464	9849661		296696	1599221	1.80	11.67	3901834
IG 70018			13178852	5455078	4368809	2109441			0.99	8.28	3644725
IG 70022	1935784		10257909	6921284	8625786		238122	1430201	1.66	12.40	4820866
IG 72933		11997141		2571922	1739721	3177196				1.82	1733538
IG 72953		8546097		4020350		2593166	100507			2.01	1448324
PI 510663		8110067		2895968	9875098			1039618	1.54	4.94	
PI 568217		3992889		949614	2088943		906164			1.90	535706
PI 599046			14581663	8282749	8588951		141074	1386528	1.59	12.34	4419374
PI 599066	2333833		10772210	8700371	11095668		241205	1820982	1.79	11.46	5910868
PI 599077		5360297		1712899	2979843		1368174		0.51	2.33	905743
PI 599109		7172556		2878611	7650840	2745024			1.13	3.29	2322192
ICCW 17148		3988841		1098947	1919780		851388			2.38	
JG 11		6961356		802709	765918					0.67	972627
KAK 2		8259146		1279065	1866531					1.23	1899780
ICC 3137		4787243			2142737	1434781				0.56	1437468
ICCL 86111		7480171		1538768	1469760	2406681				0.58	1008069
ICC 506 EB		2376851				1207956					

[#] Concentrations of identified compounds were estimated by comparing the mean peak area of standards at known concentration and represented as mg g^{-1} of sample

Table 4.28 (cont.).

Compound (Peak areas)	Unknown	Naringenin #	Genestein #	Unknown	Unknown	Unknown	Formononetin#	Biochanin A #
RT (min)	**18.85**	**19.78**	**20.39**	**21.02**	**22.00**	**22.33**	**22.76**	**25.70**
IG 599076			0.68	1680166			2.61	8.73
IG 69979		0.854	0.61	282491		942716		
IG 70006	4852850	0.159	6.73	1005441	1803795		3.12	1.15
IG 70012	5661838	0.181	8.36	1067462	2273310		2.65	1.14
IG 70018	2869790		6.97	1343718	1808823	787708	4.42	
IG 70022	5483948	0.140	9.75	1150071	3135134		3.16	1.51
IG 72933	628877		2.85	599762	899845		0.86	1.55
IG 72953	553165		1.70		639745		0.83	1.31
PI 510663	1870192		3.07	1322241		1674021	1.37	5.02
PI 568217	739726		0.95				0.74	6.13
PI 599046	4510380		8.51	1380883	2389492	981914	4.36	1.99
PI 599066	5255779	0.739	9.02	1165816	2699763		2.06	0.94
PI 599077	1031200		1.15		508409		0.77	0.24
PI 599109	2373293		3.32	1221090	933164		1.06	0.90
ICCW 17148	736217		0.88				0.59	4.55
JG 11			1.78		636209		0.57	1.50
KAK 2	446428		2.27	908534	583074		1.34	1.43
ICC 3137			1.77	596295	654792		0.60	1.07
ICCL 86111			0.86	483359	472306		0.75	6.13
ICC 506 EB			0.42	566628			0.78	2.69

Concentrations of identified compounds were estimated by comparing the mean peak area of standards at known concentration and represented as mg g^{-1} of sample

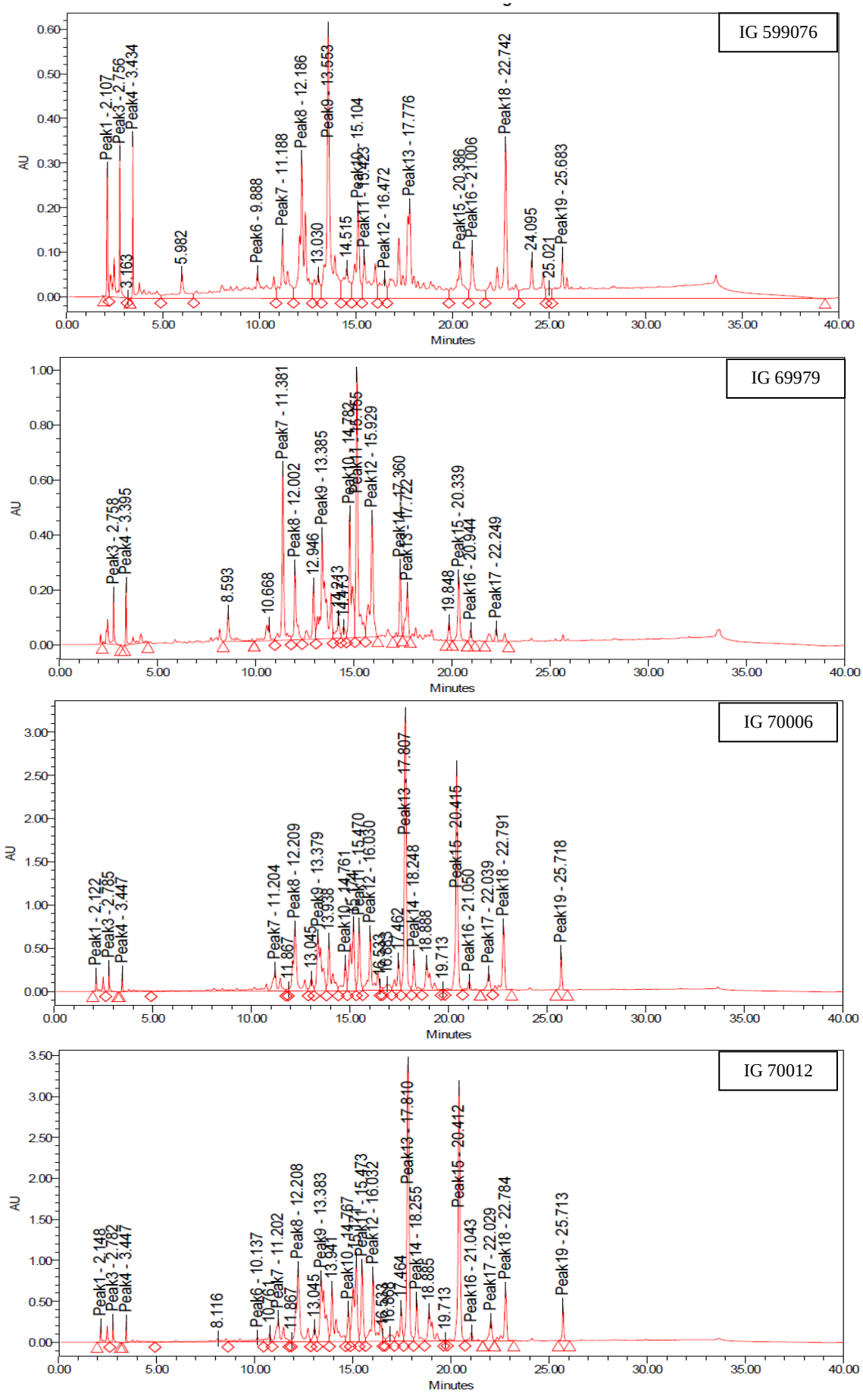


Figure 4.5. HPLC fingerprints of flavonoids in wild relatives of chickpea

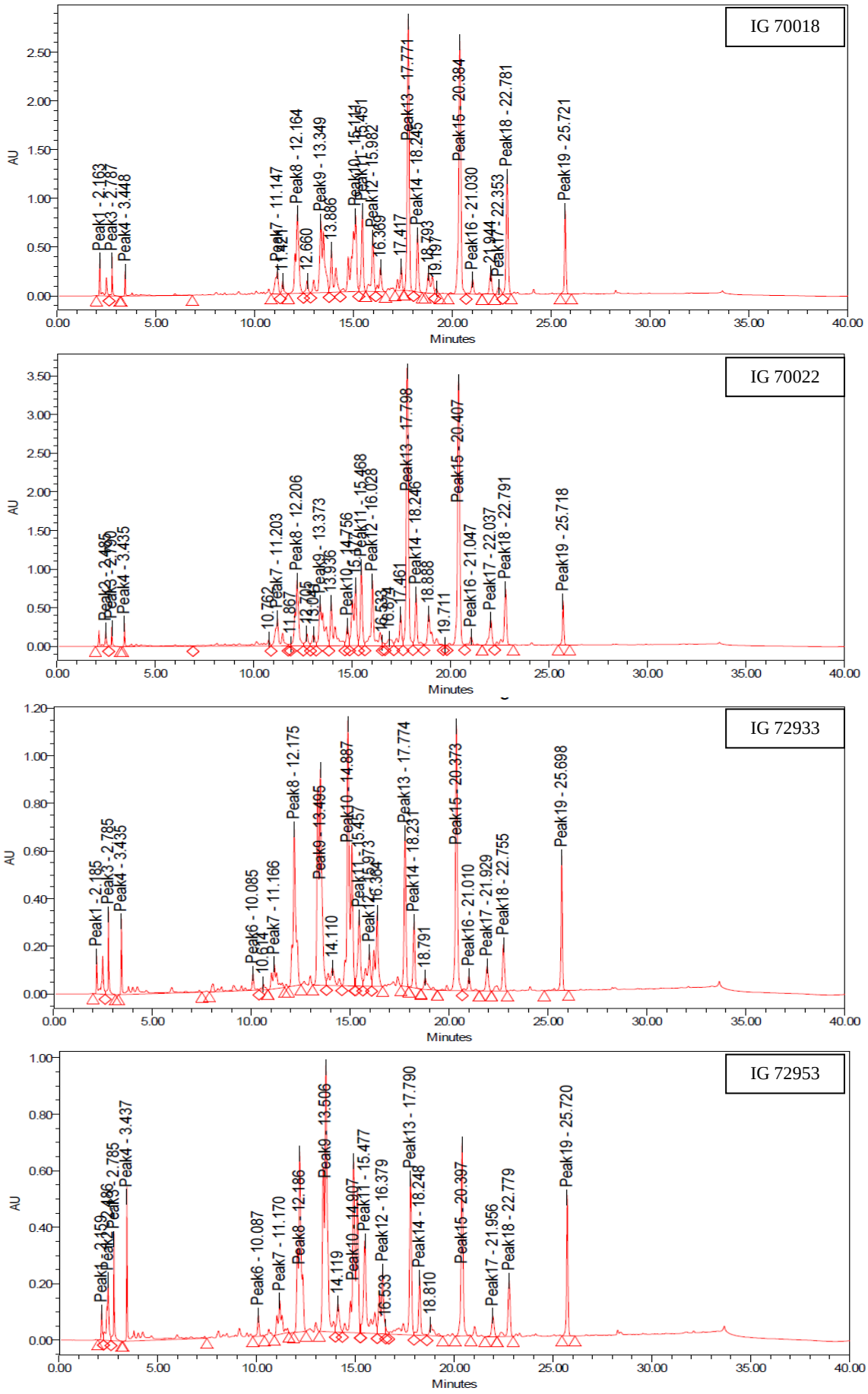


Figure 4.5. (Cont.)..

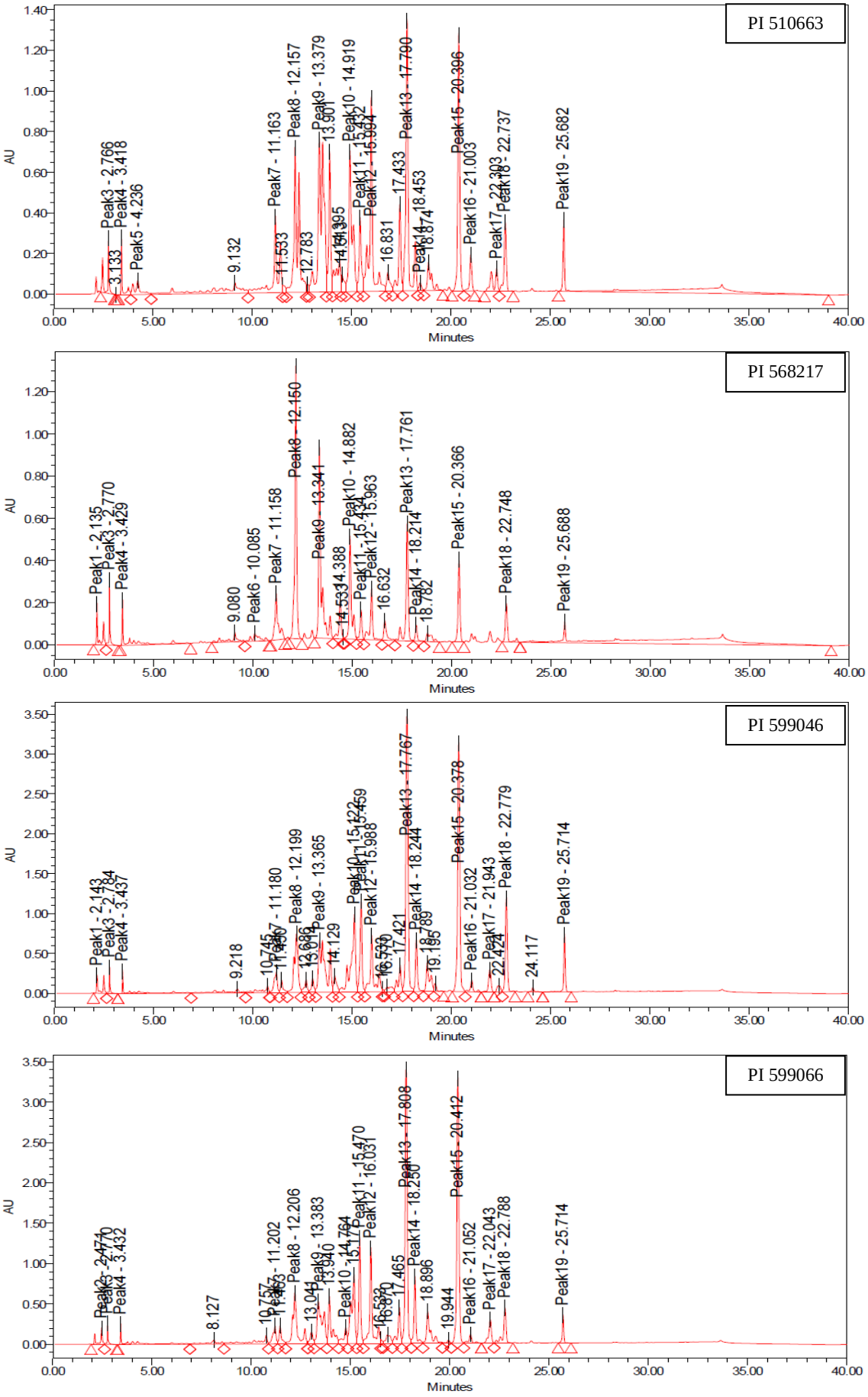


Figure 4.5. (Cont.)..

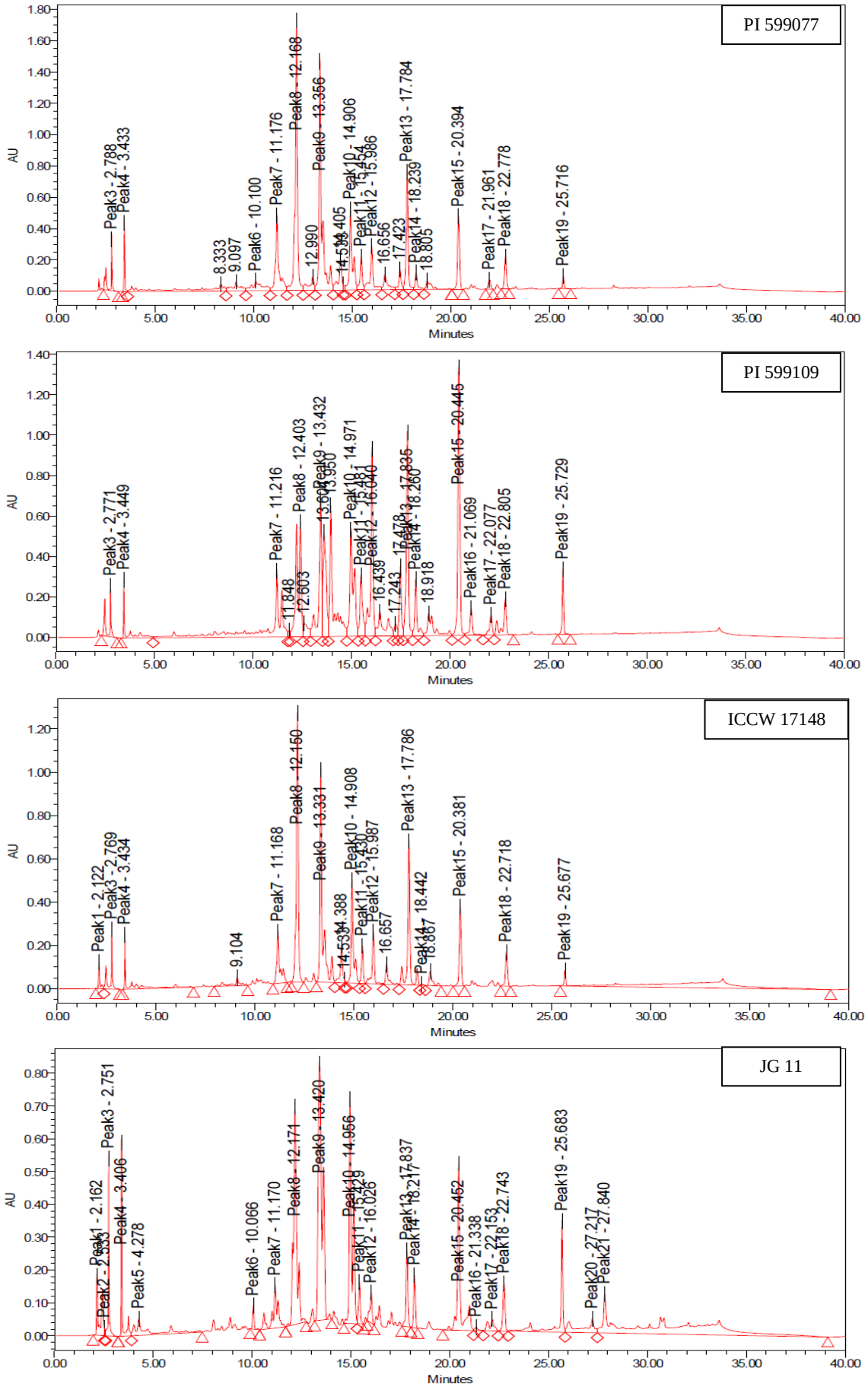


Figure 4.5. (Cont.)..

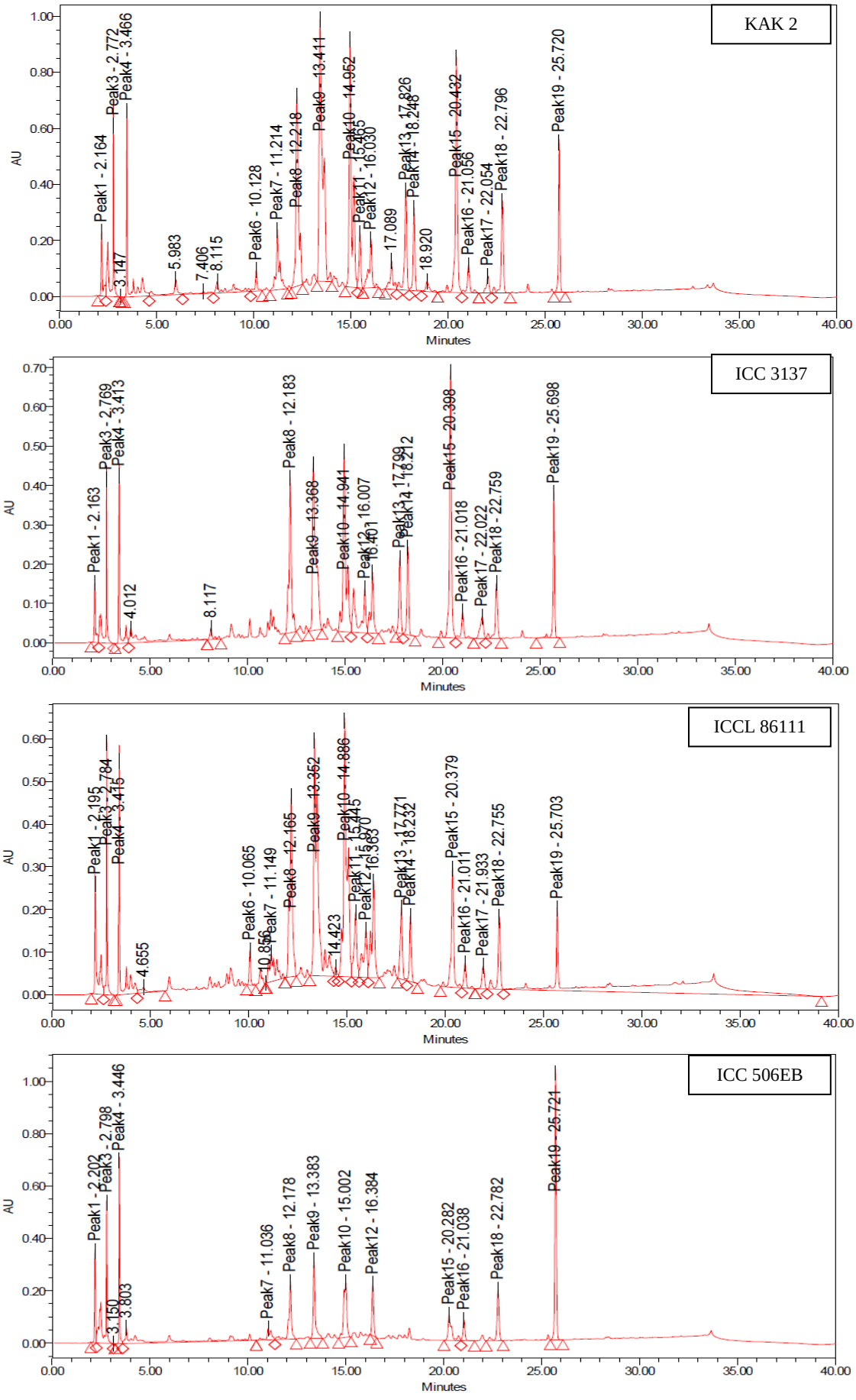


Figure 4.5. (Cont.)..

Some of the peaks were observed in all the genotypes but missed in one or two genotypes like peak at RT of 11.18 min was observed in all the genotypes except in IG 69979 (*C. cuneatum*) and ICC 506EB (resistant check), peak at RT of 12.17 min was observed in all the genotypes except in PI 599109 (*C. pinnatifidum*), peak at RT of 13.37 min was observed in all the genotypes except in *C. chrossanicum* (IG 599076) and *C. reticulatum* (IG 72933 and IG 72953), peak at RT of 15.45 min was observed in all the genotypes except in IG 69979 (*C. cuneatum*), ICC 3137 and ICC 506EB, peak at RT of 16.00 min was observed in all the genotypes except in IG 599076 (*C. chrossanicum*), IG 72953 (*C. reticulatum*) and ICC 506EB.

Among the identified compounds, Genestein was present in all genotypes, where highest concentrations were recorded in *C. bijugum* genotypes (6.73 to 9.75 mg/g) followed by *C. pinnatifidum* (3.07 to 3.32 mg/g) and lowest concentration was recorded in ICC 506EB (0.42 mg/g). Quercetin was present in all the genotypes except in ICC 506EB, where highest concentrations were recorded in *C. bijugum* genotypes (8.28 to 12.40 mg/g) followed by *C. pinnatifidum* (3.29 to 4.94 mg/g) and lowest concentration was recorded in ICC 506EB (0.42 mg/g). Formononetin was present in all the genotypes (0.57 to 4.42 mg/g) except in IG 69979, and biochanin A was present in all the genotypes (0.24 to 8.73 mg/g) except in IG 69979 and IG 70018. Among the identified compounds ferulic acid, naringin, 3,4-dihydroxy flavones and naringenin were present only in some genotypes of wild relatives of chickpea and completely absent in cultivated chickpea, whereas chlorogenic acid present in IG 70012, PI 599066, KAK 2 and ICC 3137. Among unknown compounds, peaks observed at RT of 10.73 min, 11.45 min, 11.86 min, 13.54 min, 13.92 min, 14.14 min, 14.51 min, 14.76 min, 15.14 min, 16.56 min, 16.84 min and 22.33 min were observed only in few genotypes of wild relatives of chickpea in varying concentrations but completely absent in cultivated chickpea.

4.6.1 Association of Flavonoids in Wild Relatives of Chickpea with Survival and Development of *H. armigera* in Diet Incorporation Assay

Significant association was exhibited between flavonoid content of wild relatives of chickpea and biological parameters of *H. armigera* reared on artificial diet impregnated with lyophilized leaf powder of wild relatives of chickpea resulted in antibiosis mechanism of resistance against to the pest (Table 4.29). Among the identified compounds, chlorogenic acid showed significant negative correlation with

biological parameters such as larval survival, larval weight, pupation, adult emergence and fecundity, whereas larval period and pupal period had significant positive correlation. Ferulic acid showed significant negative correlation with pupation, pupal period, adult emergence and fecundity. Naringin amount was significantly and negatively correlated with larval weight, pupal weight and fecundity. 3,4-Dihydroxy flavones had significant negative correlation with larval survival, pupation and adult emergence. Quercetin content was significantly and negatively correlated with all parameters except with larval period and pupal period where significant positive correlation was observed. Naringenin content was negatively correlated with larval weight and fecundity, while positively correlated with adult emergence. Genestein showed significant positive correlation with pupal period and negative correlation with pupation, pupal weight, adult emergence and fecundity. Formononetin showed significant positive correlation with larval and pupal period and negatively correlated with all other parameters. Among all other unidentified peaks, compounds present at RT of 2.77, 3.43, 11.45, 11.86, 13.54, 16.56 and 22.33 min showed positive association with survival and development of *H. armigera*, where all other compounds showed positive correlation with larval and pupal period, and negative correlation with larval survival, pupation, larval and pupal weight, adult emergence and fecundity.

Several isoflavones such as judaicin, judaicin 7-*O*-glucoside, and judaicin 7-*O*-(6”-*O*-malonylglucoside and pterocarpans like maakiain 3-*O*-glucoside and maackiain 3-*O*-(6’-*O*-malonyl glucoside) (Stevenson and Veitch, 1996), and 2-arylbenzofuran (Stevenson and Veitch, 1998), which have been isolated from the roots of wild chickpea, had shown antifeedant and antibiosis activity towards *H. armigera* larvae and responsible for the adverse effects of wild relatives of chickpea on *H. armigera* (Simmonds and Stevenson, 2001). Flavonoids such as chlorogenic acid, caffeic acid and protocatechuic acid were more toxic to *H. armigera* larvae and the digestive enzyme activities of the larvae were significantly reduced in flavonoid treated diets (War *et al*., 2013). Induction of flavonoids in groundnut genotypes in response to feeding of *H. armigera* and *Aphis craccivora* revealed that compounds like chlorogenic acid, syringic acid, quercetin, caffeic acid, vanillic acid and ferulic acid were observed in insect infested plants, especially in the resistant genotypes (War *et al*., 2016).

Table 4.29. Association of flavonoids in wild relatives of chickpea with survival and development of *H. armigera* in diet incorporation assay

RT (min)	Compound	Larval survival (%)	Mean larval weight (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%)	Fecundity
2.15	Unknown	-0.42	-0.06	0.05	-0.19	-0.19	0.19	-0.33	-0.39
2.49	Unknown	-0.59**	-0.94**	0.93**	-0.94**	-0.86**	0.83**	-0.90**	-0.71**
2.77	Unknown	-0.12	0.59**	-0.57**	0.63**	0.58**	-0.56*	0.73**	0.46*
3.43	Unknown	0.03	0.52*	-0.40	0.22	0.49*	-0.42	0.27	0.46*
8.11	Chlorogenic acid	-0.90**	-0.80**	0.93**	-0.84**	-0.90**	0.69**	-0.84**	-0.82**
9.12	Unknown	-0.66**	-0.54*	0.44*	-0.12	-0.31	0.72**	-0.25	-0.19
10.09	Unknown	-0.30	-0.73**	0.76**	-0.68**	-0.85**	0.87**	-0.75**	-0.71**
10.73	Unknown	-0.33	-0.10	0.85**	-0.19	-0.37	0.92**	-0.18	-0.21
11.18	Unknown	-0.04	-0.42	0.23	-0.11	-0.35	0.36	-0.33	-0.36
11.45	Unknown	0.56*	-0.92**	-0.70**	-0.11	0.47*	-0.68**	0.30	-0.32
11.86	Unknown	0.03	-0.15	-0.56*	0.63**	0.16	-0.61**	0.86**	0.04
12.17	Unknown	-0.04	-0.42	0.59**	-0.27	-0.51*	0.55*	-0.46*	-0.39
12.68	Ferulic acid	-0.29	0.36	-0.13	-0.64**	-0.17	-0.46*	-0.70**	-0.51*
13.01	Naringin	0.25	-0.50*	-0.31	-0.13	-0.44*	-0.13	-0.16	-0.71**
13.37	Unknown	-0.15	-0.27	0.47*	-0.22	-0.34	0.38	-0.34	-0.24
13.54	Unknown	0.69**	0.95**	-0.09	0.07	0.90**	-0.92**	0.07	0.95**
13.92	Unknown	-0.13	0.15	-0.44*	-0.18	0.11	-0.70**	-0.17	-0.09
14.14	Unknown	-0.74**	-0.12	0.81**	-0.79**	-0.66**	0.39	-0.98**	-0.44*
14.40	Unknown	0.42	-0.93**	0.88**	0.02	-0.45*	0.84**	-0.10	-0.34
14.51	Unknown	-0.90**	-0.30	0.11	-0.27	0.24	0.30	0.00	0.31

Table 4.29 (cont..)..

RT (min)	Compound	Larval survival (%)	Mean larval weight (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%)	Fecundity
14.92	Unknown	0.21	0.09	0.03	0.00	0.13	-0.05	-0.16	0.36
15.14	Unknown	-0.11	-0.12	0.04	-0.58**	-0.71	0.35	-0.88**	-0.66**
15.45	Unknown	-0.51*	-0.51*	0.38	-0.69**	-0.51*	0.59**	-0.73**	-0.57**
16.00	Unknown	-0.58**	-0.64**	0.45*	-0.57**	-0.56*	0.69**	-0.61**	-0.59**
16.38	Unknown	0.36	-0.51*	0.46*	-0.40	-0.38	0.21	-0.56*	-0.01
16.56	Unknown	0.27	-0.11	0.53*	0.51*	-0.10	-0.13	0.62**	-0.07
16.84	Unknown	-0.60**	-0.18	-0.28	-0.68**	-0.60**	0.00	-0.54*	-0.75**
17.43	3,4-Dihydroxy flavone	-0.58**	0.11	-0.12	-0.45*	-0.19	0.24	-0.53*	0.06
17.79	Quercetin	-0.52*	-0.51*	0.50*	-0.68**	-0.60**	0.67**	-0.77**	-0.59**
18.23	Unknown	-0.58**	-0.52*	0.46*	-0.74**	-0.63**	0.73**	-0.69**	-0.54*
18.85	Unknown	-0.69**	-0.49*	0.25	-0.66**	-0.50*	0.56*	-0.70**	-0.59**
19.78	Naringenin	0.05	-0.62**	-0.39	0.19	0.24	-0.18	0.80**	-0.62**
20.39	Genestein	-0.30	-0.37	0.40	-0.58**	-0.47*	0.58**	-0.69**	-0.48*
21.02	Unknown	-0.38	-0.49*	0.66**	-0.46*	-0.58**	0.61**	-0.47*	-0.42
22.00	Unknown	-0.45*	-0.59**	0.63**	-0.80**	-0.68**	0.75**	-0.74**	-0.67**
22.33	Unknown	0.02	0.12	-0.03	0.91**	0.59**	0.18	0.69**	0.95**
22.76	Formononetin	-0.41	-0.49*	0.53*	-0.71**	-0.57**	0.60**	-0.73**	-0.59**
25.70	Biochanin A	-0.23	-0.17	0.20	-0.13	-0.15	0.05	0.05	-0.06

*,** Correlation coefficients significant at $P \leq 0.05$ and 0.01, respectively

4.7 BIOCHEMICAL CHARACTERIZATION OF WILD RELATIVES OF CHICKPEA IN RELATION WITH EXPRESSION OF RESISTANCE TO *H. armigera*

Various biochemical components *viz*., proteins, phenols, total soluble sugars and tannins were estimated in lyophilized leaf powders of different wild relatives of chickpea genotypes and presented hereunder.

4.7.1 Protein Content

Significant differences were observed in protein content among different genotypes of wild relatives of chickpea (Table 4.30). During post-rainy season, 2014-15 protein content was observed in a range of 11.42% (ICC 3137) to 16.41% (KAK 2). Highest amount of proteins were recorded in KAK 2 (16.41%) followed by PI 599066 (15.89%), PI 599046 (15.73%), IG 70006 (15.67%), PI 510663 (15.60%), PI 599109 (15.59%), while lowest was observed in ICC 3137 (11.42%) and PI 568217 (11.99%). During post-rainy season, 2015-16 the genotypes IG 70018, PI 599046, IG 70012, PI 599066, IG 70022 and IG 70006 (*C. bijugum*) recorded higher amount of protein (15.40 to 12.38%) compared to all other genotypes, while lowest was observed in ICC 506EB (8.27%). Under glasshouse conditions, protein content in different genotypes ranged between, 8.16% in PI 568217 and 12.20% in IG 70012, with an average of 9.92% among all the genotypes.

4.7.2 Phenol Content

During post-rainy season, 2014-15 (Table 4.30), all genotypes of wild relatives of chickpea exhibited significantly higher amount of phenols (6.55 mg/g in PI 599077 to 7.97 mg/g in PI 599046) compared to cultivated chickpea (5.93 mg/g in ICCL 86111 to 6.15 mg/g in ICC 506EB) except in *C. reticulatum*, IG 72953 (4.10 mg/g) and IG 72933 (4.52 mg/g) and *C. chrossanicum*, IG 599076 (6.15 mg/g). During post-rainy season, 2015-16 highest phenol content was observed in PI 599046 (6.50 mg/g) followed by IG 70006 (6.41 mg/g), IG 70022 (6.22 mg/g) and IG 70012 (6.03 mg/g), while lowest was recorded in IG 599076 (4.07 mg/g) and ICC 3137 (4.16 mg/g). Phenol content in all other genotypes was observed in a range of 5.42 mg/g in PI 599066 and 4.28 mg/g in PI 599077. Phenol content under

Table 4.30. Biochemical characterization of wild relatives of chickpea exhibiting different levels of resistance to *H. armigera*

Species	Genotype	Post-rainy season, 2014-15				Post-rainy season, 2015-16				Glasshouse condition			
		Protein (%)	Phenols (mg/g)	TSS (%)	Tannins (mg/g)	Protein (%)	Phenols (mg/g)	TSS (%)	Tannins (mg/g)	Protein (%)	Phenols (mg/g)	TSS (%)	Tannins (mg/g)
C. chrossanicum	IG 599076	13.88	6.15	9.01	7.58	11.72	4.07	10.35	8.55	8.98	2.76	7.91	9.03
C. cuneatum	IG 69979	13.78	7.02	9.58	8.61	10.34	5.04	11.05	9.55	7.87	3.42	9.51	8.39
C. bijugum	IG 70006	15.67	7.46	10.70	9.48	12.38	6.41	12.44	9.15	9.88	5.90	12.57	10.15
C. bijugum	IG 70012	15.49	7.78	11.46	7.97	14.00	6.03	13.65	9.15	12.20	5.65	11.98	9.97
C. bijugum	IG 70018	14.96	7.94	10.23	8.88	15.40	5.40	13.41	8.30	11.99	5.42	14.96	8.97
C. bijugum	IG 70022	14.65	7.35	11.49	8.64	12.44	6.22	14.02	8.73	11.65	5.18	16.35	9.24
C. reticulatum	IG 72933	13.33	4.52	9.17	9.24	10.96	5.06	11.19	10.09	9.17	3.16	7.38	9.09
C. reticulatum	IG 72953	13.42	4.10	9.46	8.48	11.45	4.38	11.23	10.61	9.65	3.57	9.05	10.24
C. pinnatifidum	PI 510663	15.60	7.17	9.45	7.36	11.58	5.15	12.35	5.39	8.60	4.42	8.27	11.21
C. judaicum	PI 568217	11.99	6.84	8.19	9.27	10.03	4.47	11.34	9.24	8.16	3.42	10.50	11.00
C. bijugum	PI 599046	15.73	7.97	9.61	8.79	14.42	6.50	14.12	7.88	11.52	5.49	13.57	9.24
C. bijugum	PI 599066	15.89	7.76	10.58	8.82	13.44	5.42	14.70	7.09	11.93	4.87	9.67	13.64
C. judaicum	PI 599077	13.63	6.55	11.08	10.27	10.61	4.28	13.97	11.21	7.73	3.31	7.87	11.73
C. pinnatifidum	PI 599109	15.59	7.41	9.08	7.58	11.87	4.82	11.55	5.97	8.18	5.18	10.89	11.48
C. microphyllum	ICCW 17148	12.39	7.55	8.04	8.24	11.72	4.30	11.91	10.30	11.79	4.55	13.54	9.00
C. arietinum	JG 11	14.51	7.58	12.19	9.73	11.96	4.66	15.86	11.42	10.91	3.31	8.28	13.12
C. arietinum	KAK 2	16.41	5.97	13.60	8.97	13.01	4.47	17.18	10.64	10.75	4.18	9.34	16.30
C. arietinum	ICC 3137	11.42	5.99	10.59	5.97	10.92	4.16	16.05	10.79	9.44	3.06	8.90	12.55
C. arietinum	ICCL 86111	13.13	5.93	10.51	8.30	11.33	4.98	13.60	9.18	9.32	4.93	11.00	15.82
C. arietinum	ICC 506EB	13.52	6.15	11.62	7.97	8.27	4.57	16.54	7.94	8.65	3.55	10.46	12.39
	Fpr	**0.002**	**<0.001**	**<0.001**	**NS**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Mean	**14.25**	**6.76**	**10.28**	**8.5**	**11.89**	**5.02**	**13.33**	**9.06**	**9.92**	**4.27**	**10.60**	**11.13**
	SE±	**0.70**	**0.13**	**0.59**	**0.83**	**0.44**	**0.06**	**0.56**	**0.10**	**0.37**	**0.16**	**0.55**	**0.31**
	LSD (p=0.05)	**2.07**	**0.38**	**1.75**	**-**	**1.31**	**0.18**	**1.67**	**0.30**	**1.09**	**0.46**	**1.64**	**0.93**

glasshouse conditions varied significantly among genotypes, with a range of 3.06 mg/g in ICC 3137 to 5.90 mg/g in IG 70006.

4.7.3 Total Soluble Sugars (TSS)

Total soluble sugars (TSS) were ranged from 8.04% (ICCW 17148) to 13.60% (KAK 2) among genotypes during post-rainy season, 2014-15 (Table 4.30). The genotypes ICCW 17148, PI 568217, IG 599076, PI 599109, IG 72933, PI 510663, IG 72953, IG 69979 and PI 599046 exhibited significantly low amount (8.04 to 9.61%) of total soluble sugars compared to ICC 3137 (10.59%). During post-rainy season, 2015-16 significant differences were exhibited between genotypes with respect to total soluble sugars. The lowest amount of total soluble sugars observed in IG 599076 (10.35%) and was at par with IG 69979 (11.05%), IG 72933 (11.19%), IG 72953 (11.23%), PI 568217 (11.34%), PI 599109 (11.55%) and ICCW 17148 (11.91%), while highest was recorded in susceptible check, KAK 2 (17.18%). Under glasshouse condition, total soluble sugars were recorded in a range of 7.87% (PI 599077) to 16.35% (IG 70022).

4.7.4 Tannin Content

During post-rainy season, 2014-15, no significant differences were observed in tannin content among all genotypes (Table 4.30). During post-rainy season, 2015-16 highest amount of tannins were observed in JG 11 (11.42 mg/g) followed by PI 599077 (11.21 mg/g), whereas lowest was recorded in PI 510663 (5.39 mg/g) and PI 599109 (5.97 mg/g). Significant differences were observed in tannin content in different genotypes under glasshouse condition, with an average of 11.13 mg/g. The genotype KAK 2 had more tannin content (16.30 mg/g) compared to all other genotypes, whereas IG 69979 had the least (8.39 mg/g) tannin content.

4.7.5 Association of Biochemical Components in Wild Relatives of Chickpea with Survival and Development of *H. armigera* in Diet Incorporation Assay

Among the biochemical components, proteins showed a significant negative association with larval weight (r= -0.26), pupation (r= -0.31) and adult emergence (r= -0.26) of *H. armigera* reared on artificial diet impregnated with lyophilized leaf powders of wild relatives of chickpea genotypes (Table 4.31). The negative effects of proteins on survival and development of *H. armigera* could be due to presence of higher amounts of insecticidal proteins such as protease inhibitors or lectins in these

Table 4.31. Association of biochemical components in wild relatives of chickpea with survival and development of *H. armigera* in diet incorporation assay

	Larval survival (%)	Mean larval weight (mg)	Larval period (days)	Pupation (%)	Mean pupal weight (mg)	Pupal period (days)	Adult emergence (%)	Fecundity	Protein (%)	Phenols (mg/g)	Total soluble sugars (%)	Tannins (mg/g)
Larval survival (%)	1.00											
Mean larval weight (mg)	0.47**	1.00										
Larval period (days)	-0.43**	-0.69**	1.00									
Pupation (%)	0.56**	0.72**	-0.66**	1.00								
Mean pupal weight (mg)	0.51**	0.82**	-0.75**	0.75**	1.00							
Pupal period (days)	-0.51**	-0.74**	0.79**	-0.59**	-0.75**	1.00						
Adult emergence (%)	0.66**	0.78**	-0.65**	0.86**	0.80**	-0.68**	1.00					
Fecundity	0.46**	0.70**	-0.62**	0.54**	0.71**	-0.61**	0.63**	1.00				
Protein (%)	0.05	-0.26*	0.01	-0.31*	-0.14	0.16	-0.26*	-0.11	1.00			
Phenols (mg/g)	-0.03	-0.35**	0.13	-0.41**	-0.25*	0.27*	-0.37**	-0.30*	0.78	1.00		
Total soluble sugars (%)	-0.03	0.15	-0.21*	0.35**	0.25*	-0.08	0.11	0.06	0.13	0.06	1.00	
Tannins (mg/g)	0.07	0.28*	-0.06	0.25*	0.19	-0.15	0.25*	0.17	-0.42	-0.41	-0.13	1.00

*,** Correlation coefficients significant at $P \leq 0.05$ and 0.01, respectively

wild relatives of chickpea. Phenols showed significant negative correlation with larval weight, pupation, pupal weight, adult emergence and fecundity (r= -0.35, -0.41, -0.25, -0.37 and -0.30, respectively), while positive and significant correlation was showed with pupal period (r= 0.27). The total soluble sugars and tannins showed positive effects on survival and development of *H. armigera*. Significant positive correlation was observed between pupation (r= 0.35), pupal weight (r= 0.25) and total soluble sugars, while with larval period it had shown negative correlation (r= -0.21). Tannins showed significant positive association with larval weight, pupation and adult emergence (r= 0.28, 0.25 and 0.25, respectively). From the above results it was evidenced that, proteins and phenols were associated with resistance to *H. armigera*, whereas total soluble sugars and tannins were associated with susceptibility.

The results are in agreement with Girija *et al*. (2008) who reported that total phenols exhibited highly significant negative association with percent pod damage by *H. armigera* in chickpea. Among the chickpea lines, BG256 exhibited higher phenols and less pod damage than Annigeri and ICCV 2. Rupalighodeswar *et al*. (2003) reported that chickpea cultivars resistant to *H. armigera* found to contain significantly higher total phenolics, chlorogenic acid, silica and malic acid. Sahoo and Patnaik (2003) observed that low sugar and high phenol content was recorded in resistant cultivars of pigeonpea against pod borer attack. Expression of resistance to *H. armigera* was associated with low amounts of sugars and high amounts of tannins and polyphenols in wild relatives of pigeonpea (Sharma *et al*., 2009). On contrary, Kanchan *et al*. (2005) reported that protein content had positive correlation with pod damage; these differences could be explained with presence of higher amounts of protease inhibitors in wild relatives compared to cultivated genotypes.

4.8 GC-MS PROFILES OF THE LEAF SURFACE CHEMICALS IN WILD RELATIVES OF CHICKPEA WITH EXPRESSION OF RESISTANCE TO *H. armigera*

4.8.1 Hexane Extracts of Leaf Surface Chemicals

The GC-MS profiles of 20 genotypes (both wild relatives and cultivated chickpea) altogether showed 56 peaks with varying retention times (RT) from 3.87 to 29.20 min (Table 4.32. and Figure 4.6). Of the 56 peaks, 19 peaks were observed

in all the genotypes with varying peak areas at RT of 7.34, 7.96, 8.05, 11.20, 11.29, 11.55, 12.22, 13.26, 14.11, 14.46, 14.58, 14.82, 15.16, 15.88, 17.08, 17.25, 17.74, 19.63 and 29.20 min. The peak at RT of 10.30 min was present in all the genotypes except in JG 11, the peak at RT of 11.67 min was present in all the genotypes except in ICC 506EB, the peak at RT of 14.70 min was present in all the genotypes except in JG 11 and ICC 506EB, the peak at RT of 20.06 min was present in all the genotypes except in IG 69979 and PI 510663. The peak at RT of 7.19 min was present only in different genotypes of wild relatives and completely absent in cultivated chickpea genotypes, whereas peak at RT of 27.56 and 27.85 min were present only in cultivated chickpea genotypes and absent in genotypes of wild relatives. Peak at RT of 10.39 min, 11.01 min, 11.88 min13.98 min and 19.94 min were observed in susceptible checks, ICC 3137 and KAK 2, whereas completely absent in resistant checks, ICC 506EB and ICCL 86111. Peak at RT of 19.32 min, 22.38 min and 27.13 min were observed in resistant checks, ICC 506EB and ICCL 86111 and completely absent in susceptible checks, ICC 3137 and KAK 2. The peaks at RT of 13.38 min, 16.95 min and 24.96 min were observed only in resistant check, ICC 506EB and some wild relatives, whereas it was absent in all other cultivated chickpea genotypes.

4.8.2 Methanol Extracts of Leaf Surface Chemicals

The GC-MS profiles of 20 genotypes (both wild relatives and cultivated chickpea) altogether showed 107 peaks with varying retention times (RT) from 3.06 to 29.77 min (Table 4.33 and Figure 4.7). Of the 107 peaks only two peaks i.e. RT of 10.29 min and 21.95 min were observed in all the genotypes with varying peak areas. The peak at RT of 24.90 min was present in all the genotypes except in IG 69979, IG 72933 and JG11, the peak at RT of 25.07 min was present in all the genotypes except in JG 11, the peaks at RT of 26.74 min and 28.67 min were present in all the genotypes except in IG 72933 and ICC 3137. Among all the peaks, 33 peaks were present only in few genotypes of wild relatives and completely absent in cultivated chickpea genotypes. The peaks at RT of 11.25 and 12.13 min were present only in cultivated chickpea genotypes and absent in genotypes of wild relatives. Peaks at RT of 3.06 min, 3.14 min and 10.83 min were observed in susceptible checks, ICC 3137 and KAK 2 along with some genotypes of wild relatives, whereas completely absent in resistant checks, ICC 506EB and ICCL

Table 4.32. GC-MS profiles (peak areas) of hexane extracts of leaf surface chemicals in wild relatives of chickpea

Species	RT (min) / Genotype	3.87	7.19	7.34	7.96	8.05	8.71	10.30	10.39	10.52	10.64	11.01
C. chrossanicum	IG 599076	1178103	945298	1335150	4340045	1416893	1047076	1456851		976192	1082639	
C. cuneatum	IG 69979	1004047	1264620	3604033	1134428	907885	1039735	5718676				
C. bijugum	IG 70006	1673534		1829927	5606270	1870801		1866688		2117073	1508522	
C. bijugum	IG 70012	1157134		1338629	4771937	1479994	1134544	1713181		1002383	1066681	
C. bijugum	IG 70018	1191720	859407	1324712	4382888	1372209	1018796	1385207	724046	775691	779802	735331
C. bijugum	IG 70022	1232544		1388695	4598957	1442545		1717344				
C. reticulatum	IG 72933	1097098		1225304	4195181	1304807	984950	1237193		860119	937071	
C. reticulatum	IG 72953	1023679	701846	1080089	3612206	1129595	830889	1343398	696059		700296	628072
C. pinnatifidum	PI 510663			1244423	4425879	1408210		1455717		1120625	1060174	1103916
C. judaicum	PI 568217			1504848	4476408	1378065	1085091	1409153		1170076	1057682	
C. bijugum	PI 599046	1399384		1680238	5700188	1879113	1423993	1922779				
C. bijugum	PI 599066	1325941		1540998	5239008	1677211	1261665	1767750				
C. judaicum	PI 599077	906517	795036	1332900	4006206	1189047	971933	1187117			878389	
C. pinnatifidum	PI 599109	917533	798970	1138284	3379469	1053948	760398	1084366				
C. microphyllum	ICCW 17148	1038254		1413560	5110999	1622318	1241362	1864566	921720	1046090	1118117	1021010
C. arietinum	JG 11	1490141		1626417	4993381	1646438				1962313		
C. arietinum	KAK 2	996392		1393562	4847691	1467746	1164807	1400285	891452	950935	1020115	888718
C. arietinum	ICC 3137			1475974	5206426	1619691	1185829	1729620	868878	761288	921726	930391
C. arietinum	ICCL 86111	843601		1081469	3920452	1176983	899315	1057037		773558	711174	
C. arietinum	ICC 506 EB	1099859		1274578	4346557	1344093		1353327				

Table 4.32 (cont.).

Species	RT (min) / Genotype	11.20	11.29	11.55	11.67	11.76	11.88	12.22	13.26	13.38	13.58	13.98	14.11
C. chrossanicum	IG 599076	6 607787	4 245406	9 486962	2 133160	1191105	1272929	1616746	2303208		1467834		2445680
C. cuneatum	IG 69979	1850489	3808367	935759	924622		1152676	1668792	1011898	998121	1417374		1088844
C. bijugum	IG 70006	7551004	2830108	6072335	1569857	1449447	1576703	1916035	2981430		1738913		2735733
C. bijugum	IG 70012	7001869	2592001	5626039	1500204	1353034	1412940	1799996	3015566			959911	2602930
C. bijugum	IG 70018	6267539	2065925	4625393	1133709	1017758	1114375	1388585	2279761			722838	1979308
C. bijugum	IG 70022	6811613	2468687	5287945	1403209	1233013	1322159	1699227	2775319				2467924
C. reticulatum	IG 72933	6412207	2227115	5104496	1354132	1179752	1225655	1616572	2507528				2316874
C. reticulatum	IG 72953	5552477	1780553	4192961	1065806		720004	1227017	2146631				1842158
C. pinnatifidum	PI 510663	7147121	2302928	4642359	1137911			1465212	2416293	1787252	1342505		1990735
C. judaicum	PI 568217	6446375	2301382	4764372	1208024		1069944	1472177	2266511	1825124	1410672		2065677
C. bijugum	PI 599046	7950982	3318089	6601642	1875288	1728757	1843541	2231681	3455825		2039464		3159838
C. bijugum	PI 599066	6952399	2414942	5463439	1349529	1229741	1355040	1732881	2667401				2512360
C. judaicum	PI 599077	5954169	1890046	3957404	959125	849728	909360	1204943	1846564		987072		1571345
C. pinnatifidum	PI 599109	4959167	1555049	3444994	741092			1020819	1597783		806358		1404606
C. microphyllum	ICCW 17148	7269771	2835467	5793647	1630764	1418468	1431792	1927970	3150054			957624	2638617
C. arietinum	JG 11	6740073	2691989	5481693	1720335	1551368	1640888	1828511	2559618		1541840		2094040
C. arietinum	KAK 2	6858873	2232253	5334269	1452879	1248914	1330242	1644193	2673360			850752	2393094
C. arietinum	ICC 3137	6817426	2374334	5390026	1403525		948626	1714860	2925223			854505	2387731
C. arietinum	ICCL 86111	5058945	1761456	4051917	972811			1204346	2171576				1987698
C. arietinum	ICC 506 EB	5767008	2012306	4314638				1287622	2244229	1752585	1297018		1997303

Table 4.32 (cont.).

Species	RT (min) / Genotype	14.46	14.50	14.58	14.70	14.82	15.16	15.88	16.95	17.08	17.25	17.35
C. chrossanicum	IG 599076	1553404	1270429	6511817	1208389	2069129	2092808	1327587	1920640	2036934	4575974	970820
C. cuneatum	IG 69979	976588	5086087	944797	1619081	1804700	1229112	973962	1811531	3028650	1148193	
C. bijugum	IG 70006	1940802	1483252	8219933	1562842	2733718	3251641	1727674	1805968	2712715	5396672	
C. bijugum	IG 70012	1707330	1513278	7786383	1830724	2137555	2394226	2014907		1308611	4455277	
C. bijugum	IG 70018	2342395		5961279	1391713	1626015	1731341	1458156		928242	3450473	
C. bijugum	IG 70022	2915595		7108729	1292173	2368714	2119981	2006693	1314683	2436403	5356308	1111939
C. reticulatum	IG 72933	1550203	1345284	6871565	1249217	2249219	2074866	1719439	1922783	1615745	5133750	1100088
C. reticulatum	IG 72953	2178163		5477221	1166591	1545924	1600355	1444258		1084641	3217178	
C. pinnatifidum	PI 510663	1243957	1143765	6037234	1129322	1920860	2276962	1738362	1280470	2167568	3696718	
C. judaicum	PI 568217	1348199	1058153	6166645	1121954	2110517	2483423	1530116	1254651	2184976	3881181	
C. bijugum	PI 599046	2131958	1829725	9156259	1820578	3152910	3546326	2283856	1982457	3319005	5788019	1499203
C. bijugum	PI 599066	1689330	3169785	6980066	1327935	2428582	2201358	1610100	1408498	1849576	5402858	1200365
C. judaicum	PI 599077	1017236	835769	4697220	834905	1633102	1408163	1363699		1375055	2815145	
C. pinnatifidum	PI 599109	855252		4159632	731329	1497995	1362713	928566	697093	1188351	2618558	
C. microphyllum	ICCW 17148	1802441	1510981	7729576	1447482	2550529	2327147	2000738	2058295	1784447	5460551	1191980
C. arietinum	JG 11	1744066	1661480	7843421		2363182	3222334	2238523		3331281	4238766	
C. arietinum	KAK 2	2860240		7028232	1677870	2155965	2130697	1952843		1165568	4196414	946331
C. arietinum	ICC 3137	2977878		7304113	1761465	2064421	2210870	1896669		1371047	4324776	
C. arietinum	ICCL 86111	2230904		5569001	1220874	1454232	1266738	1748959		1314288	3097751	
C. arietinum	ICC 506 EB	1253061	1102873	5868545		2058955	2351389	1607566	1238694	2068381	3878210	

Table 4.32 (cont.).

Species	RT (min) / Genotype	17.74	18.23	19.32	19.50	19.63	19.94	19.99	20.06	20.38	21.36	21.63
C. chrossanicum	IG 599076	1235452		1776470		2378925			982545		2152063	
C. cuneatum	IG 69979	1047651		1298592		2364358		1389384				1068908
C. bijugum	IG 70006	1641840		2309225		3478681		1862686	1476022			1851301
C. bijugum	IG 70012	1521780				3116232	1131919	2664634	1349978			
C. bijugum	IG 70018	1160390				2469121		1470001	889723			
C. bijugum	IG 70022	1420017			1716652	3051888		1450304	1374465			1686245
C. reticulatum	IG 72933	1382730			1262656	3032234	1421911	2023032	1440291			2039418
C. reticulatum	IG 72953	1071315	919513	1515868		2339776		748145	806796	779640		
C. pinnatifidum	PI 510663	1223880		1545831		3028049		1189798			2171121	1511504
C. judaicum	PI 568217	1138178		1552074		2507189		1065083	1057334		1789509	1446437
C. bijugum	PI 599046	2053312		2200522		3492893		2172659	1653436			2030425
C. bijugum	PI 599066	1532811			1325929	2967276		6003357	1516154	2367119		1848399
C. judaicum	PI 599077	861179		1080811		1702347		1014063	790710			1008657
C. pinnatifidum	PI 599109	816060		991515		1705819		1157399	752594			936920
C. microphyllum	ICCW 17148	1482096				2936185		1397099	1110965			
C. arietinum	JG 11	2079620	1517347	2665214		2728722		2297547	1760801		1825989	1719706
C. arietinum	KAK 2	1381381				2972076	2501451		1177929			
C. arietinum	ICC 3137	1467729	791409		1382461	3105497	2643550		1283141			2220517
C. arietinum	ICCL 86111	1045716	1153091	1933663		2121574			811200	994723	744822	
C. arietinum	ICC 506 EB	1327661		1501978		2507088		1520744	1122256			1480741

Table 4.32 (cont.).

Species	RT (min) / Genotype	21.77	22.38	23.86	24.91	24.96	26.74	27.13	27.56	27.85	28.67	29.20
C. chrossanicum	IG 599076		1156783			25080340		2271494				4337324
C. cuneatum	IG 69979	2213163	1656602	1127237	16496407			2265890				7182107
C. bijugum	IG 70006	3596864		9611688		16578603	2421373				6613635	12607963
C. bijugum	IG 70012		1071646		15185396	17527803		1959414			4341523	9179325
C. bijugum	IG 70018		1023169		8297729		1753120	1829319			5447039	9406970
C. bijugum	IG 70022	3490147	1398879	11243661	18965628		2280068	2733877			5868020	9129262
C. reticulatum	IG 72933	4041978	1179134		14316906							10589007
C. reticulatum	IG 72953		786296	3571252	17890281		2289525	2197530			2720459	10609350
C. pinnatifidum	PI 510663	2709062			11342684	22529966	1352579	1357745			2996529	9806941
C. judaicum	PI 568217	2557339			12105229			1099002			2726595	5961314
C. bijugum	PI 599046	3980789	1351547	14685954		21872170	1891353	9161632				10127432
C. bijugum	PI 599066	3430415			12249852	13093142	2889666	9218449				9809646
C. judaicum	PI 599077	1990455	947427		17791220		848910	1112935			3846641	7454602
C. pinnatifidum	PI 599109	1916541	842292	3215333	16093103		1437376	1117691			2360466	4820627
C. microphyllum	ICCW 17148		968296		10311817	8032541						5337884
C. arietinum	JG 11	2805715	2424719				4191742	4858562		1725752	2186139	13573777
C. arietinum	KAK 2				19180177		8464285		2131199	1552544	4748093	25058420
C. arietinum	ICC 3137	4004982			16500616		12430003		3221741		4319388	31468631
C. arietinum	ICCL 86111		895172	5715365	19892611		5854539	2072394			1612265	16496817
C. arietinum	ICC 506 EB	2857235	1090034		13839456	12991823	10978938	1159621	2591870	2327692	5334724	37475419

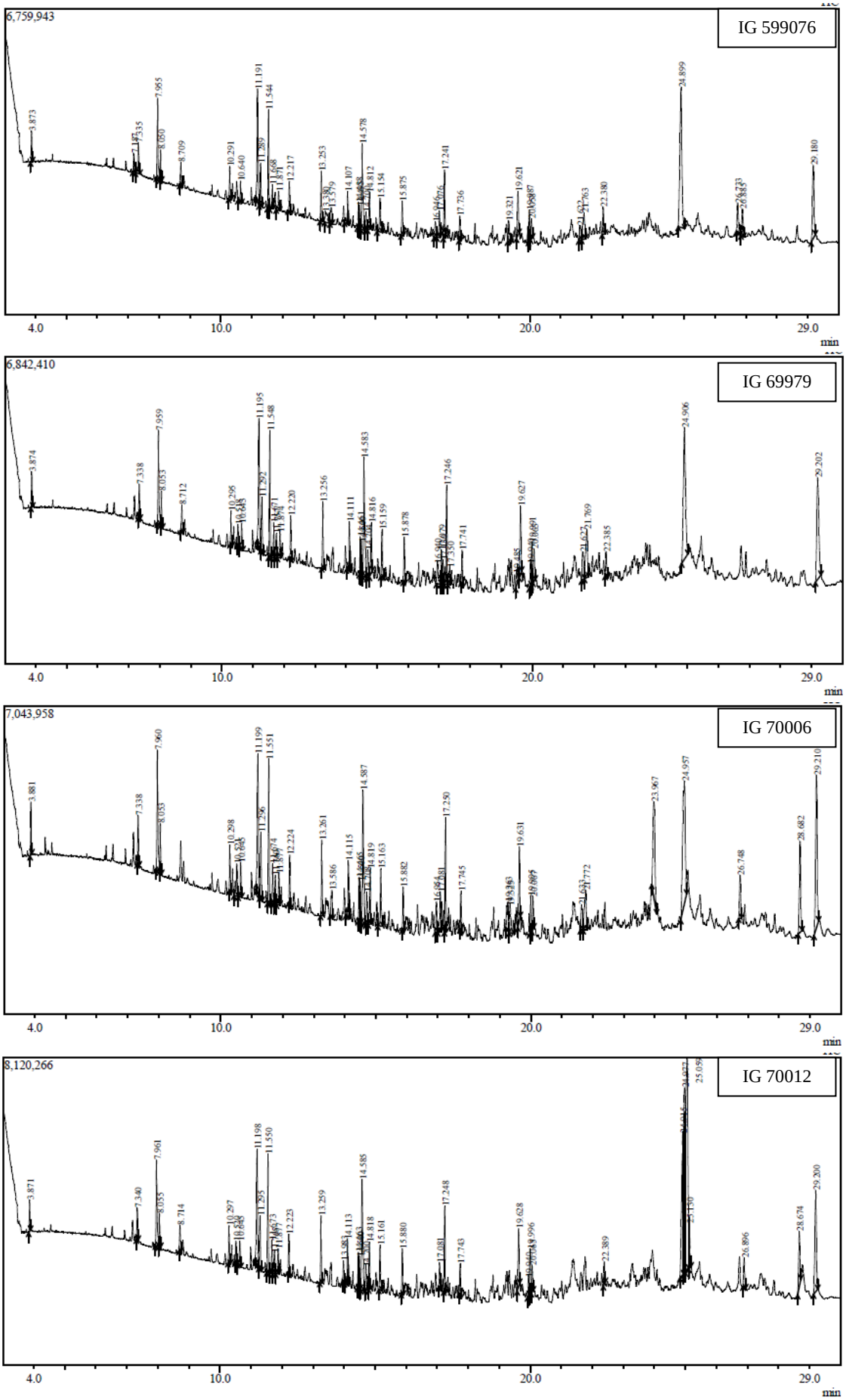


Figure 4.6. GC-MS profile of hexane extracts of leaf surface chemicals in wild relatives of chickpea

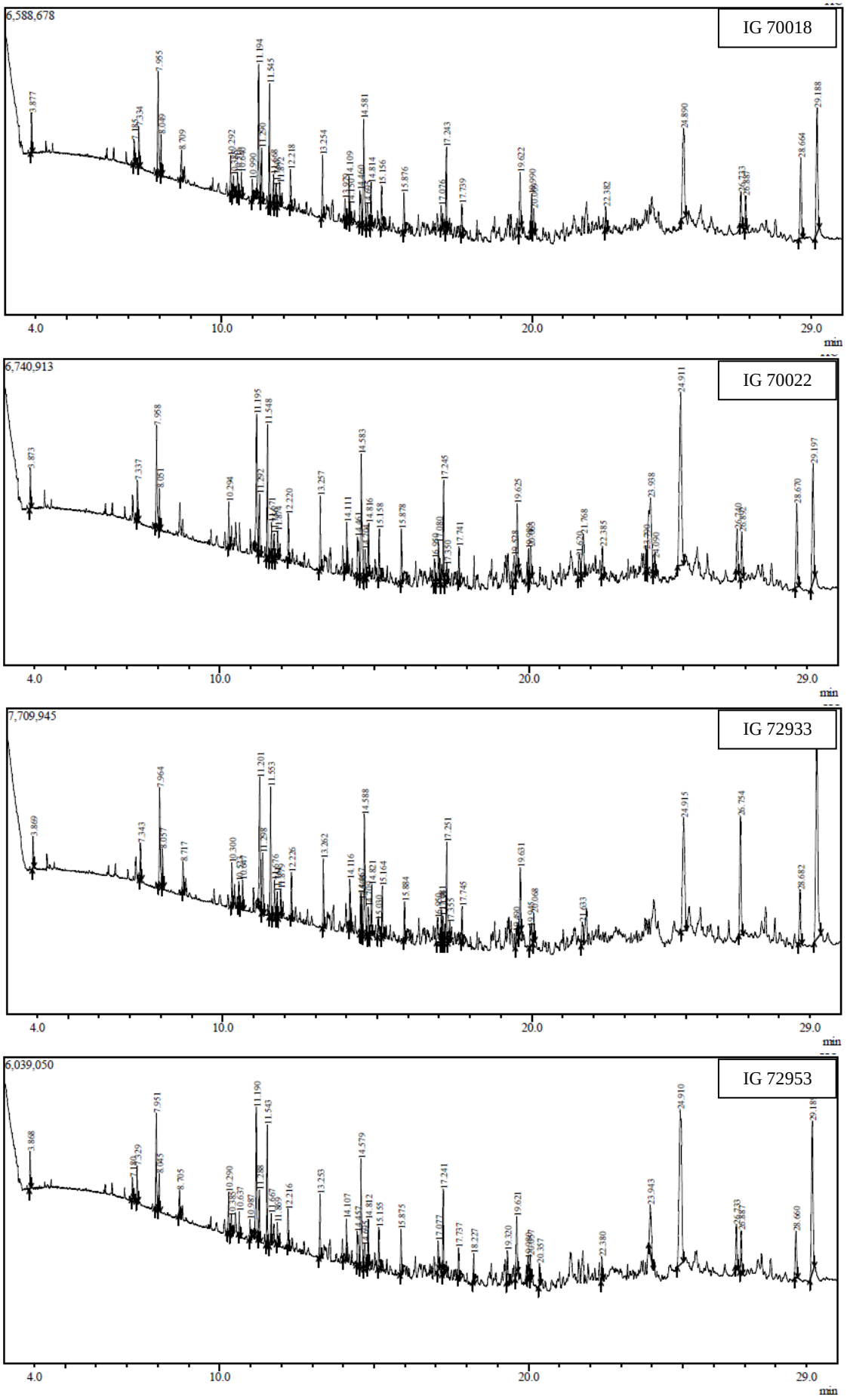


Figure 4.6. (cont.)..

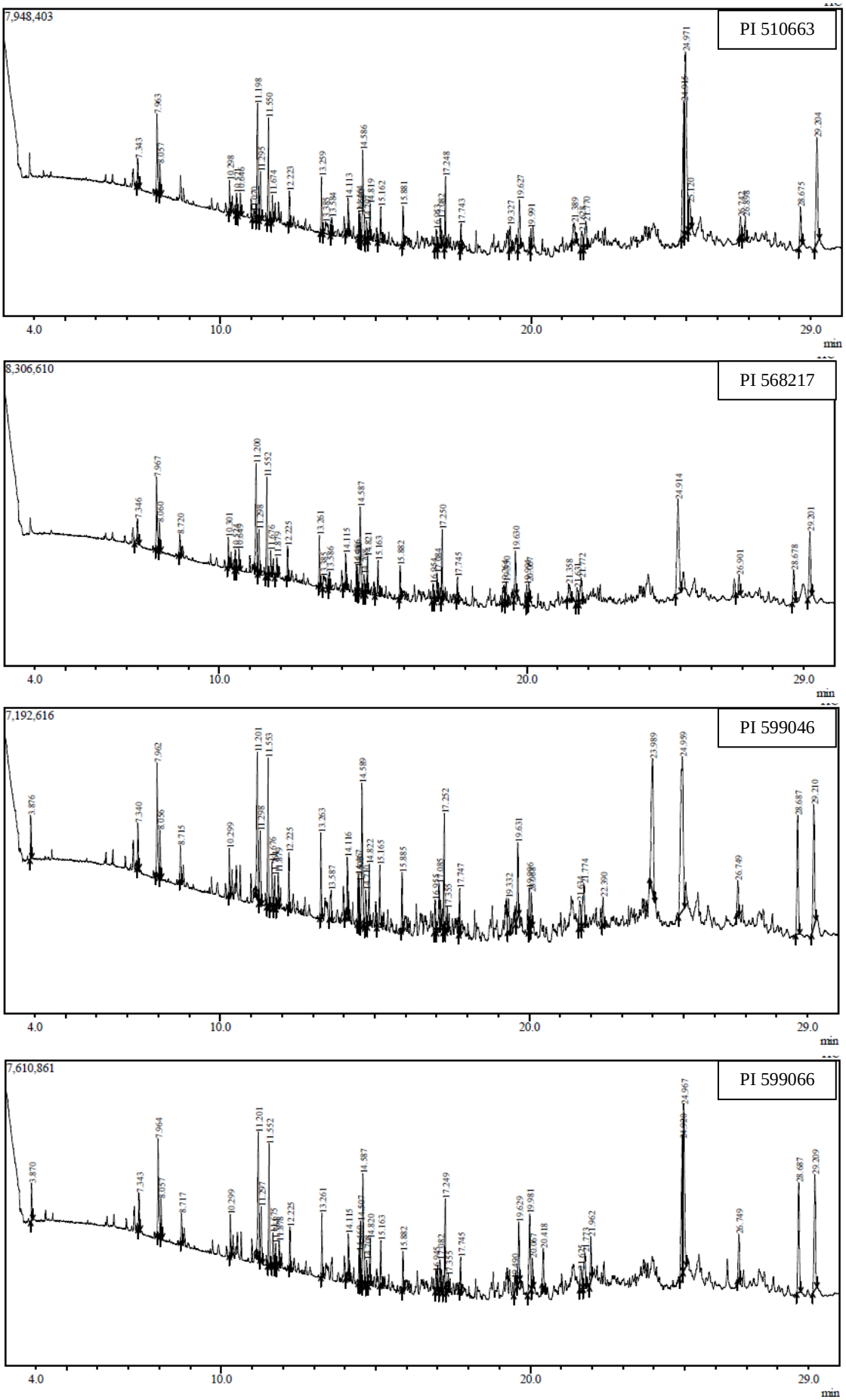


Figure 4.6. (cont.)..

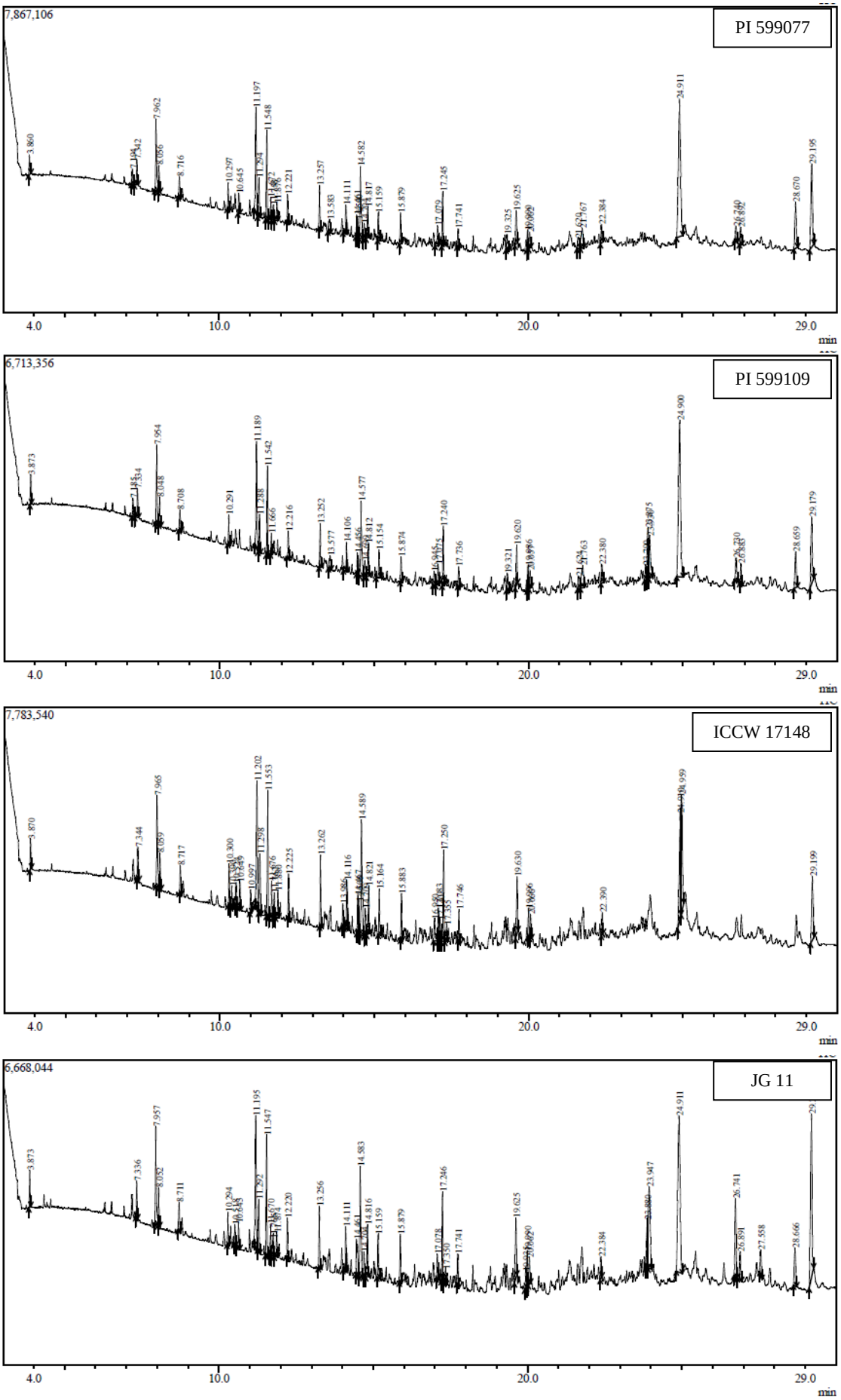


Figure 4.6. (cont.)..

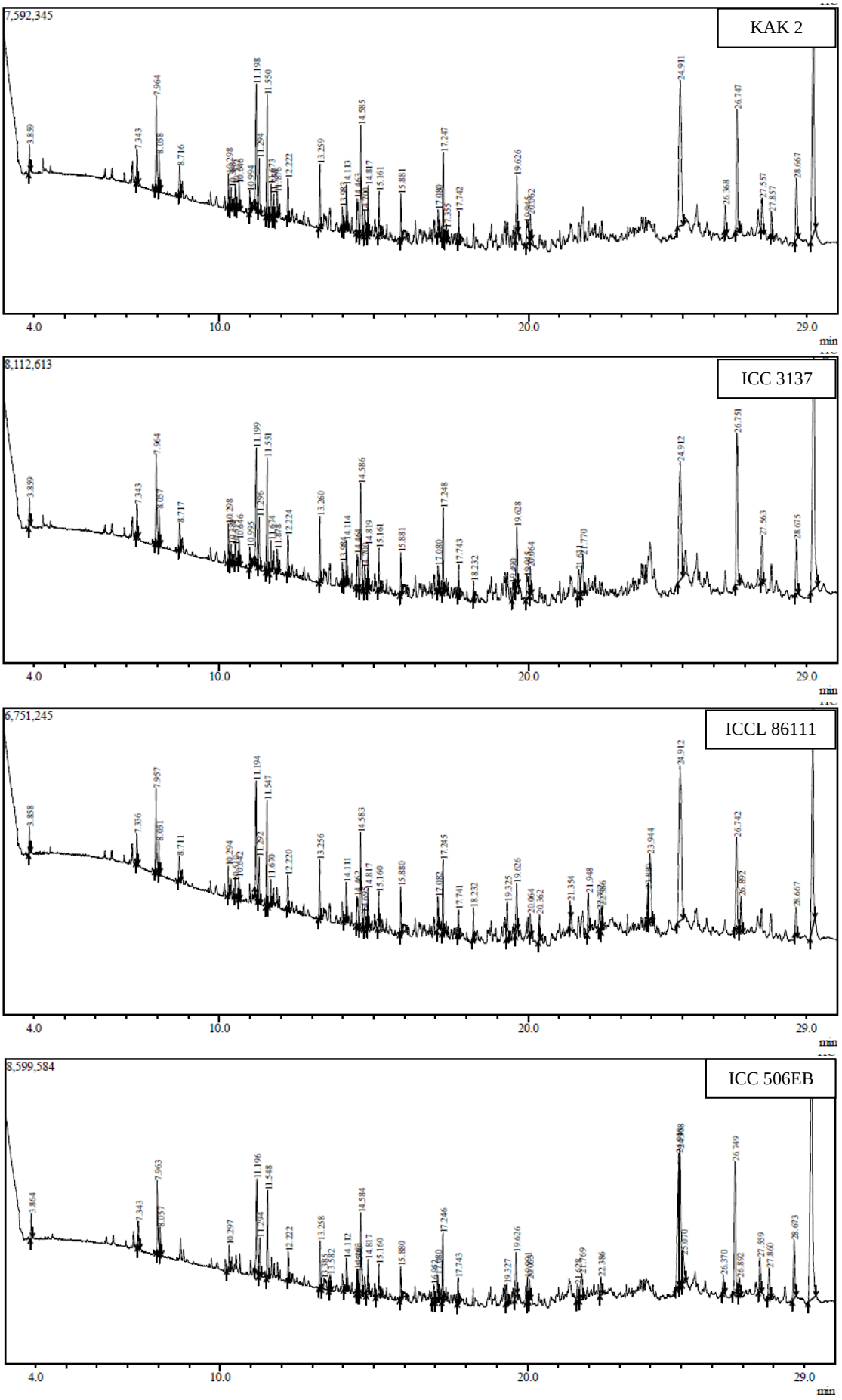


Figure 4.6. (cont.)...

Table 4.33. GC-MS profiles (peak areas) of methanol extracts of leaf surface chemicals in wild relatives of chickpea

Species	RT (min) / Genotype	3.06	3.09	3.14	3.32	3.92	4.12	4.54	4.62	4.67	4.72	4.76	4.92
C. chrossanicum	IG 599076	444546	2443593		133189	110041							
C. cuneatum	IG 69979		2922903						1269160				
C. bijugum	IG 70006		2779479										130735
C. bijugum	IG 70012									1015173			
C. bijugum	IG 70018		3104422							1874468			
C. bijugum	IG 70022			2692928					279350				
C. reticulatum	IG 72933	2747921				140124	116105				1443987	285798	454105
C. reticulatum	IG 72953	375816		2442577									
C. pinnatifidum	PI 510663							179387	913263			152211	142299
C. judaicum	PI 568217	309224	2700282		103476			426169			131264		
C. bijugum	PI 599046		2992588										
C. bijugum	PI 599066		3359855							5577276	359708		
C. judaicum	PI 599077	493359	2604918						1482739	81529	180197	65510	
C. pinnatifidum	PI 599109	4796069			149935								
C. microphyllum	ICCW 17148		2952705		184583	185235		369791					
C. arietinum	JG 11												
C. arietinum	KAK 2	1573546		3817289			267069				577831	247383	
C. arietinum	ICC 3137	1608799	1174702	2516909						2000443		1397451	
C. arietinum	ICCL 86111		3213368							2378296		450379	
C. arietinum	ICC 506 EB		3059393				932128		2819905			442503	

Table 4.33 (cont.).

Species	RT (min) / Genotype	5.08	5.26	6.09	6.25	6.45	6.60	6.94	7.34	7.43	7.70	8.70	8.78	8.90
C.chrossanicum	IG 599076		109226									558609		
C. cuneatum	IG 69979								617310				2183316	
C. bijugum	IG 70006		94252					145259						
C. bijugum	IG 70012							470868	462581		182161		1314345	651370
C. bijugum	IG 70018				477280	383203		717432	399707					812072
C. bijugum	IG 70022	167554						167196				717146		
C. reticulatum	IG 72933			135101	190751	659981	213412	297004	1214086	208438	197229			596544
C. reticulatum	IG 72953	519197												654349
C. pinnatifidum	PI 510663								577626		150049		1427161	526504
C. judaicum	PI 568217	46815							162026	92780		173961	186984	
C. bijugum	PI 599046											244967		
C. bijugum	PI 599066							504870	815306					382448
C. judaicum	PI 599077								254908		65762	510377		
C. pinnatifidum	PI 599109											1985164		
C. microphyllum	ICCW 17148		140901						203799			259961	146145	
C. arietinum	JG 11				226967	275952			361246					
C. arietinum	KAK 2					471425			628932					1042079
C. arietinum	ICC 3137			260942		770533	338311	449717	1145794	248772	251960			328692
C. arietinum	ICCL 86111			248858		660331	518848	286997	1210568	352499	221558			571230
C. arietinum	ICC 506 EB								405366					

Table 4.33 (cont.).

Species	RT (min) / Genotype	8.96	9.02	9.10	9.19	9.34	9.46	9.51	9.57	9.68	9.79	10.10
C. chrossanicum	IG 599076					105626					110016	107321
C. cuneatum	IG 69979	553513		630405			7729099	908827	561444			610591
C. bijugum	IG 70006					133488						
C. bijugum	IG 70012						8178468		1260987			
C. bijugum	IG 70018	986422							23389965	410589		
C. bijugum	IG 70022	236614				1237584	183672					
C. reticulatum	IG 72933		1177390		2479192	1611364	3028335			35075883	2262281	
C. reticulatum	IG 72953				925331		4387618	632248				277708
C. pinnatifidum	PI 510663		366361			2765775	185808					
C. judaicum	PI 568217	243322				1194212	170460					
C. bijugum	PI 599046						230884					
C. bijugum	PI 599066			835584	1176552			16486345		405917		
C. judaicum	PI 599077		463954			3120257		622498				
C. pinnatifidum	PI 599109					596393						
C. microphyllum	ICCW 17148	198567				699007	146149					
C. arietinum	JG 11	1095804		615039	739685		5618297	675664	225888			
C. arietinum	KAK 2				3240576				18308735	1653126		
C. arietinum	ICC 3137		439310		5960605	1692212				38210514	1615651	
C. arietinum	ICCL 86111				2474828	5072770	4770100			38785249	842022	
C. arietinum	ICC 506 EB		537064		7519123		10197364		574530			

Table 4.33. (cont.).

Species	RT (min) / Genotype	10.18	10.29	10.61	10.67	10.75	10.83	11.08	11.15	11.25	12.13	16.75	17.01
C. chrossanicum	IG 599076	220573	1253476									447264	
C. cuneatum	IG 69979		1018094				8265497					451663	3682937
C. bijugum	IG 70006		863023										
C. bijugum	IG 70012		587186		3633884		2106032						
C. bijugum	IG 70018		948548		932055	994756							
C. bijugum	IG 70022		678611	739977									
C. reticulatum	IG 72933	510252	1032048		515142				17814570				
C. reticulatum	IG 72953		793225		920014	736166	502304						
C. pinnatifidum	PI 510663		898294		2707674		152519						
C. judaicum	PI 568217		612093	825359									
C. bijugum	PI 599046		781928										
C. bijugum	PI 599066		1052577		2465321		8382410	805279					
C. judaicum	PI 599077		716292			3640684	244854						
C. pinnatifidum	PI 599109		778561		312238							632841	1148108
C. microphyllum	ICCW 17148		1004189	614571	162646	192901						411749	2511625
C. arietinum	JG 11		907007			568645	2179835				432654		
C. arietinum	KAK 2	596644	1040903		1234713		8595749	6631007	868109				
C. arietinum	ICC 3137	686276	1046523		542665		9531076		13025105	7380084	302442		
C. arietinum	ICCL 86111	507841	999868		461461				20103400	9646391	258636		
C. arietinum	ICC 506 EB	279790	762807					38418283	6374768	2060363			

Table 4.33 (cont.).

Species	RT (min) / Genotype	17.08	17.16	17.25	17.38	17.99	18.24	19.66	19.95	19.97	20.00	20.40	21.35
C. chrossanicum	IG 599076		7568472						233693		100179	106918	
C. cuneatum	IG 69979	1722085							2079869			2775505	389466
C. bijugum	IG 70006					1609374		94154	340976	308925			367198
C. bijugum	IG 70012						401040	864241		1362240	166950	2520272	
C. bijugum	IG 70018						492122	1972720		1777514		2703059	
C. bijugum	IG 70022		1625986			307751				330031			411831
C. reticulatum	IG 72933							790956		2242308			
C. reticulatum	IG 72953	1080844		1726794	2705700	5738698			1091089				
C. pinnatifidum	PI 510663		1867790						525632		192619	149676	
C. judaicum	PI 568217	523060		3342509			67832	66623	453641	504857			
C. bijugum	PI 599046	643545	2750397		3860453	1263163			442483	404991			841757
C. bijugum	PI 599066							1075512	694435			2062201	
C. judaicum	PI 599077	2196172		3983967					287912				
C. pinnatifidum	PI 599109	1528569	2045982	2158154	6027933				329882				
C. microphyllum	ICCW 17148	2775574	1980254						426965		221870		
C. arietinum	JG 11								1372804			445772	1782611
C. arietinum	KAK 2								1078431			744949	
C. arietinum	ICC 3137							592988				810415	
C. arietinum	ICCL 86111			3691747					1176509			519751	
C. arietinum	ICC 506 EB	4042355						251590	1555136			484084	250624

Table 4.33 (cont.).

Species	RT (min) / Genotype	21.69	21.77	21.95	22.04	22.20	22.32	22.38	22.65	22.71	23.02	23.22	23.28
C. chrossanicum	IG 599076	106830		102486									
C. cuneatum	IG 69979		2450620	2489494				6281971	8666282				507267
C. bijugum	IG 70006	142141		236732			435302				243284		1141793
C. bijugum	IG 70012			822926	373650		435735	1344080		19346908	295964		2810007
C. bijugum	IG 70018	987823	459734	1062400	593477		1115305	1408249		29113098			663251
C. bijugum	IG 70022			240218			165073				340367	368246	1343541
C. reticulatum	IG 72933	3264505		2358942				1286757	888135				
C. reticulatum	IG 72953		1219288	669208									
C. pinnatifidum	PI 510663			300678		421189				1046597			
C. judaicum	PI 568217		267563	318202		254006		150136					
C. bijugum	PI 599046			292463	409982		1786099				674557		3727566
C. bijugum	PI 599066	377442		392208	373164		429124	975949		17400132			
C. judaicum	PI 599077	332726		201760		89050							
C. pinnatifidum	PI 599109			170276		206983						133234	
C. microphyllum	ICCW 17148			177874		224248							
C. arietinum	JG 11	216686	389987	1736381									
C. arietinum	KAK 2	1002905	787932	558613					637157			340438	
C. arietinum	ICC 3137	3522830		1837309			1989901		514586				
C. arietinum	ICCL 86111		2217689	1290245					255803				
C. arietinum	ICC 506 EB	1031978		1212637									

Table 4.33. (cont.)...

Species	RT (min) / Genotype	23.39	23.54	23.77	23.98	23.93	24.09	24.18	24.26	24.47	24.62	24.77	24.90
C. chrossanicum	IG 599076		345910									120464	316194
C. cuneatum	IG 69979		521253					1842222		3911471			
C. bijugum	IG 70006	101551				89028	91438	453118				608103	717554
C. bijugum	IG 70012	476737	883465				1049288	1824375		4059440		580711	1301197
C. bijugum	IG 70018		1534561				2529367	848108		6806257	414744	373737	391432
C. bijugum	IG 70022				601436	900932	574700	332502	257252		186094	589331	2037844
C. reticulatum	IG 72933				2995746								
C. reticulatum	IG 72953				488059								322665
C. pinnatifidum	PI 510663		771421									328319	918816
C. judaicum	PI 568217		65315	49018								167869	469190
C. bijugum	PI 599046	741178			321770	522215	383840	3568882	404411			1095710	3312820
C. bijugum	PI 599066		438803		1086030			1494069	401582	3700015	315530		1195048
C. judaicum	PI 599077		57111	210032					159872			224053	543487
C. pinnatifidum	PI 599109				562583	493855						219880	1624298
C. microphyllum	ICCW 17148			140041	544605	259511						190378	1420480
C. arietinum	JG 11					18695136			438191			385797	
C. arietinum	KAK 2				722615	845104							1563995
C. arietinum	ICC 3137				1189926				691538				2651610
C. arietinum	ICCL 86111												580836
C. arietinum	ICC 506 EB				408011								1343626

Table 4.33. (cont.)...

Species	RT (min) / Genotype	24.98	25.07	25.21	25.42	25.60	25.67	25.80	25.98	26.37	26.74	26.90	27.02
C. chrossanicum	IG 599076	192148	1130743				185998				442646	106859	174069
C. cuneatum	IG 69979		6356439		2356363				3492188	2161605	3327592		1196235
C. bijugum	IG 70006		2898406	225086	153046			254591	359612	104717	420364	125558	
C. bijugum	IG 70012		6662266	976698					553621	786372	962238		
C. bijugum	IG 70018		2808921							918047	585909		
C. bijugum	IG 70022		2777447	387725				269691	275880	254139	1269651		189652
C. reticulatum	IG 72933		2651284	664295									
C. reticulatum	IG 72953	336832	985406						261771	453565	2633801	215309	294331
C. pinnatifidum	PI 510663		1271658	743379	227381	712418					952268	209641	
C. judaicum	PI 568217		524304								394556	99803	
C. bijugum	PI 599046		9684482	1362465			199716	1264982	1531225		701128	1324760	367002
C. bijugum	PI 599066		1156349							946598	848450		
C. judaicum	PI 599077	260959	1144997	131204	120616	143474			101066		323260		238113
C. pinnatifidum	PI 599109		505928	188891	128029	597056					825890		133632
C. microphyllum	ICCW 17148		1089148						140751		792826		
C. arietinum	JG 11	37641718			2479609		2230880		2278795	4002664	5743257		1307365
C. arietinum	KAK 2		1172823						543103	574285	1757940		243791
C. arietinum	ICC 3137		2407999										
C. arietinum	ICCL 86111		969098						493025	473807	1163506		
C. arietinum	ICC 506 EB		1724557			739326	276124			736831	3245063	630047	243668

Table 4.33. (cont.)...

Species	RT (min) / Genotype	27.25	27.41	27.56	27.63	27.76	27.86	28.67	29.01	29.19	29.23	29.77
C. chrossanicum	IG 599076							573792		702587		
C. cuneatum	IG 69979	934848		1062154				1149269	324452		5782175	
C. bijugum	IG 70006	5083955	97287			193195	128139	1257536	4206330	948410		973116
C. bijugum	IG 70012	5217771						1537641	4342069	1606832		1319471
C. bijugum	IG 70018	6083932	811702					1569108	2244260			1297295
C. bijugum	IG 70022	2473134						3160510	3325189	1715321		1942136
C. reticulatum	IG 72933										2578946	
C. reticulatum	IG 72953			753266				1213954			1944539	
C. pinnatifidum	PI 510663	243879		125920	647902	374500	315789	1957300	602837	2469067		380526
C. judaicum	PI 568217							970949	1731246	1242585		
C. bijugum	PI 599046	8480636			393112			2100925	11041797	1291461		3101544
C. bijugum	PI 599066	3674363	534170					3019510	5270901	1022424		2259027
C. judaicum	PI 599077							1196869		1177449		
C. pinnatifidum	PI 599109				511791	131816	184223	1533502	305679	1544939		1257737
C. microphyllum	ICCW 17148							1345349	901996	1684138		352927
C. arietinum	JG 11	1704294	3352541	1245509	777911		671496	891317	2334802		1994066	
C. arietinum	KAK 2	333046	381123	1209965	428739			537445			764725	
C. arietinum	ICC 3137										2882161	
C. arietinum	ICCL 86111			1004809		161568		752890		450945	214670	
C. arietinum	ICC 506 EB		437948	1474276	1268305			1303421		3456826		480549

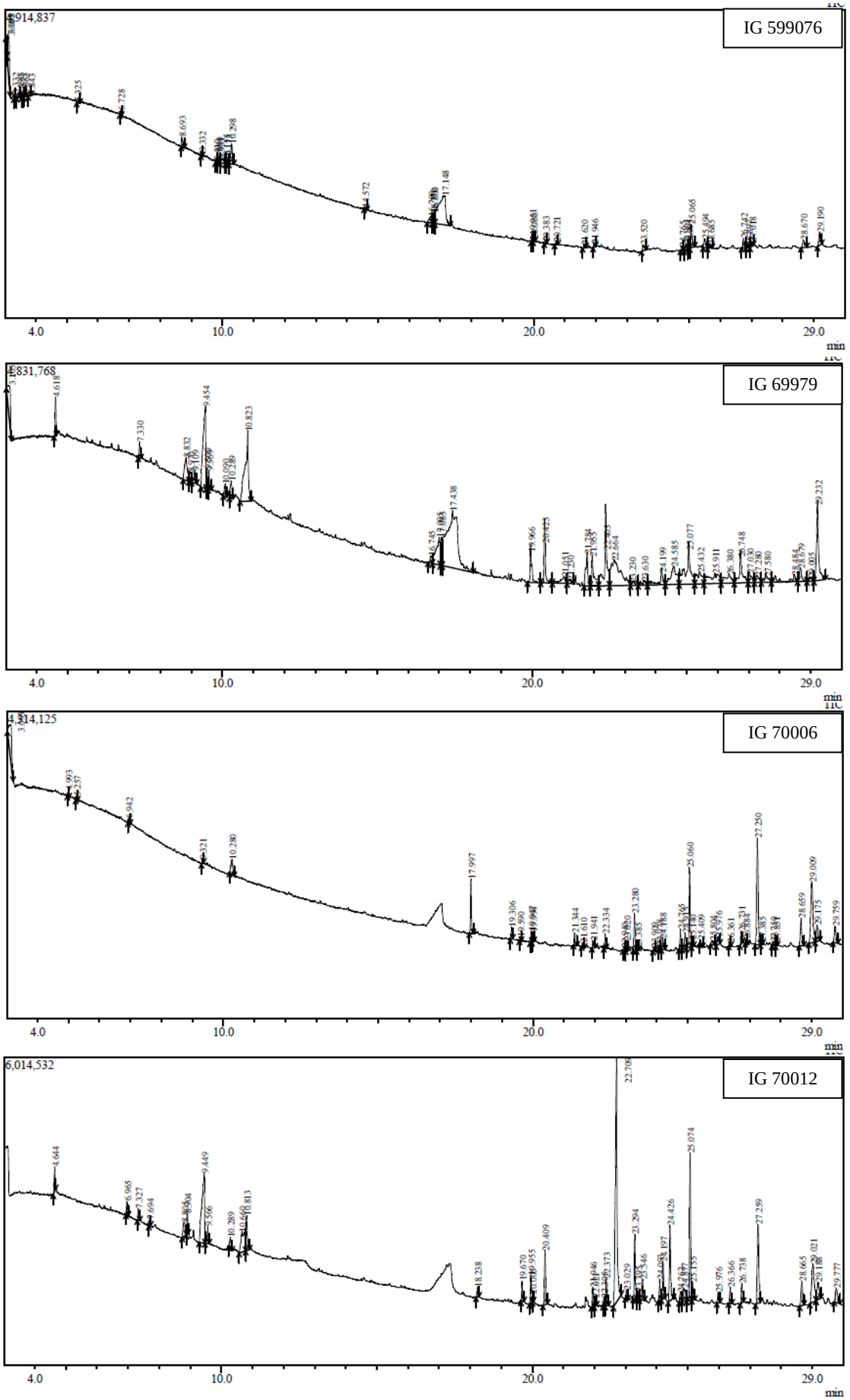


Figure 4.7. GC-MS profile of methanol extracts of leaf surface chemicals in wild relatives of chickpea

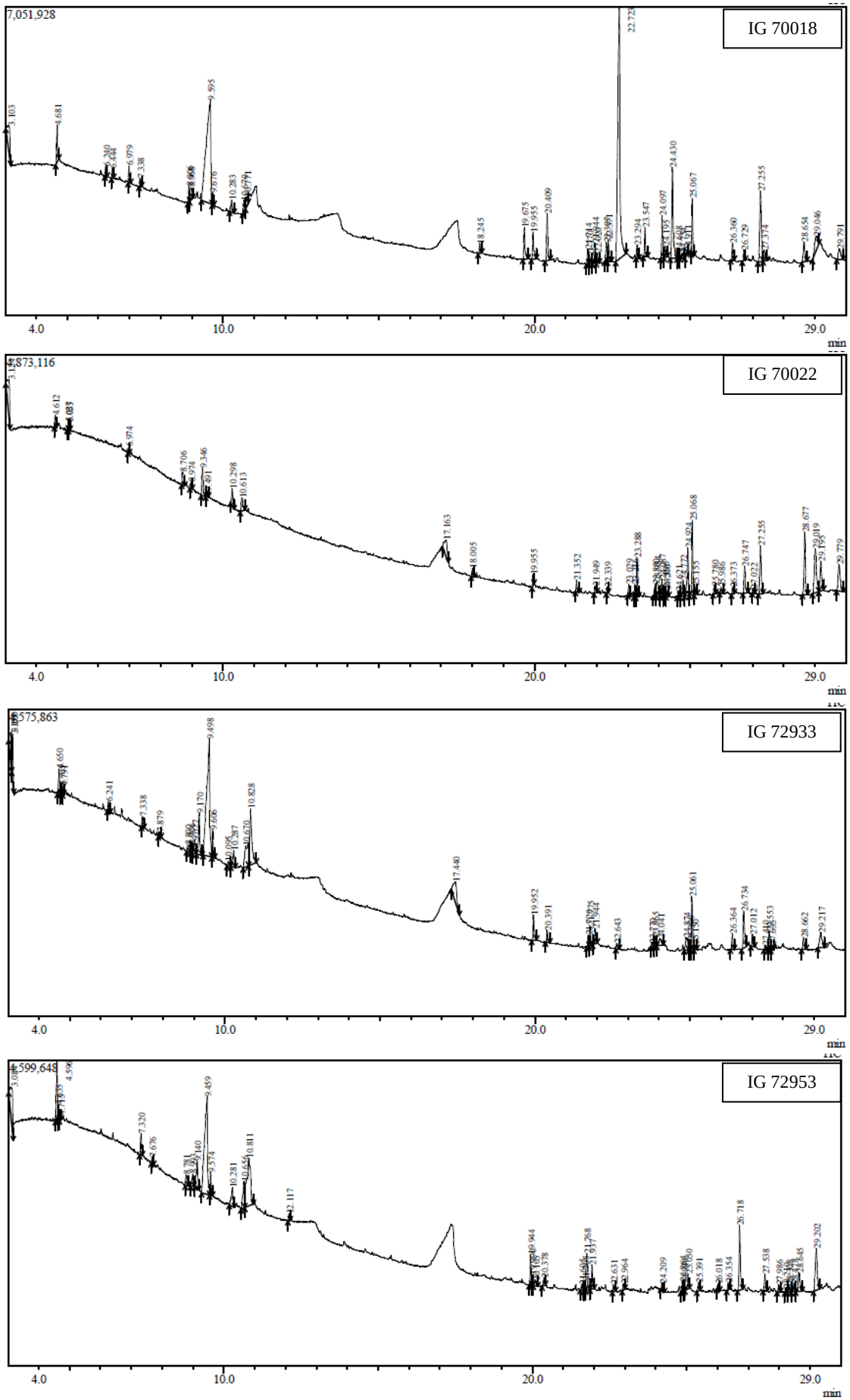


Figure 4.7. (cont.)..

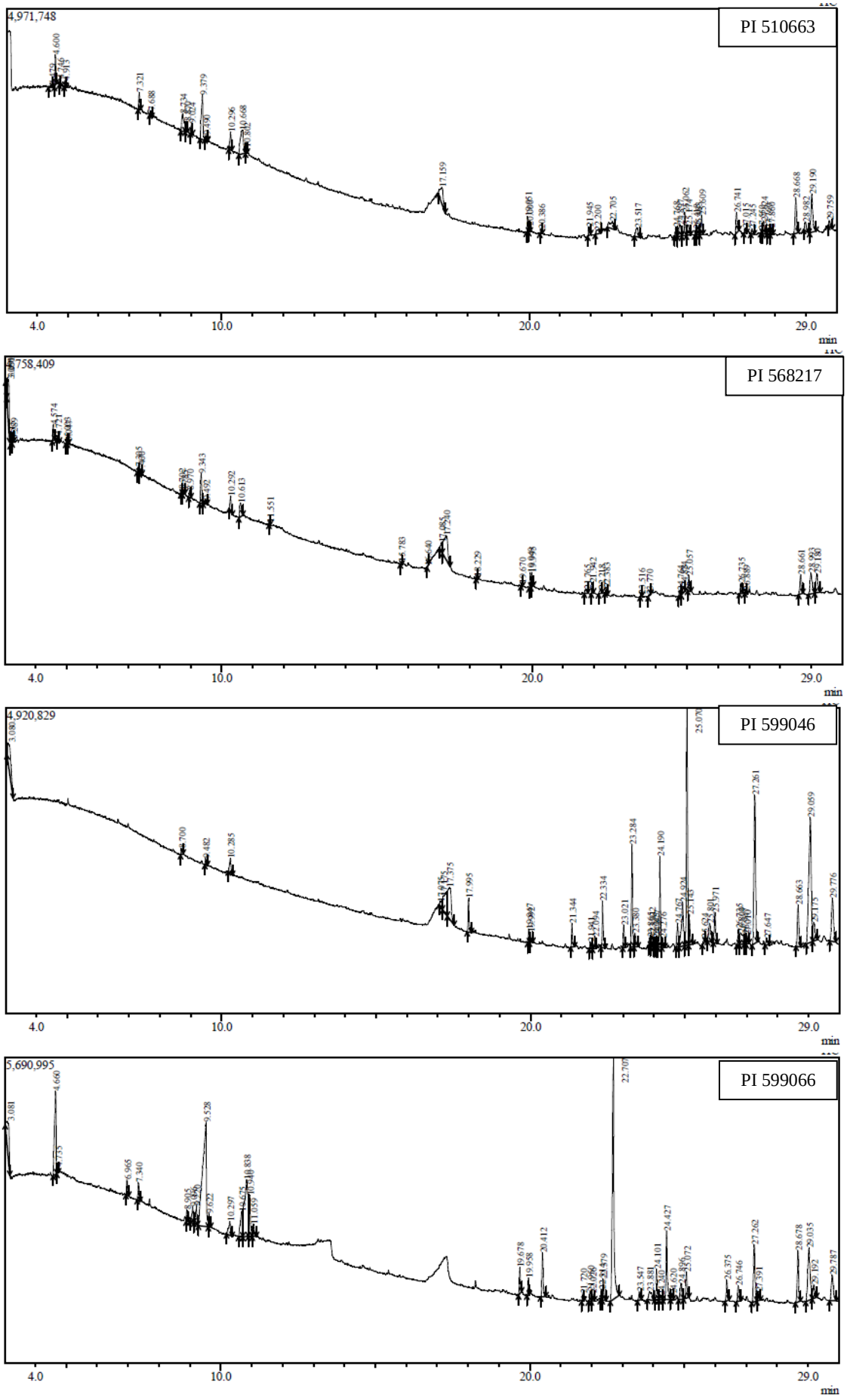


Figure 4.7. (cont.)..

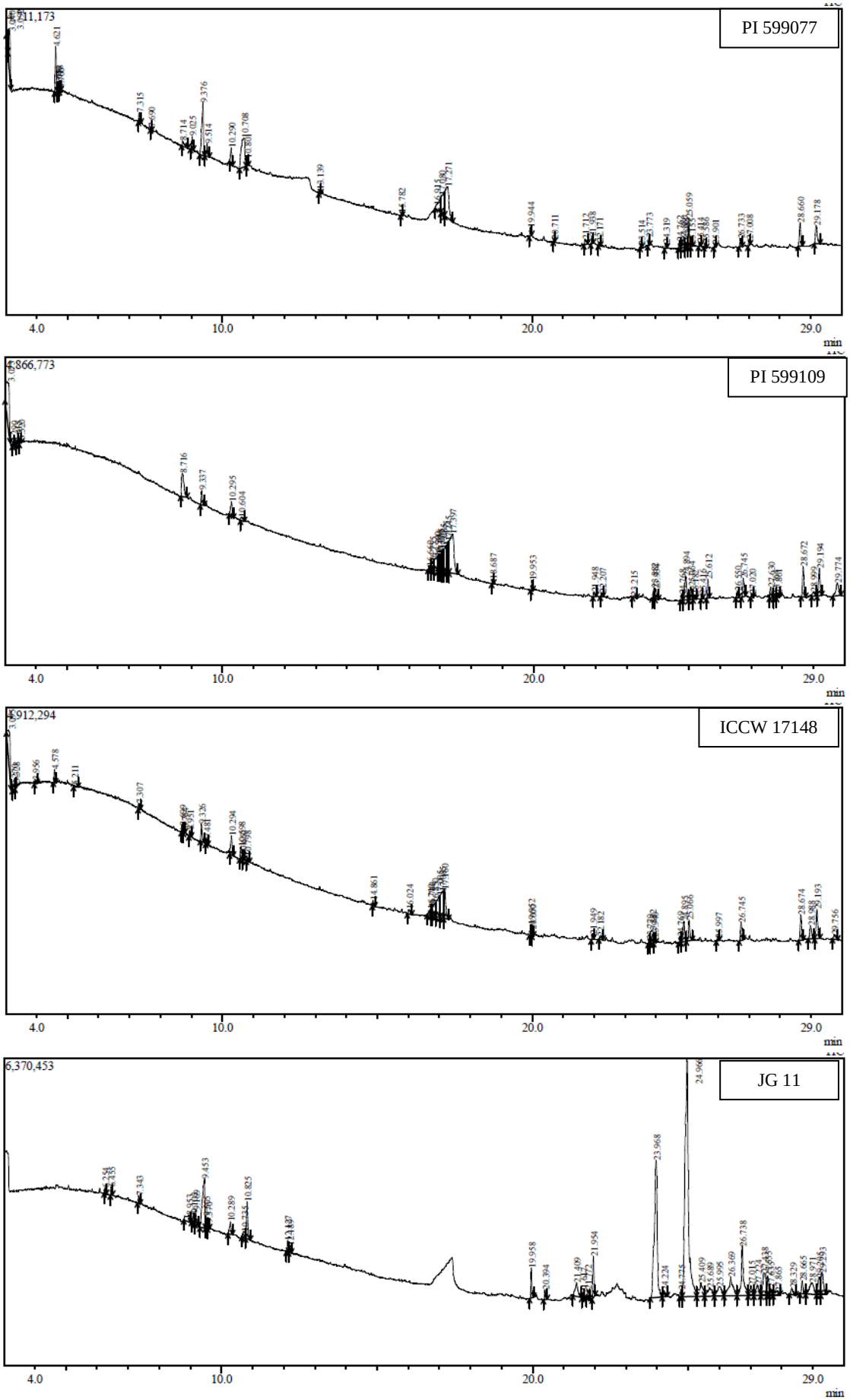


Figure 4.7. (cont.)..

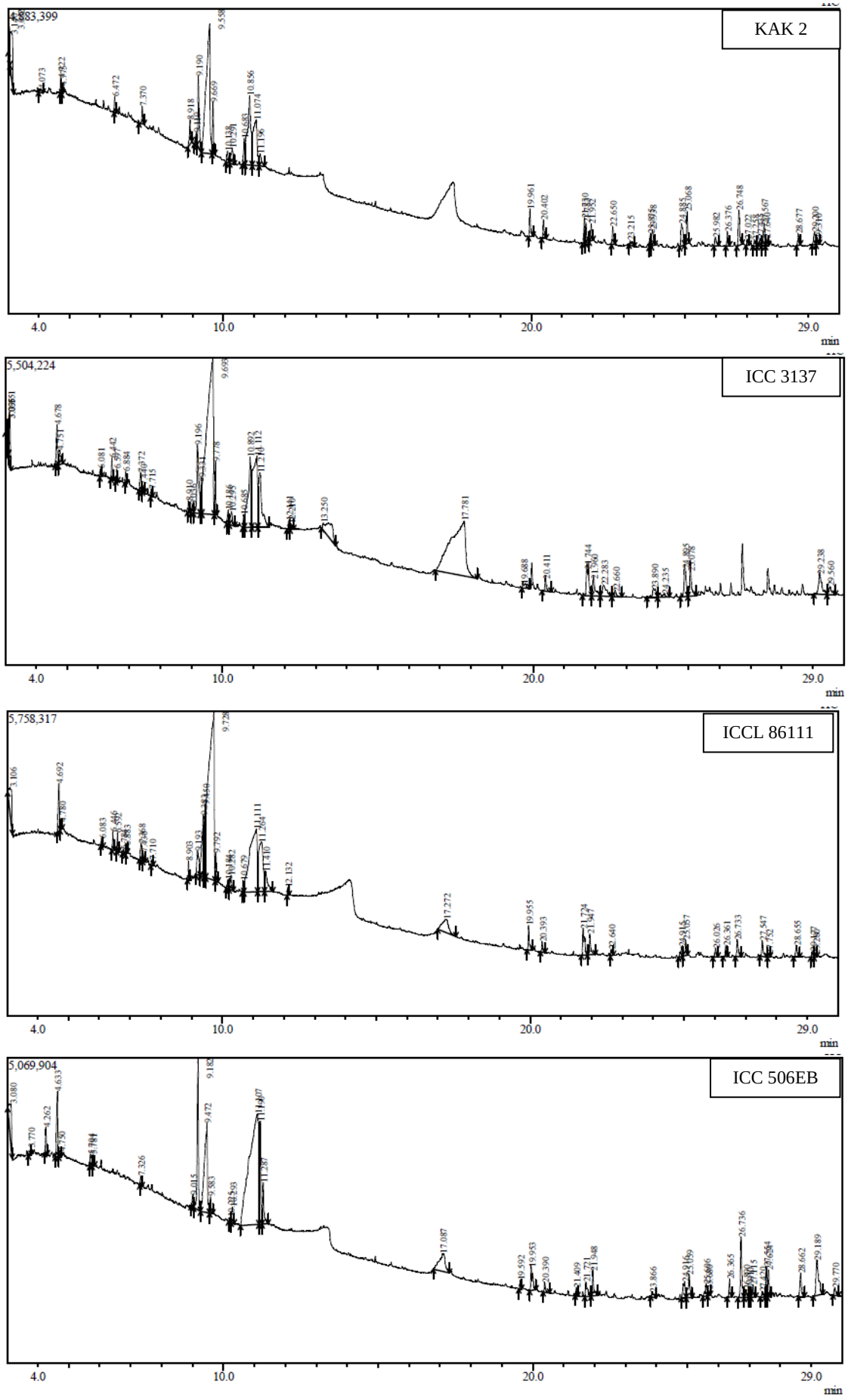


Figure 4.7. (cont.)..

86111. Peak at RT of 9.46 min was observed in resistant checks, ICC 506EB and ICCL 86111 and completely absent in susceptible checks, ICC 3137 and KAK 2.

4.8.3 Association of Hexane Extracts of Leaf Surface Chemicals in Wild Relatives of Chickpea with Oviposition Preference and Detached Leaf Assay for Resistance to *H. armigera*

Among all the compounds, peaks identified at RT of 19.50 and 27.85 min showed significant negative association with damage rating, whereas peak at RT of 16.95, 19.94 and 21.36 showed significant positive association (Table 4.34). Peaks identified at RT of 7.19, 10.30, 11.29, 13.98, 18.23 and 27.85 min exhibited significant negative association with larval survival, while peaks at RT of 11.20, 13.38, 14.46 and 19.94 min exhibited significant positive correlation. The compounds identified at RT of 7.19, 11.01, 13.98, 17.35, 20.38, 27.56 and 27.85 negatively and significantly correlated with larval weight, and compounds at RT of 18.23, 19.94, 26.74 and 29.20 positively associated with larval weight. These observations indicate the presence of some feeding repellents as well as phago-stimulants on the plant surface which affect the feeding behaviour of *H. armigera* on wild relatives of chickpea. The peaks at RT of 13.58, 18.23, 19.32, 23.86 and 27.85 min showed significant negative association with oviposition under mutli-choice condition and peaks at RT of 10.39, 11.01, 19.94 and 21.36 min showed significant positive association. The compound at 27.85 min retention time exhibited significant negative association with oviposition under no-choice condition, while compounds at RT of 10.39, 11.01, 13.98, 19.50 and 21.36 min showed significant positive association.

4.8.4 Association of Methanol Extracts of Leaf Surface Chemicals in Wild Relatives of Chickpea with Oviposition Preference and Detached Leaf Assay for Resistance to *H. armigera*

Of the 107 peaks identified with methanol extracts of leaf surface chemicals, only few peaks performed significant correlation with oviposition non-preference assay and detached leaf assay (Table 4.35). Among all the compounds, 18 compounds at different retention times showed significant positive correlation with damage rating, while 14 peaks showed significant negative correlation. Association of larval survival with methanol extracts exhibited 28 peaks with significant positive correlation, whereas 20 peaks showed significant negative correlation at different retention times. Similarly, larval weight showed negative correlation with 21 peaks and positive

Table 4.34. Association of hexane extracts of leaf surface chemicals in wild relatives of chickpea with oviposition preference and detached leaf assay for resistance to *H. armigera*

Peak at RT (min)	Detached leaf assay			Oviposition preference	
	Damage Rating	Larval Survival (%)	Mean larval weight (mg)	Multichoice condition	Nochoice condition
3.87	-0.03	-0.04	-0.12	-0.41	-0.04
7.19	-0.18	-0.72**	-0.47*	-0.20	-0.06
7.34	-0.03	-0.44*	-0.17	-0.03	0.10
7.96	0.09	0.40	-0.01	-0.01	0.13
8.05	0.12	0.22	-0.10	-0.07	0.12
8.71	0.14	0.20	-0.31	0.05	0.28
10.30	-0.04	-0.45*	-0.24	-0.03	0.09
10.39	0.31	-0.08	-0.22	0.97**	0.83**
10.52	-0.08	-0.33	0.06	-0.23	-0.02
10.64	-0.15	-0.28	-0.40	0.03	0.23
11.01	-0.10	-0.11	-0.61**	0.69**	0.50**
11.20	0.12	0.44*	-0.08	0.03	0.19
11.29	0.22	-0.46*	-0.18	-0.08	0.03
11.55	0.31	0.07	0.03	-0.06	-0.02
11.67	0.32	-0.11	0.07	-0.11	-0.03
11.76	0.10	-0.03	-0.18	-0.31	-0.22
11.88	-0.12	-0.07	-0.23	-0.37	-0.16
12.22	0.15	-0.04	-0.23	-0.09	0.19
13.26	0.07	0.32	-0.13	-0.06	0.16
13.38	-0.34	0.80**	0.12	0.14	0.02
13.58	0.20	-0.06	-0.01	-0.70***	-0.23
13.98	-0.14	-0.81**	-0.58**	0.07	0.86**
14.11	0.09	0.19	-0.15	-0.16	0.06
14.46	0.26	0.44*	0.11	0.04	0.16
14.50	0.10	-0.37	-0.31	-0.16	-0.10
14.58	0.11	0.32	0.00	-0.12	0.07
14.70	0.09	0.00	-0.01	-0.17	0.10
14.82	0.05	0.00	-0.22	-0.13	0.08
15.16	0.04	0.10	-0.03	-0.21	0.02

Table 4.34. (cont.)..

Peak at RT (min)	Detached leaf assay			Oviposition preference	
	Damage Rating	Larval Survival (%)	Mean larval weight (mg)	Multichoice condition	Nochoice condition
15.88	0.07	0.26	0.04	-0.09	0.12
16.95	0.46*	-0.43	0.27	0.11	-0.05
17.08	-0.06	-0.37	-0.17	-0.27	-0.08
17.25	0.07	0.18	-0.17	-0.11	0.03
17.35	-0.41	0.14	-0.49*	-0.38	-0.01
17.74	0.15	0.06	0.03	-0.27	-0.07
18.23	-0.29	-0.65**	0.85**	-0.64**	-0.43
19.32	0.22	-0.06	0.32	-0.63**	-0.31
19.50	-0.80**	0.14	-0.73**	-0.27	0.83**
19.63	0.06	0.16	-0.20	-0.16	0.05
19.94	0.90**	0.86**	0.61**	0.97**	0.29
19.99	0.11	0.01	-0.20	-0.39	-0.24
20.06	0.07	-0.06	-0.09	-0.32	-0.08
20.38	-0.04	0.39	-0.91**	-0.41	0.41
21.36	0.62**	0.28	-0.35	0.75**	0.48*
21.63	0.41	0.26	0.18	-0.24	-0.27
21.77	0.28	0.17	0.09	-0.33	-0.29
22.38	0.20	-0.22	0.14	-0.19	-0.03
23.86	-0.24	0.38	-0.34	-0.83**	0.30
24.91	0.21	-0.26	0.29	-0.12	-0.09
24.96	0.43	0.00	0.08	-0.35	-0.28
26.74	0.21	-0.07	0.56*	0.26	-0.23
27.13	0.35	0.18	-0.18	-0.43	-0.21
27.56	-0.09	-0.31	-0.93**	0.16	0.16
27.85	-0.92**	-0.86**	-0.54*	-0.81**	-0.97**
28.67	-0.29	-0.07	-0.11	-0.13	0.25
29.20	0.07	0.07	0.56*	0.03	-0.29

*,** Correlation coefficients significant at $P \leq 0.05$ and 0.01, respectively

correlation with 34 peaks. Oviposition preferences revealed that, 27 peaks at different retention times showed significant negative correlation with oviposition under multi-choice condition, while 14 peaks showed positive correlation. Under no-choice conditions, 29 peaks exhibited significant negative correlation with oviposition on different genotypes, whereas 13 peaks showed significant positive association. The results indicated that methanol extracts of leaf surface chemicals in wild relatives of chickpea had higher amount of phagostimulants than antifeedants and higher amounts of oviposition repellents than oviposition attractants. Since larvae of *H. armigera* would contact the compounds present on the plant surface before feeding or laying eggs, it is likely that they would play a role in oviposition attraction, food selection and initiation of feeding (Green *et al*., 2002).

Sujana *et al*. (2012) reported that methanol washed pods of wild relatives of pigeonpea were less preferred for feeding by *H. armigera* larvae than the unwashed pods, but the hexane washed pods were preferred more than the unwashed pods which suggested that methanol extracted the phagostimulants from the pod surface, while hexane removed the antifeedants. Green *et al*. (2002) reported that solvent extraction of pod surfaces affected the feeding preference of *H. armigera* in wild and cultivated pigeonpea as the larvae preferred unextracted pods of *C. cajan*, the extracted pods of *C. scarabaeoides* (first and second instar) or the unextracted pods of *C. scarabaeoides* (fourth and fifth instar). Glass fibre disc bioassays showed that the methanol, hexane and water extracts from the pod surface of *C. cajan* stimulated the feeding of fifth instars. Acetone extracts from pods of pigeonpea and its wild relatives, *C. cajan* and *C. platycarpus* had a significant feeding stimulant effect on *H. armigera* larvae whereas extracts from pods of *C. scarabaeoides* had no effect. Water extract of C. *scarabaeoides* pods had a significant antifeedant effect, whereas extracts from *C. cajan* and *C. platycarpus* pods had no effect (Shanower *et al*., 1997). A complete understanding of the nature and number of compounds present in plant surface of wild relatives of chickpea would facilitate the selection of wild relatives with diverse mechanism of resistance to *H. armigera*. Hence, further studies are necessary to isolate the compounds and study their effect on food selection and oviposition preference by *H. armigera*.

Table 4.35. Association of methanol extracts of leaf surface chemicals in wild relatives of chickpea with oviposition preference and detached leaf assay for resistance to *H. armigera*

Peak at RT (min)	Detached leaf assay			Oviposition preference	
	Damage Rating	Larval Survival (%)	Mean larval weight (mg)	Multichoice condition	Nochoice condition
3.06	-0.18	0.23	-0.26	-0.09	-0.36
3.09	-0.59*	-0.10	-0.33	-0.53*	-0.35
3.14	0.42	0.90**	0.37	0.45*	0.19
3.32	0.18	-0.05	-0.22	0.74**	0.11
3.92	-0.90**	0.97**	-0.71**	0.90**	0.75**
4.12	-0.79**	-0.51*	0.47*	-0.60**	-0.31
4.54	-0.68**	-0.60**	-0.48*	0.24	0.96**
4.62	-0.34	-0.48*	0.93**	-0.19	-0.75**
4.67	0.35	0.26	-0.49*	-0.30	-0.67**
4.72	0.32	-0.26	0.56*	-0.34	-0.86**
4.76	0.27	0.09	0.12	0.22	0.08
4.92	0.71**	-0.21	0.88**	-0.15	-0.92**
5.08	0.90**	-0.25	0.83**	-0.36	-0.91**
5.26	0.18	0.87**	-0.84**	0.97**	0.84**
6.09	0.05	0.41	-0.97**	0.35	0.65**
6.25	-0.90**	0.90**	-0.95**	0.40	0.86**
6.45	0.01	-0.29	-0.18	0.30	-0.22
6.60	-0.62**	-0.29	-0.82**	-0.35	-0.01
6.94	0.18	0.79**	0.04	0.16	0.17
7.34	0.30	-0.10	0.28	-0.18	-0.60**
7.43	0.09	-0.67**	0.70**	-0.40	-0.65**
7.70	0.27	0.14	0.12	-0.22	-0.48*
8.70	-0.09	0.17	-0.15	-0.12	-0.37
8.78	-0.01	-0.65**	0.54*	-0.56*	-0.86**
8.90	0.13	0.47*	0.39	0.10	0.22
8.96	0.50*	0.13	0.90**	-0.48*	-0.87**
9.02	0.07	-0.42	0.51*	-0.47*	-0.63**
9.10	-0.06	0.65**	-0.72**	-0.80**	-0.66**
9.19	-0.39	-0.18	0.28	0.25	0.01
9.34	-0.22	0.03	0.34	-0.11	-0.22
9.46	-0.30	-0.78**	0.51*	-0.50*	-0.44*
9.51	-0.06	0.43	-0.75**	-0.51*	-0.36
9.57	0.33	0.89**	0.13	0.58**	0.40
9.68	-0.10	-0.74**	0.46*	0.03	-0.46*
9.79	-0.15	0.64**	0.93**	0.28	-0.07
10.10	-0.98**	-0.41	-0.79**	0.29	0.97**
10.18	0.37	0.79**	0.19	0.68**	0.57**
10.29	0.63**	-0.11	0.34	0.30	-0.39
10.61	-0.68**	0.81**	-0.05	-0.69**	-0.53**
10.67	-0.33	-0.02	-0.50*	-0.51*	0.20

Table 4.35 (cont.).

Peak at RT (min)	Detached leaf assay			Oviposition preference	
	Damage Rating	Larval Survival (%)	Mean larval weight (mg)	Multichoice condition	Nochoice condition
10.75	-0.36	-0.39	0.30	0.14	0.47*
10.83	0.38	0.09	-0.02	0.24	-0.06
11.08	-0.81**	-0.79**	0.61**	-0.44*	-0.77**
11.15	-0.33	-0.59**	-0.71**	-0.43	-0.49*
11.25	0.49*	0.38	-0.96**	0.25	0.28
12.13	0.55*	0.13	0.97**	-0.11	0.22
16.75	-0.35	0.58**	-0.32	-0.37	-0.40
17.01	0.04	-0.94**	0.78**	-0.08	0.17
17.08	-0.57**	-0.56*	0.51*	0.16	-0.27
17.16	0.83**	-0.87**	0.88**	-0.17	-0.59**
17.25	-0.75**	-0.68**	0.19	0.29	0.37
17.38	-0.96**	0.77**	-0.86**	0.54*	0.90**
17.99	0.84**	0.21	0.86**	0.80**	-0.70**
18.24	-0.31	0.12	0.53*	-0.60**	-0.87**
19.66	0.11	0.63**	-0.11	-0.11	0.04
19.95	-0.08	-0.34	0.41	-0.28	-0.41
19.97	0.22	0.09	0.66**	0.09	-0.50*
20.00	-0.47*	0.81**	-0.85**	0.57**	0.76**
20.40	-0.41	-0.02	-0.57**	-0.33	0.48*
21.35	0.85**	0.44*	0.43	0.14	-0.05
21.69	0.18	0.23	0.28	0.37	-0.13
21.77	-0.19	-0.77**	-0.14	-0.37	-0.60**
21.95	0.06	-0.32	0.45*	-0.11	-0.39
22.04	-0.16	0.86**	0.97**	0.77**	0.19
22.20	0.23	0.85**	-0.69**	-0.26	-0.57**
22.32	0.76**	0.57**	0.69**	0.60**	-0.16
22.38	0.14	-0.78**	0.01	0.10	-0.12
22.65	-0.35	-0.69**	-0.90**	-0.20	0.20
22.71	-0.47*	0.10	0.56*	-0.52*	0.36
23.02	0.84**	0.89**	0.02	-0.12	-0.48*
23.22	0.08	0.08	0.14	-0.09	0.91**
23.28	0.19	0.26	-0.29	-0.84**	-0.07
23.39	0.48*	0.96**	-0.64**	-0.54**	-0.01
23.54	-0.35	0.44*	-0.10	-0.46*	-0.18
23.77	0.71**	-0.91**	0.81**	0.45*	0.99**
23.98	0.17	-0.25	0.33	0.01	-0.41
23.93	0.31	-0.22	0.60**	-0.20	-0.40
24.09	-0.18	0.75**	0.31	0.41	0.24
24.18	0.57**	0.07	0.01	-0.35	-0.39
24.26	0.80**	0.71**	0.28	0.24	-0.41
24.47	-0.26	0.72**	0.88**	0.36	0.28
24.62	0.40	0.92**	0.97**	0.85**	-0.52*
24.77	-0.23	0.09	-0.20	-0.68**	-0.20

Table 4.35 (cont.).

Peak at RT (min)	Detached leaf assay			Oviposition preference	
	Damage Rating	Larval Survival (%)	Mean larval weight (mg)	Multichoice condition	Nochoice condition
24.90	0.11	0.27	-0.22	0.01	0.11
24.98	0.06	0.20	0.90**	-0.47*	-0.23
25.07	-0.19	-0.17	-0.25	-0.47*	0.04
25.21	0.18	0.35	-0.20	-0.59**	-0.24
25.42	0.62**	-0.49*	0.39	-0.45*	-0.36
25.60	-0.35	0.23	-0.27	-0.74**	-0.89**
25.67	0.28	0.20	0.59**	0.28	0.34
25.80	0.94**	0.76**	0.15	-0.98**	-0.57**
25.98	0.14	-0.42	0.04	-0.21	-0.31
26.37	0.29	-0.18	0.28	0.08	-0.09
26.74	0.18	-0.23	0.49*	-0.16	-0.37
26.90	-0.09	0.22	-0.04	-0.70**	-0.29
27.02	0.09	-0.38	0.17	-0.15	-0.11
27.25	-0.39	0.05	-0.22	-0.71**	-0.05
27.41	0.31	-0.05	0.40	-0.09	-0.04
27.56	-0.14	-0.37	0.76**	-0.35	-0.29
27.63	-0.72**	-0.87**	0.49*	-0.43	-0.89**
27.76	0.63**	0.49*	-0.41	0.58**	0.39
27.86	0.97**	0.06	0.77**	-0.14	-0.84**
28.67	-0.33	0.22	-0.69**	-0.30	0.15
29.01	0.08	0.10	-0.04	-0.61**	-0.26
29.19	-0.43	0.13	0.06	0.03	-0.09
29.23	-0.20	-0.60**	-0.75**	0.06	0.27
29.77	0.28	0.26	-0.36	-0.55*	-0.03

*,** Correlation coefficients significant at $P \leq 0.05$ and 0.01, respectively

4.9 PROTEOLYTIC ACTIVITIES IN MIDGUT EXTRACT OF *H. armigera* LARVAE FED ON WILD RELATIVES OF CHICKPEA IN DETACHED POD ASSAY

4.9.1 Total Protease Activity

Significant differences were observed among the total protease activities in midgut extracts of *H. armigera* larvae fed on different genotypes of wild relatives of chickpea (Figure 4.8). Highest total protease activity was observed gut of larvae fed on the genotype IG 70022 (0.060 U mg^{-1}) followed by IG 69979 (0.048 U mg^{-1}) and IG 70006 (0.043 U mg^{-1}), while lowest was observed in larval gut fed on PI 599066 (0.012 U mg^{-1}) and was on par with JG 11 (0.013 U mg^{-1}).

4.9.2 Trypsin Activity

Trypsin activity in midgut extract of *H. armigera* larvae fed on different accessions of wild relatives of chickpea was indicated in Figure 4.9. Trypsin activity was found to be maximum in gut of larvae fed on genotypes, IG 69979 (0.331 U mg^{-1}), which was on par with IG 70022 (0.327 U mg^{-1}) and IG 70006 (0.321 U mg^{-1}). However, the enzyme activity was significantly reduced in larvae fed on the genotypes PI 599066 and IG 70018 (0.080 and 0.092 U mg^{-1}, respectively).

4.9.3 Chymotrypsin Activity

Chymotrypsin activity in midgut extracts of *H. armigera* larvae fed on different accessions of wild relatives of chickpea are shown in Figure 4.10. Chymotrypsin activity was higher in the gut extract of larvae fed on IG 70022 (0.642 U mg^{-1}) and IG 69979 (0.598 U mg^{-1}), while lowest activity was observed in the gut of larvae fed on genotypes, PI 599066 (0.089 U mg^{-1}) and JG 11(0.121 U mg^{-1}).

4.9.4 Aminopeptidase Activity

Aminopeptidase activity was more in the gut of larvae fed on the genotype IG 70022 (0.042 U mg^{-1}) and was similar to that of IG 599076 (0.041 U mg^{-1}) and IG 69979 (0.037 U mg^{-1}) (Figure 4.11). Aminopeptidase activity was lowest in larvae fed on susceptible checks, KAK 2 and ICC 3137 (0.016 U mg^{-1}), which was on par with commercial cultivar, JG 11 (0.018 U mg^{-1}) and in resistant checks, ICCL 86111 and ICC 506EB (0.019 U mg^{-1}).

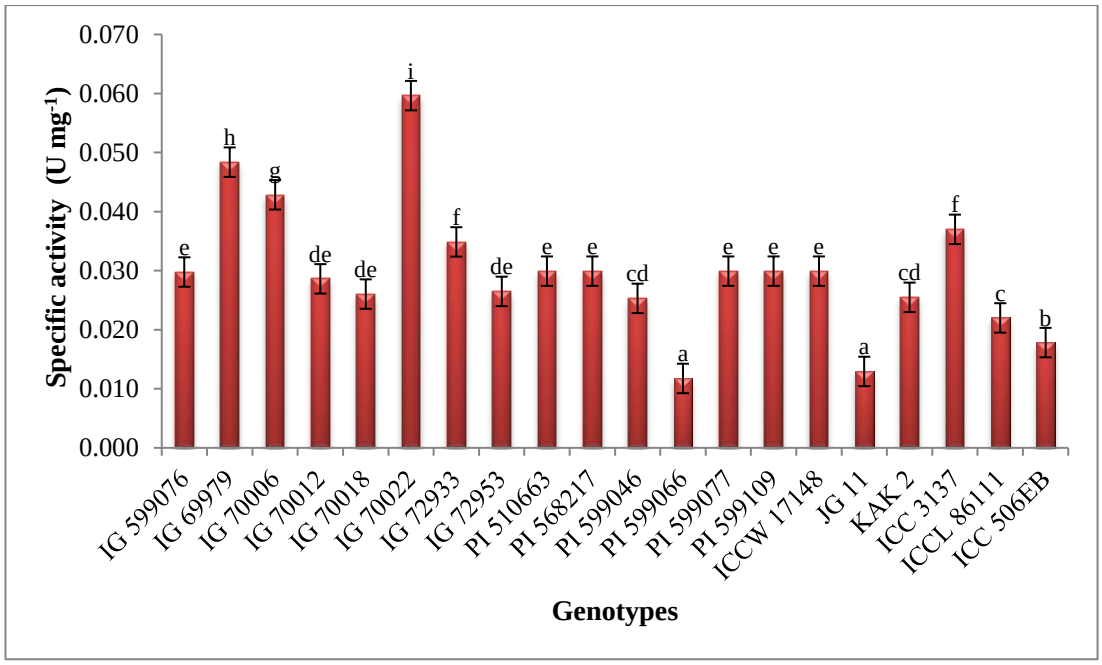


Figure 4.8. Total protease activity (Mean ± SE) in the mid gut extracts of *H. armigera* larvae fed on different genotypes of wild relatives of chickpea.

Means followed by same alphabet did not differ significantly at LSD, P≤0.01

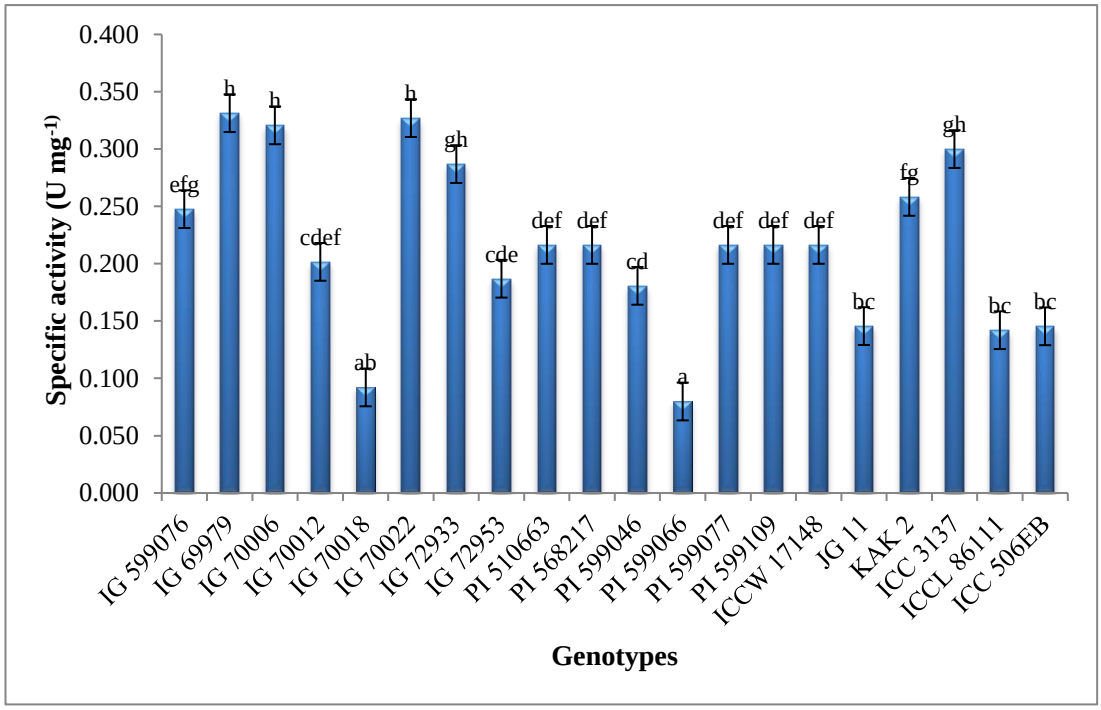


Figure 4.9. Trypsin activity (Mean ± SE) in the mid gut extracts of *H. armigera* larvae fed on different genotypes of wild relatives of chickpea.

Means followed by same alphabet did not differ significantly at LSD, P≤0.01

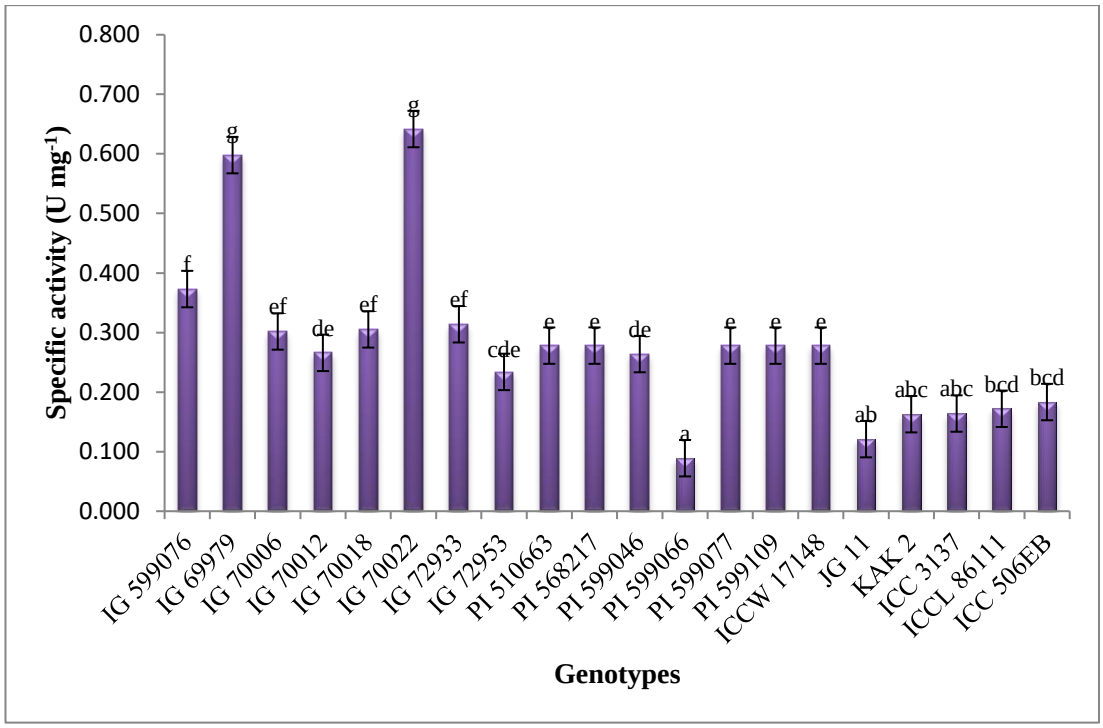


Figure 4.10. Chymotrypsin activity (Mean ± SE) in the mid gut extracts of *H. armigera* larvae fed on different genotypes of wild relatives of chickpea.

Means followed by same alphabet did not differ significantly at LSD, P≤0.01

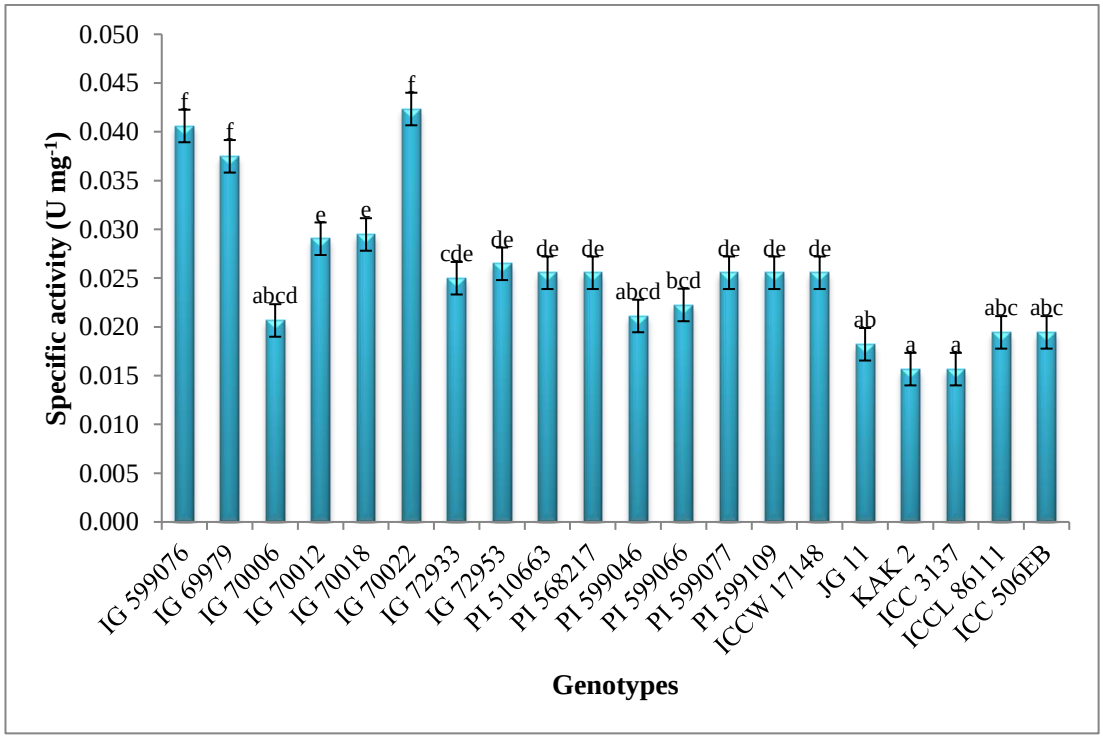


Figure 4.11. Aminopeptidase activity (Mean ± SE) in the mid gut extracts of *H. armigera* larvae fed on different genotypes of wild relatives of chickpea.

Means followed by same alphabet did not differ significantly at LSD, P≤0.01

Activities of digestive enzymes in insect depend on either the quality of food sources or consumed chemical compounds and enzyme inhibitors (Slansky, 1982 and Mendiola-Olayab *et al.*, 2000). Any interference in the activity of digestive enzymes by enzyme-inhibitors of host plant can result in poor nutrient utilization and developmental retardation (Gatehouse and Gatehouse, 1999 and Jongsma and Bolter, 1997). Larvae fed on wild relatives of chickpea showed higher total protease activity though they have recorded lesser weight gain percentage, it could be because of hyperproduction of proteases to overcome the effect of ingested PI from host plant (Broadway, 1996).

Lowest levels of trypsin and chymotrypsin activities were observed in the larval gut of *H. armigera* fed on PI 599066 (*C. bijugum*), this could be explained that, inhibitors were active in the gut and inhibited the proteinase activity and thus the larvae suffered due to dietary PIs and showed stunted growth (Harsulkar *et al*., 1999). When the larvae fed on genotype IG 69979 (*C. cuneatum*) and IG 70022 (*C. bijugum*), the gut extract showed increased activity of trypsin and chymotrypsin though weight gain by larvae was very low when fed on these genotypes. This may be due to hyper production of trypsin and chymotrypsin to overcome the effects of protease inhibitor factors present in the genotypes. Varietal differences in trypsin and chymotrypsin inhibitors in chickpea have been reported by Sastry and Murray (1987). Patankar *et al*. (1999) reported that wild relatives of chickpea exhibited diversity of TI isoforms with respect to both number and activity as compared to cultivated chickpea. Larvae fed on genotypes, IG 70018 and PI 599046 (*C. bijugum*) showed high chymotrypsin activity and low trypsin activity.

Increased chymotrypsin activity was due to the compensation of inhibitory effects of trypsin inhibitors of these genotypes. Increased activity of chmotrypsin and elastase like enzymes to compensate the inhibitory effect of trypsin has been reported when larvae reared on corn (Baghery *et al*., 2014), soybean (Naseri *et al*., 2010), and giant taro trypsin inhibitor (Wu *et al*., 1997). Larvae fed on wild relatives of chickpea recorded high activity of aminopeptidase compared to cultivated chickpea, which might be due to high PI activity in wild relatives. Results are consistent with our previous findings where in *H. armigera* fed on PI showed higher aminopeptidase activity (Lomate and Hivrale, 2011 and Hivrale *et al*., 2013).

Correlation studies (Table 4.36) showed that, there was negative association of weight gain percentage of larvae with digestive enzymes whereas the association is significant with chymotrypsin and aminopeptidase activity and non-significant with trypsin and total protease activity. As per these relationships between midgut proteases and diet complexity of the host plant, it looks that there is an insect mechanism to exactly discover the food contents and regulate the levels of these essential digestive enzymes (Kotkar *et al*., 2009). Insects can adapt to proteinase inhibitors by over expressing proteinase inhibitor-insensitive proteinases, or by regulating the level of existing serine proteinases, or by degrading the proteinase inhibitor (Broadway, 1996., Giri *et al*., 1998 and Dunse *et al*., 2010). Hyper secretion of additional proteinases in response to the inhibitors requires the utilization of valuable amino acid pools that could starve the insects (Broadway, 1995). Therefore, it is worth to study the exact biochemical mechanisms underlying this phenomenon to develop PI based insect control strategy.

Table 4.36. Association of protease activity in larval gut and pod wall thickness of wild relatives of chickpea with resistance to third instar larvae of *H. armigera* using detached pod assay

	Damage rating	**Weight gained by larvae (%)**	**Pod damage (%)**
Total protease activity (U mg^{-1})	-0.35	-0.39	-0.29
Trypsin activity (U mg^{-1})	0.02	-0.08	-0.17
Chymotrypsin activity (U mg^{-1})	-0.49*	-0.56**	-0.35
Aminopeptidase activity (U mg^{-1})	-0.57**	-0.70**	-0.33
Pod wall thickness (mm)	-0.61**	-0.40	-0.53*

*,** Correlation co-efficient significant at $P \leq 0.05$ and 0.01, respectively

The correlation studies of pod wall thickness with detached pod assay showed that, there was significant negative association of pod wall thickness with damage rating and pod damage percentage (Table 4.36). The genotypes having thick pods were less damaged with larval feeding. However, percentage of weight gained by larvae was negatively associated with pod wall thickness but the relation was not significant. The present results are in conformity with the earlier findings which indicated the negative association of pod damage by *H. armigera* with pod wall thickness in chickpea (Kanchana *et al*., 2005., Girija *et al*., 2008 and Hossain *et al*., 2008b) and pigeonpea (Shanower *et al*., 1997).

4.10 PROTEASE INHIBITORS (PIS) IN WILD RELATIVES OF CHICKPEA AGAINST *H. armigera*

4.10.1 Electrophoretic Visualization of *H. armigera* Gut Isoforms

Electrophoretic visualization of *H. armigera* gut isoforms revealed that a total of 10 isoforms were observed ranging with a molecular weight of 3.0 to 43.0 kDa (Figure 4.12). Six to ten isoforms of gut proteases were earlier reported in *H. armigera* (Harsulkar *et al*., 1998 and Udamale *et al*., 2013). Proteolytic activity of insect gut comprises of many isoforms of proteinases having diverse properties and specificities (Johnston *et al*., 1991). The presence of isoproteinases of different specificities in the midgut has a great significance for the survival and adaptation of phytophagous insects on several host plants. Presence of multi-isoforms of HGP thus supported the polyphagous nature of *H. armigera*.

4.10.2 Dot-Blot Assay

In vitro screening of protease inhibitors (PI) using dot-blot assay at three concentrations of trypsin and the inhibitor of plant sample extract (3:1, 1:1 and 1:3) revealed that, at 1:3 concentration, all plant samples showed complete inhibition of trypsin resulted in non hydrolysis of gelatine on the X-ray film (Table 4.37). Complete inhibition of trypsin was observed even at 1:1 concentration of trypsin and PI extract, except in PI 510663, PI 599109 (*C. pinnatifidum*), PI 568217 (*C. judaicum*) and susceptible checks (KAK 2 and ICC 3137). Accessions belonging to *C. bijugum* (IG 70012, IG 70018, IG 70022, PI 599046 and PI 599066), *C. chrossanicum* (IG 599076), *C. cuneatum* (IG 69979), *C. reticulatum* (IG 72953), *C. judaicum* (PI 599077) had shown complete inhibition of trypsin at low concentrations of PI (3:1), whereas genotypes IG 70006, IG 72953, PI 568217, ICCW 17148, KAK 2, ICCL 86111 and ICC 506EB had shown partial inhibition of trypsin resulted in partial hydrolysis of gelatine on X-ray film and other genotypes PI 510663, PI 599109 (*C. pinnatifidum*), JG11 and ICC 3137 has not shown inhibition of trypsin resulted in complete hydrolysis of gelatine on X-ray film.

4.10.3 Zymogram Analysis of Trypsin Inhibitor (TI) Isoforms

Electrophoretic visualization of trypsin inhibitor isoforms showed a significant variability in terms of number and band pattern in wild relatives of chickpea in a range of 3.0 to 43.0 kDa (Figure 4.13). The genotypes IG 70018,

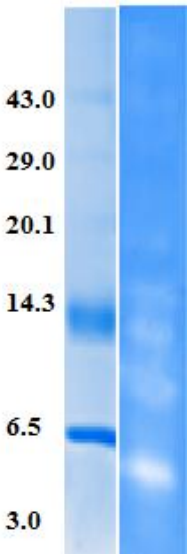


Figure 4.12. Zymogram analysis for the detection of *H. armigera* gut proteinases.
Lane 1 - molecular weight markers (3.0 to 43.0 kDa) and lane 2- *H. armigera* gut proteinases

Table 4.37. *In-vitro* screening of trypsin inhibitory (TI) activity in wild relatives of chickpea using dot blot assay

Species	Genotype	Concentration of trypsin : seed extract (Total volume of 20 µl)		
		3:1	1:1	1:3
C. chrossanicum	IG 599076	T	T	T
C. cuneatum	IG 69979	T	T	T
C. bijugum	IG 70006	P	T	T
C. bijugum	IG 70012	T	T	T
C. bijugum	IG 70018	T	T	T
C. bijugum	IG 70022	T	T	T
C. reticulatum	IG 72933	T	T	T
C. reticulatum	IG 72953	P	T	T
C. pinnatifidum	PI 510663	N	P	T
C. judaicum	PI 568217	P	P	T
C. bijugum	PI 599046	T	T	T
C. bijugum	PI 599066	T	T	T
C. judaicum	PI 599077	T	T	T
C. pinnatifidum	PI 599109	N	P	T
C. microphyllum	ICCW 17148	P	T	T
C. arietinum	JG 11 (C)	N	T	T
C. arietinum	KAK 2 (S)	P	P	T
C. arietinum	ICC 3137(S)	N	P	T
C. arietinum	ICCL 86111 (R)	P	T	T
C. arietinum	ICC 506EB (R)	P	T	T

C- Commercial cultivar, S- Susceptible check, R- Resistant check
N – No TI activity as evidenced by complete hydrolysis of gelatine by trypsin on x-ray film
P – Partial or moderate TI activity as evidenced by partial hydrolysis of gelatine by trypsin on x-ray film
T –Total or Higher TI activity as evidenced by no hydrolysis of gelatine by trypsin on x-ray film

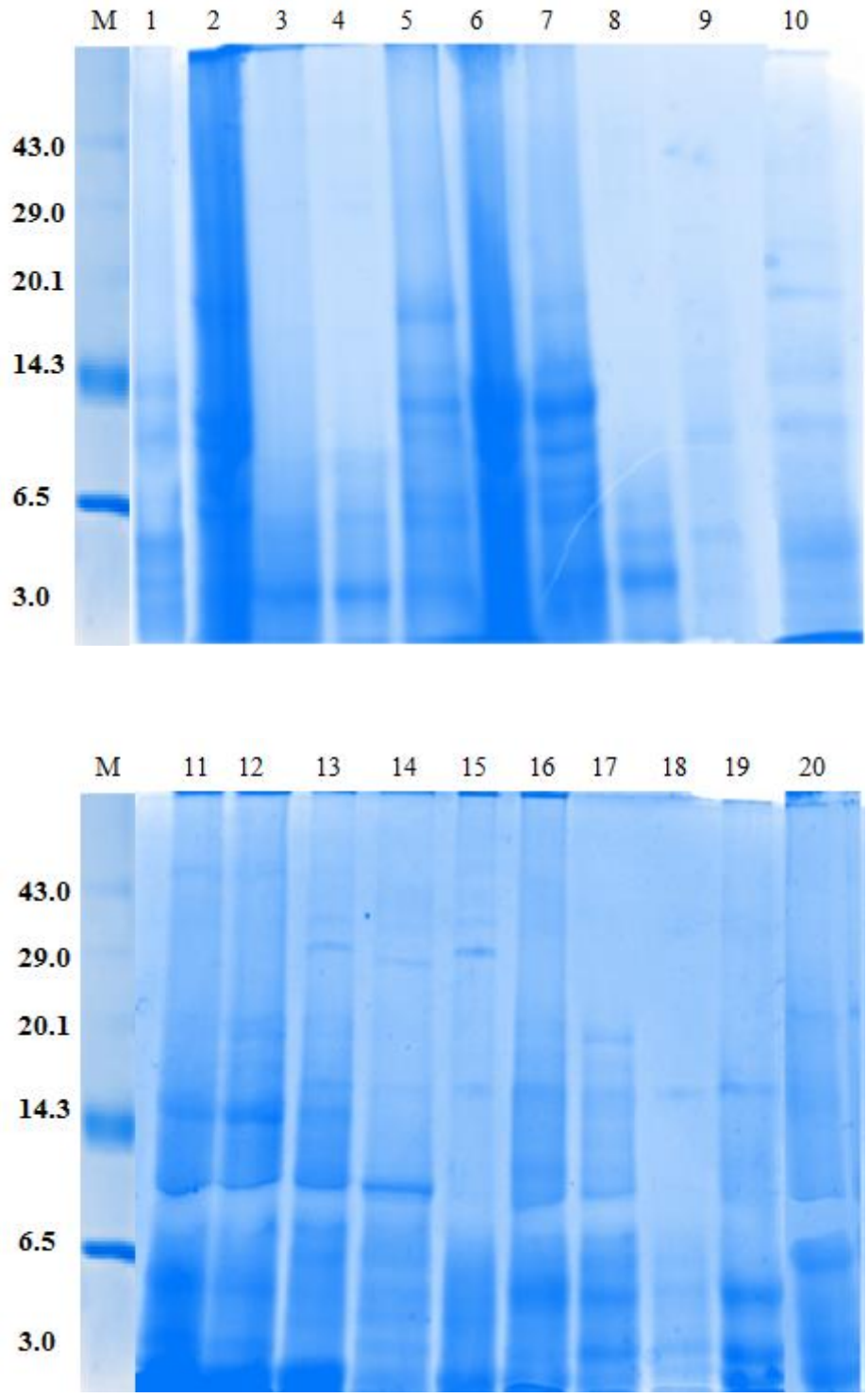


Figure 4.13. Zymogram analysis for the detection of trypsin inhibitor isoforms in wild relatives of chickpea genotypes.

Lane 1 - IG 599076, lane 2 - IG 69979, lane 3 -IG 70006, lane 4 - IG 70012, lane 5 - IG 70018, lane 6 - IG 70022,lane 7 - IG 72933, lane 8 - IG 72953, lane 9 - PI 510663, lane 10 - PI 568217, lane 11 - PI 599046, lane 12 - PI 599066, lane 13 - PI 599077, lane 14 - PI 599109, lane 15 - ICCW17148, lane 16 - JG 11, lane 17 - KAK 2, lane 18 - ICC 3137, lane 19 - ICCL 86111, lane 20 - ICC 506EB; M- standard molecular weight marker (3.0 to 43.0 kDa).

PI 599066 (*C. bijugum*) and IG 72933 (*C. arietinum*) showed a maximum of seven isoforms, whereas PI 599046 (*C. bijugum*) and PI 599077 (*C. judaicum*) showed six isoforms and IG 70022 (*C. bijugum*), PI 568217 (*C. judaicum*), IG 599076 (*C. chrossanicum*), and IG 69979 (*C. cuneatum*) showed five isoforms. Minimum of two isoforms were observed in PI 510663 (*C. pinnatifidum*). Remaining all genotypes exhibited four isoforms except in PI 599109 (*C. pinnatifidum*), ICCW 17148 (*C. microphyllum*) and susceptible check ICC 3137 (*C. arietinum*) showed three isoforms. The differential appearance of TI may be attributed to temporal expression of different genes or may be due to posttranslational modification of inhibitors or their pro-proteins (Giri *et al*., 1998).

Patankar *et al*. (1999) also observed the diversity in TI isoforms with respect to both number and activity as compared to cultivated chickpea and reported that *C. bijugum* showed two major bands and one minor activity band, while *C. reticulatum* showed two bands, *C. judaicum* revealed two TI activity bands and *C. cuneatum* showed a single TI band. However, no TI band was observed in *C. pinnatifidum*. The variation observed in wild *Cicer* species is considered significant, as the TIs are known to serve as defense proteins against herbivores (Ryan, 1990). Patankar *et al*. (2001) also observed significant variation in the TI isoforms from wild *Cicer* species. However, they have observed great conservation of TI isoforms in the mature seeds of the chickpea cultivars. A similar observation existed in pigeonpea where TIs and chymotrypsin inhibitors were conserved in matured seeds of the cultivated pigeonpea, whereas high levels of diversity existed in uncultivated species of *Cajanus* (Kollipara *et al*., 1994 and Pichare and Kachole, 1994). Progressive increase in PI activity throughout seed development had also been observed (Giri *et al*., 1998) and reported that three TI bands were detected at 24 days after flowering (DAF), while seven TI bands were detected at 36 DAF and it was further observed that insect feeding also increased production of PI activity where seven different TI bands were present in seeds at 36 DAF, the time of maximum *H. armigera* attack.

4.10.4 Inhibitory Potential of *H. armigera* Gut (HG) Proteases in Wild Relatives of Chickpea

Significant variations were observed in terms of inhibitory potential of *H. armigera* gut (HG) proteases in different wild relatives of chickpea under *in vitro* condition (Figure 4.14). Highest HG total protease inhibitory activity (interms of Units g^{-1} sample)

was observed in IG 70018 (17.65), IG 72933 (14.97), IG 70006 (14.43) and IG 70012 (14.15) compared to resistant check, ICC 506EB (9.88), whereas lowest values were observed in *C. pinnatifidum*, PI 599109 (1.90) and PI 510663 (3.51), *C. judaicum*, PI 568217 (4.88) and PI 599077 (7.24) and *C. microphyllum*, ICCW 17148 (7.24). Inhibitory activity of HG trypsin (Units g^{-1}) was observed significantly highest in *C. reticulatum*, IG 72933 (76.67) and *C. bijugum*, IG 70012 (64.14), IG 70018 (63.46) and IG 70006 (62.78), and significantly low in *C. pinnatifidum*, PI 599109 (4.73) and PI 510663 (11.24), *C. microphyllum*, ICCW 17148 (13.94) and PI 568217 (26.70) compared to resistant check, ICC 506EB (32.60).

HG chymotrypsin inhibitor activity (Units g^{-1}) in different genotypes of wild relatives of chickpea showed significant variations. Highest inhibitory activity was observed in *C. bijugum*, IG 70018 (35.29) whereas lowest was observed in *C. chrossanicum* (IG 599076) with 9.64 Units g^{-1}. As an overall view, the genotypes, *C. bijugum*, *C. reticulatum* and *C. cuneatum* showed higher inhibitory activity of HG proteases, while *C. pinnatifidum* and *C. judaicum* had low inhibitory activity compared to cultivated chickpea. The present results are similar to the findings of (Patankar *et al*., 1999) who observed that highest inhibition of HGP was effected by *C. bijugum* PIs, followed by *C. echinospermum* and *C. arietinum*, while the lowest HGP inhibition was detected in *C. pinnatifidum* and *C. cuneatum*. The amount of PI activity increased several fold when seeds were injured by *H. armigera* feeding. Insect damage resulted in a six fold increase in *H. armigera* gut PI activity and a two fold increase in TI activity (Giri *et al*., 1998).

However, earlier reports observed that PI insensitive and inhibitor digestive proteases produced by *H. armigera* and none of the TIs from chickpea and its wild relatives inhibited gut protease activity totally in *H. armigera* (Giri *et al*., 1998). Laskowski *et al*. (1988) have proposed that structural compatibility between the plant PIs and the insect proteinases determines the level of inhibitory activity against specific proteinases. Structural variation occurring in gut proteinases followed by selection against host plant PIs may modify insect proteinases that, although of the same class are insensitive to host plant PIs. An alteration in an insect proteinase isozyme may result in less inhibitor binding, leading to successful predation. In order to survive, plants also must evolve their inhibitor proteins to effectively inhibit insect proteinases. Both pests and plants have therefore been evolving new forms of enzymes and inhibitors to counteract each other's defense mechanisms (Bown *et al*., 1997).

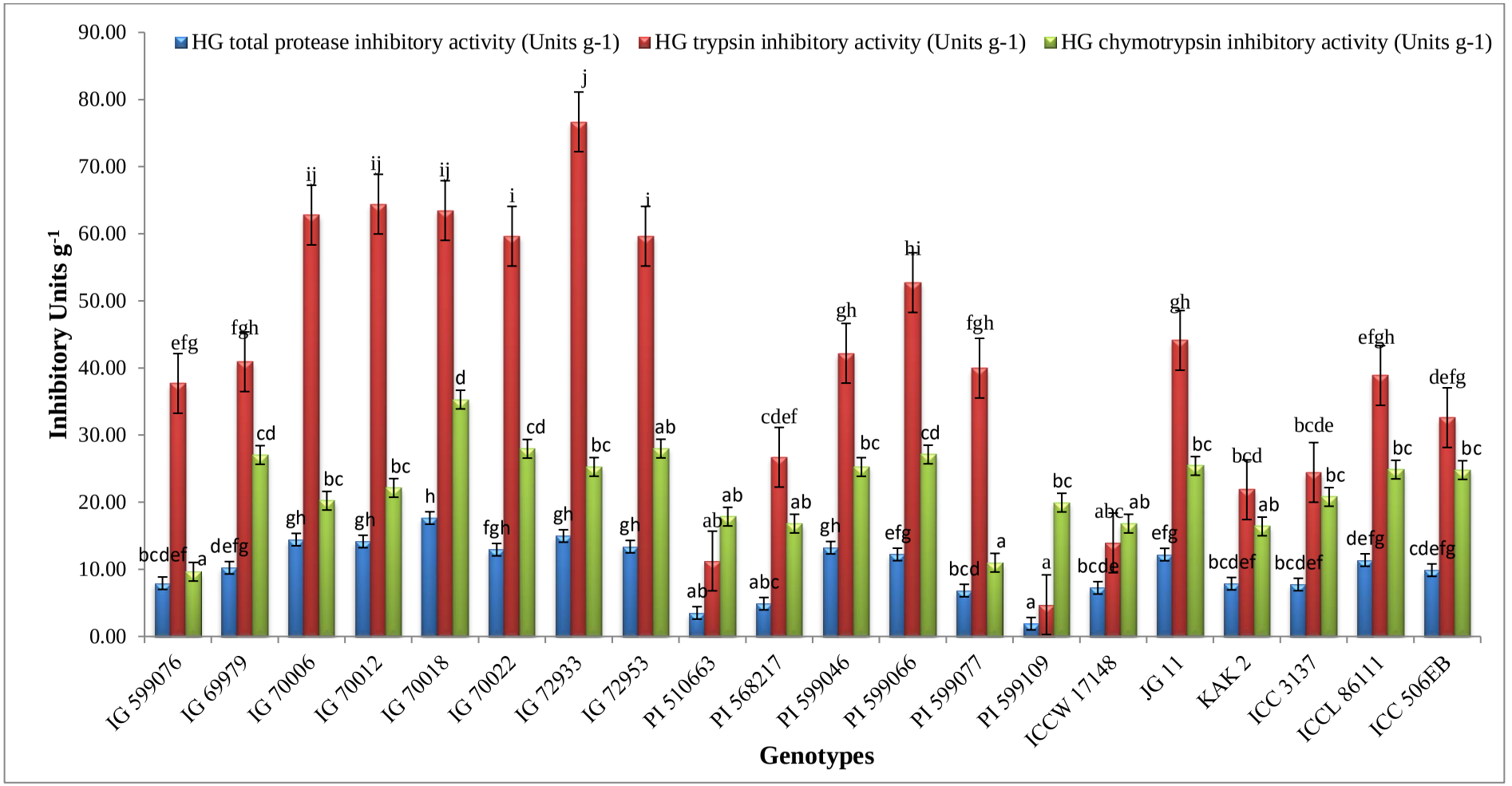


Figure 4.14. Inhibitory activities of *H. armigera* gut (HG) proteases (Mean ± SE) in wild relatives of chickpea under *in-vitro* conditions.
Means followed by the same alphabet did not differ significantly at LSD, P<0.05

4.11 LECTIN ACTIVITY IN WILD RELATIVES OF CHICKPEA

4.11.1 Hemagglutination of Lectins

Hemagglutination test involves agglutination of red blood cells or erythrocytes with lectin (Lin *et al*., 1981 and Yeasmin *et al*., 2001). The agglutination intensity increased with increase in the concentration of lectin in the plant sample (Table 4.38). However, in some genotypes the agglutination was not possible which indicates the absence or very little concentration of lectin. In some genotypes, ICCW 17148 (*C. microphyllum*), PI 599077, PI 568217 (*C. judaicum*), PI 599046, PI 599066, IG 70006 and IG 70012 (*C. bijugum*) the agglutination was more even at low concentration of plant sample. Among cultivated chickpea, agglutination activity was observed in KAK 2, but the intensity was less when compared to wild relatives of chickpea, whereas in other genotypes the agglutination was not visible in JG 11 and ICC 506EB and very little intensity was observed in ICCL 86111 and ICC 3137 even at higher concentrations. The amount of lectins was predicted by the visual grading of agglutination. Lectins are carbohydrate binding proteins known for their ability to agglutinate erythrocytes. They are abundant in the seeds of legumes, constitute up to 10% of the soluble protein in the seed extracts (Van Damme *et al*., 1998). Pedroche *et al*. (2005) have reported pa2 albumin which induced hemagglutination *in vitro*. Similarly, high levels of potent lectins were detected through hemagglutination in reproductive organs, leaves, shoots and roots of mulberry species (Zahoor *et al*., 2009). Castillo *et al*. (2007) achieved purification of the lectin from *Phaseolus acutifolius* var. escumite by agglutination of blood group O erythrocytes. Khan *et al*. (2011) revealed that cultivars of chickpea, KK-1 and Hassan-2K showed more phyto-agglutination of human erythrocytes, which shows the presence of potent lectins.

4.11.2 Zymogram Analysis of Lectins

Schiff's base staining for the detection of lectins in the wild relatives of chickpea indicated that only one type of isoform with a molecular weight of 29 kDa was observed in some of the wild relatives of chickpea genotypes (Figure 4.15). However, the intensity of band varied among the genotypes. The genotypes ICCW 17148 (*C. microphyllum*), PI 599077, PI 568217 (*C. judaicum*), PI 599046, PI 599066, IG 70006 and IG 70012 (*C. bijugum*) exhibited more intense lectin band.

Table 4.38. Agglutination of erythrocytes for the detection of lectins in seed extracts of wild relatives of chickpea.

Species	Genotype	Seed extract used against 2% erythrocyte suspension				
		10µl	20µl	30µl	40µl	50µl
C. chrossanicum	IG 599076	-	+	+	++	+++
C. cuneatum	IG 69979	-	-	-	-	-
C. bijugum	IG 70006	+	+	++	+++	+++++
C. bijugum	IG 70012	+	+	++	+++	+++++
C. bijugum	IG 70018	+	+	++	++	+++
C. bijugum	IG 70022	+	+	++	++	+++
C. reticulatum	IG 72933	-	-	-	-	+
C. reticulatum	IG 72953	-	-	+	++	+++
C. pinnatifidum	PI 510663	+	+	++	++	+++
C. judaicum	PI 568217	+	+	++	++	+++
C. bijugum	PI 599046	+	++	+++	++++	+++++
C. bijugum	PI 599066	+	++	+++	++++	+++++
C. judaicum	PI 599077	+	++	+++	++++	+++++
C. pinnatifidum	PI 599109	-	+	+	++	+++
C. microphyllum	ICCW 17148	+	++	+++	++++	+++++
C. arietinum	JG 11 (C)	-	-	-	-	-
C. arietinum	KAK 2 (S)	-	-	+	++	+++
C. arietinum	ICC 3137(S)	-	-	-	-	+
C. arietinum	ICCL 86111 (R)	-	-	-	-	+
C. arietinum	ICC 506EB (R)	-	-	-	-	-

- = Nil, + = satisfactory, ++ = fair, +++ = good, ++++ = very good, and +++++ = excellent
C- Commercial cultivar, S- Susceptible check, R- Resistant check

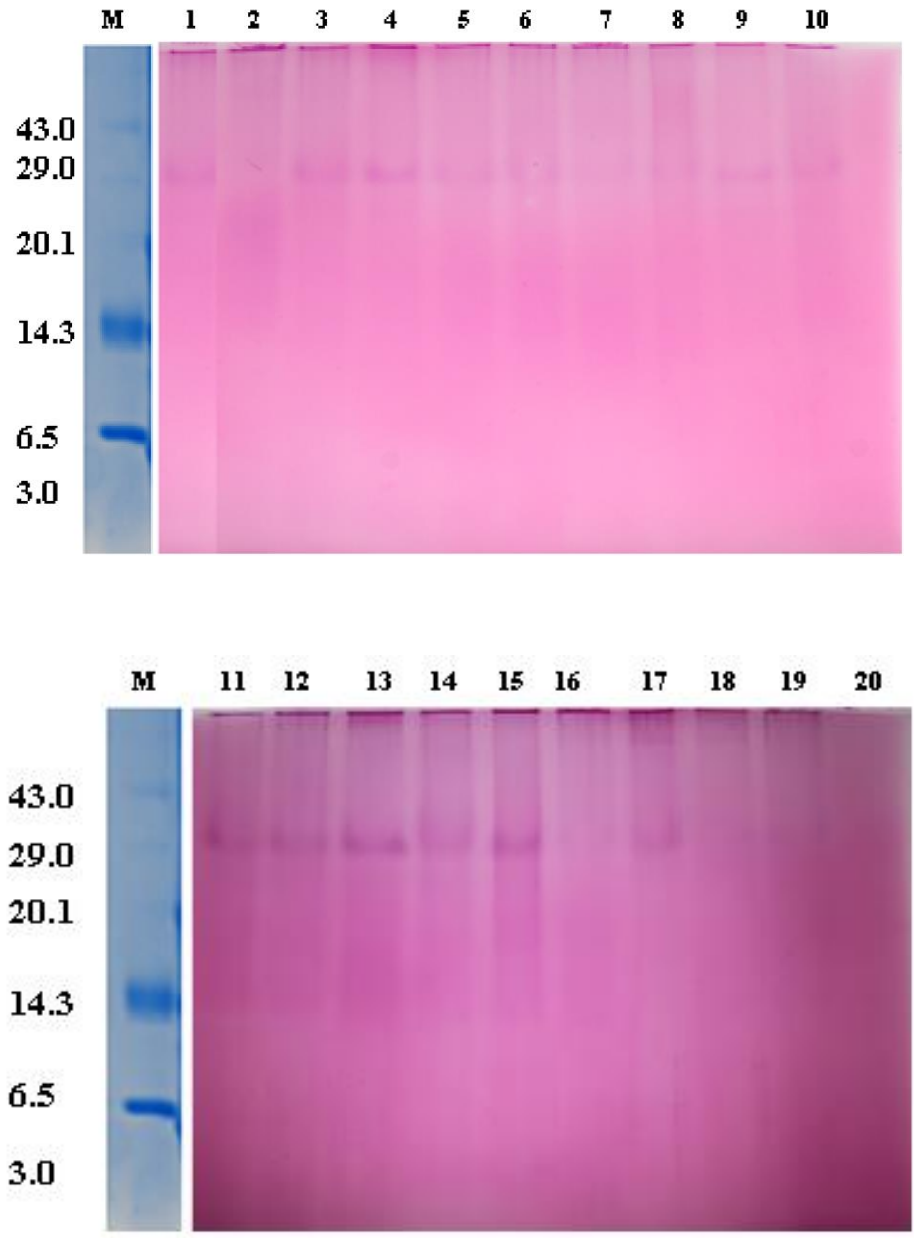


Figure 4.15. Zymogram analysis for the detection of lectin isoforms in the wild relatives of chickpea genotypes.

Lane 1 - IG 599076, lane 2 - IG 69979, lane 3 - IG 70006, lane 4 - IG 70012, lane 5- IG 70018, lane 6 - IG 70022, lane 7 - IG 72933, lane 8 - IG 72953, lane 9 - PI 510663, lane 10 - PI 568217, lane 11 - PI 599046, lane 12 - PI 599066, lane 13 - PI 599077, lane 14 - PI 599109, lane 15 - ICCW17148, lane 16 - JG 11, lane 17 - KAK 2, lane 18 - ICC 3137, lane 19 - ICCL 86111, lane 20 - ICC 506EB; M - molecular weight markers (3.0 to 43.0 kDa.

Lectins were not observed in some wild relatives of chickpea genotypes i.e., IG 69979 (*C. cuneatum*) and IG 72933 (*C. reticulatum*) as well as in cultivated chickpea genotypes except in KAK 2 which showed a less intensity band of lectin.

Qureshi *et al*. (2006) purified lectin in *C. arietinum* with an apparent mass of 30 kDa on SDS-PAGE and native PAGE. Kolberg *et al*. (1983) also isolated a potent lectin from seed extracts of *C. arietinum*, which had a molecular mass of about 44 kDa, as determined by ultracentrifugation and gel filtration, whereas SDS-PAGE showed one band corresponding to molecular mass of 26 kDa. Bashir *et al*. (2010) reported that soybean lectin did not show specificity towards any blood group and the purified soybean lectin showed a single band but on contrary, they observed molecular weight of 130 kDa on SDS-PAGE, whereas in native-PAGE it showed a band of 110 kDa. Soybean leaf glycoprotein has molecular mass of 120 kDa with subunits having molecular masses of 28 and 33 kDa (Spilatro and Anderson, 1989). These variations could be due to differences in plant species. The purified lectin from red kidney bean was observed as a single band with a molecular mass of about 30 kDa in SDS-PAGE electrophoresis (Hou *et al*., 2010). Induced production of lectins was also observed with synthesis of a19 kDa lectin as a result of jasmonic acid application which was absent in untreated leaves of tobacco (Lannoo *et al*., 2006).

Lectins bind to the glycan receptors present on the surface lining of the insect gut (Pusztai and Bardocz, 1996) and interfere with the formation and integrity of the peritrophic membrane of the midgut (Harper *et al*., 1998) resulting in harmful effects on insect. Lectins also interfere with the digestive enzymes in the midgut of insects and inhibited carbohydrases and proteinases (War *et al*., 2013). Reduced larval survival and weight, pupal weight, pupal period, pupation and adult emergence were also observed in *H. armigera* larvae fed on artificial diet impregnated with different lectins from chickpea, garlic, fieldbean, pigeonpea, jackfruit and wheat germ agglutinin (Arora *et al*., 2005., Gupta *et al*., 2005 and Shukla *et al*., 2005). These highly potent lectins could be isolated and characterized according to their molecular weight, specificity to carbohydrates binding moieties and toxicity to *H. armigera* can be used as resistant sources against pest.

4.12 GENETIC DIVERSITY OF WILD RELATIVES OF CHICKPEA BASED ON SSR MARKERS

The twenty six SSR markers used for assessing genetic diversity of wild relatives of chickpea detected a total of 186 alleles with an average of 7.15 alleles per marker, where the number of alleles ranged from 2 (CaM0244 and CaM2064) to 12 (CaM0958 and ICCM0249) (Table 4.39). The polymorphic information content (PIC) values for these markers varied from 0.21 (CaM2064) to 0.89 (CaM0958, ICCM0249 and TAA58) with an average of 0.70. Most of the markers had high PIC (>5) considered to be more informative, whereas markers CaM2064 (0.21), CaM0244 (0.33), CaM1451 (0.45) and CaM0799 (0.49) showed low polymorphism. The mean gene diversity was 0.74, which varied from 0.24 (CaM2064) to 0.90 (CaM0958, ICCM0249 and TAA58). The observed heterozygosity varied from 0.00 (TA142, CaM0244, GAA47, CaM2064, ICCM0130a and TR42) to 0.62 (CaM1515) with an average of 0.20. The markers CaM0958, ICCM0249 and TAA58 were most informative with most alleles, high gene diversity and the highest PIC value.

The 26 SSR markers placed the genotypes into three groups suggesting that there was considerable genetic diversity among the genotypes used in this study. Neighbour-joining tree based on simple matching dissimilarity matrix between 19 accessions of wild relatives of chickpea were broadly clustered into three groups (Figure 4.16). Cluster I contained total of seven accessions, which was dominated by all five genotypes of cultivated chickpea including resistant and susceptible checks for *H. armigera*. In this cluster, two wild relatives of chickpea were also grouped together with the cultivated chickpea, of which IG 72933 (*C. reticulatum*) was associated close to ICCL 86111 and ICCW 17148 (*C. microphyllum*) was associated close to JG 11. Cluster II consisted of 3 genotypes of wild relatives of chickpea, PI 510663, PI 599109 (*C. pinnatifidum*) and IG 72953 (*C. reticulatum*). Cluster III represented by nine genotypes and was dominated with six genotypes of *C. bijugum* (IG 70018, IG 70012, PI 599066, IG 70006, IG 70022 and IG 599046) along with two genotypes of *C. judaicum* (PI 568217 and PI 599077) and IG 69979 (*C. cuneatum*).

According to dendrogram (Figure 4.17) of genetic relationships among different species based on unweighted pair group method with arithmetic mean (UPGMA), the cultivated chickpea *C. arietinum* showed a closer genetic relation with the *C. reticulatum*, which is considered to be progenitor of cultivated chickpea.

Table 4.39. Information of SSR markers used in the diversity analysis of 19 wild relatives of chickpea genotypes and their properties.

Marker	Major allele frequency	Allele number	Gene diversity	Heterozygosity	PIC
H2E13	0.33	8	0.79	0.33	0.76
CaM1515	0.31	9	0.83	0.62	0.81
CaM0958	0.17	12	0.90	0.47	0.89
ICCM0249	0.14	12	0.90	0.29	0.89
ICCM0120a	0.18	9	0.86	0.09	0.85
TAA58	0.17	12	0.90	0.47	0.89
GA6	0.24	9	0.84	0.16	0.82
TA21	0.27	11	0.86	0.27	0.84
NCPGR21	0.47	6	0.69	0.11	0.64
TA71	0.18	8	0.86	0.21	0.84
TA200	0.39	6	0.73	0.21	0.70
TA142	0.22	10	0.86	0.00	0.84
CaM0244	0.71	2	0.42	0.00	0.33
GAA47	0.55	4	0.63	0.00	0.58
CaM2064	0.86	2	0.24	0.00	0.21
ICCM0130a	0.36	4	0.69	0.00	0.63
CaM0799	0.62	5	0.54	0.23	0.49
CaM1451	0.64	3	0.52	0.17	0.45
TA116	0.43	6	0.74	0.57	0.71
STMS11	0.34	6	0.74	0.11	0.70
CaM2036	0.56	4	0.61	0.17	0.56
NCPGR19	0.31	7	0.79	0.06	0.76
TA59	0.50	5	0.66	0.13	0.62
TR42	0.27	8	0.84	0.00	0.82
GA16	0.21	9	0.86	0.33	0.85
TA30	0.24	9	0.85	0.24	0.83
Mean	**0.37**	**7.15**	**0.74**	**0.20**	**0.70**
Minimum	**0.14**	**2**	**0.24**	**0.00**	**0.21**
Maximum	**0.86**	**12**	**0.90**	**0.62**	**0.89**

*Observations were missing on genotype IG 599076 (*C. chrossanicum*)

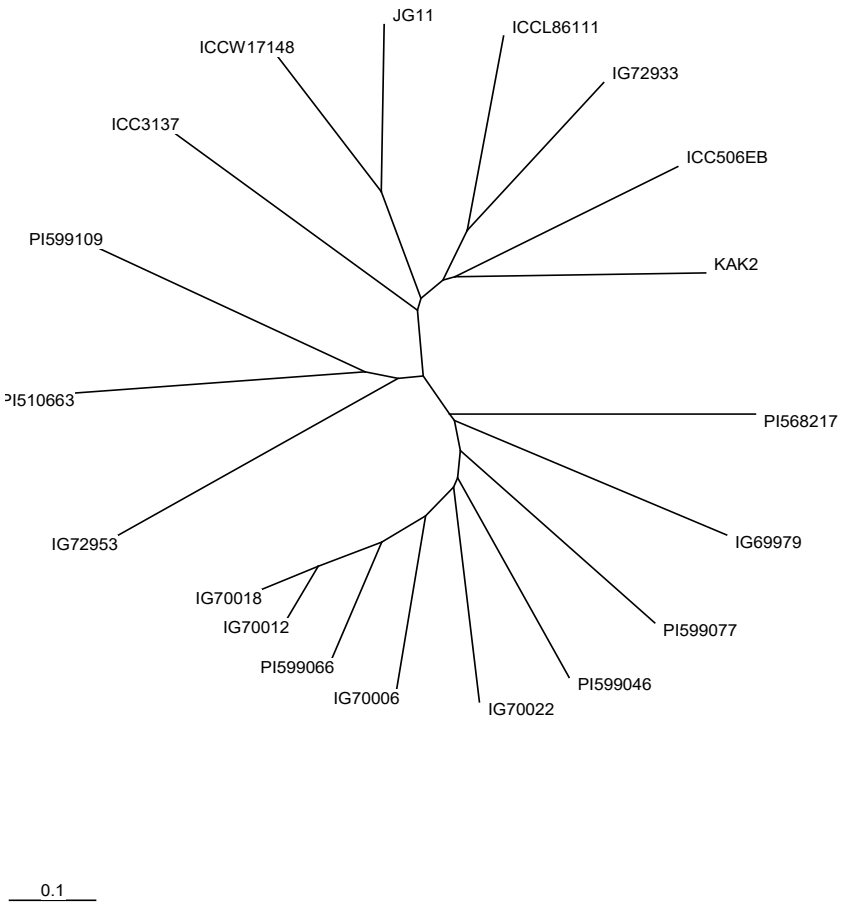


Figure 4.16. Radial tree showing the distance (dissimilarity) between different genotypes of wild relatives of chickpea using UPGMA method.

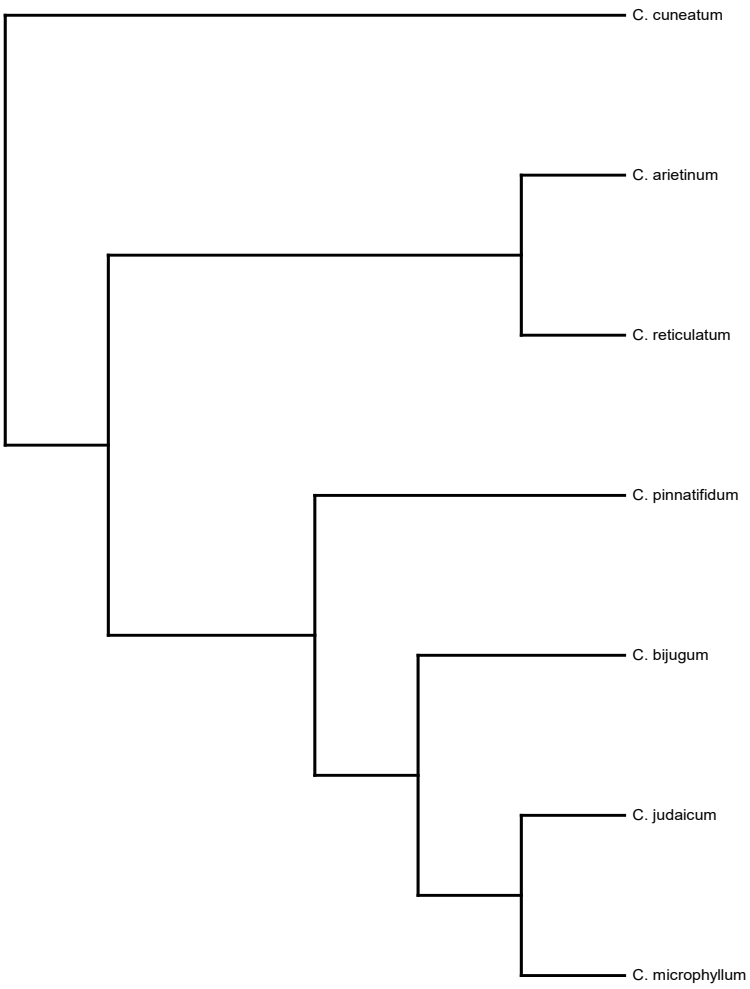


Figure 4.17. Dendrogram showing the distance (dissimilarity) between different species of wild relatives of chickpea using UPGMA method.

While, the other species *C. microphyllum*, *C. judaicum*, *C. bijugum* and *C. pinnatifidum* which were placed in other cluster showed high genetic distance with the cultivated chickpea. The other species *C. cuneatum* was placed in separate cluster indicated that it is distantly related to species in other two clusters.

Modern plant breeding and agricultural systems have narrowed the base for the genetic diversity in cultivated chickpea (Robertson *et al*., 1997) and only moderate levels of resistance to *H. armigera* are available in the cultivated chickpea. Therefore, it is time to explore wild relatives that might be used in plant breeding programs for development of resistant cultivars for *H. armigera*. The knowledge of genetic relationships between the cultivated chickpea and its wild relatives is a prerequisite to track the evolution of cultivated species and also to determine the close relatives which can be exploited for introgression of useful traits into the cultigen in plant breeding programmes. Among the different classes of molecular markers, SSRs have been proven useful for a variety of applications in plant genetics and breeding because of their reproducibility, multi allelic nature, codominant inheritance, relative abundance and genome wide coverage (Gupta and Varshney, 2000). Hence, the present study was carried out to find out the genetic diversity of wild relatives of chickpea using 26 SSR markers.

The number of allele per marker is considered to be a good indicator of genetic variability (Nevo, 1978). The results showed that a range of 2 to 12 alleles were present with an average of 7.15 alleles per locus which is in agreement with Huttel *et al*. (1999)., Choudhary *et al*. (2006)., Sethy *et al*. (2006a) and Castro *et al*. (2011) but less compared to Upadhyaya *et al*. (2008) and Naghavi *et al*. (2012) who reported average number of alleles per locus was 35 and 19.31, respectively. The differences in SSR allelic richness could be explained by several factors such as diversity range of the germplasm, number of accessions used, number of SSR loci and SSR repeat type (Yang *et al*., 2010).

In the present study, observed heterozygosity showed a wide variation from 0.00 to 0.62 with an average of 0.20. Torutaeva *et al*. (2014) also reported that heterozygosity ranged from 0.05 to 0.43 with an average of 0.13 based on genetic diversity in 23 genotypes of chickpea using nine SSR markers, whereas Choudhary *et al*. (2009) observed the average heterozygosity of 0.16. However, high level of

heterozygosity (0.76) was also observed using 16 microsatellite loci in 307 land races from Northern Iran (Naghavi *et al*., 2012).

The relative informativeness of each marker can be evaluated on the basis of its polymorphic information content (PIC) value. Similar estimates of PIC values of present study were observed in case of earlier microsatellite studies in chickpea in a range of 0.45 to 0.89 by Castro *et al*. (2011), 0.36 to 0.91 by Naghavi *et al*. (2012), 0.26 to 0.91 by Aggarwal *et al*. (2015b). Gupta *et al.* (2003) reported increased PIC with greater number of markers. They obtained PIC of 0.469 with 65 SSRs markers compared to 0.210 with 20 SSRs on 52 wheat genotypes. Although, the number of SSR marker in this study was limited, high polymorphism was revealed indicating wide diversity among accessions.

Based on cluster analysis of different wild relatives of chickpea, it was observed that *C. arietinum* and *C. reticulatum* were placed in one cluster. The other species *C. microphyllum*, *C. judaicum*, *C. bijugum* and *C. pinnatifidum* were placed in other cluster while *C. cuneatum* was placed in separate cluster. The grouping was similar as found with other studies using SSR markers (Sethy *et al*., 2006b), RAPD markers (Sudupak *et al*., 2002 and Talebi *et al*., 2009) and AFLP markers (Sudupak *et al*., 2004 and Shan *et al*., 2005).

The genotypes with different levels of resistance to *H. armigera* placed in different groups can be used to increase the level and broaden the genetic base of resistance to pod borer in chickpea. The pod borer resistance and the morphological and biochemical traits that exhibited direct effects on the resistance can be used to select pod borer resistant chickpeas. Hence, discovery and use of alien genes for resistance from wild species provide the way for sustaining crop improvement.

Chapter ~ V

Summary & Conclusions

Chapter V

SUMMARY AND CONCLUSIONS

The present research was contemplated to study the "**Biochemical and molecular mechanisms of resistance to *Helicoverpa armigera* (Hubner) in wild relatives of chickpea**". These studies were carried out at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana State, India during 2014-16 and focussed on the identification of resistance mechanisms against *H. armigera* in wild relatives of chickpea.

In the present study, a total of 20 accessions (15 wild relatives and five varieties of cultivated chickpea) were used to evaluate the relative resistance or susceptibility to *H. armigera*. Of the 15 accessions of wild relatives of chickpea, six accessions belong to *Cicer bijugum*, two accessions belong each to *C. reticulatum*, *C. judaicum* and *C. pinnatifidum*, and one accession belong each to *C. chrossanicum, C. microphyllum* and *C. cuneatum.* Five cultivars belonging to cultivated chickpea were JG 11(Commercial cultivar), KAK 2 and ICC 3137 (susceptible checks) and ICCL 86111 and ICC 506EB (moderately resistant checks).

Under multi-choice field conditions, observations were recorded on abundance of pod borers on different genotypes of wild relatives of chickpea at fortnight intervals during post-rainy season 2014-15 and 2015-16. There were no significant differences in number of *H. armigera* eggs per five plants among different genotypes throughout cropping period except 75 days after emergence (DAE) during post-rainy season, 2014-15 and 15 DAE after during post-rainy season, 2015-16. Highest oviposition was observed at 30 DAE followed by 60 DAE during post-rainy season, 2014-15, but differences were non-significant across genotypes. Among all the genotypes, larvae of *H. armigera* showed significant preference towards susceptible checks, ICC 3137 and KAK 2, whereas all the wild relatives recorded less number of larvae compared to cultivated chickpea. The genotypes, PI 510663, PI 599109 (*C. pinnatifidum*), PI568217, PI 599077 (*C. judaicum*), ICCW 1748 (*C. microphyllum*) and IG 69979 (*C. cuneatum*) recorded less number of *H. armigera* larvae. The genotypes of *C. bijugum* (IG 70006, IG 70012, IG 70018, IG 70022, PI 599046 and PI 599066) also showed less damage rating along with other wild genotypes of chickpea. Oviposition by *S. exigua* had not

shown any significant differences among genotypes. Observations on number of *S. exigua* larvae were not consistant throughout crop growing season. Highest larval count was observed at 45 DAE during post-rainy season, 2014-15. Among all the genotypes, *C. chrossanicum* (IG 599076) recorded highest number of *S. exigua* larvae. Highest number of *C. chlorideae* cocoons was observed at 105 DAE, during post-rainy season, 2014-15 and parasitization of *C. chlorideae* was not observed on any genotype during post-rainy season, 2015-16.

Under multi-choice field conditions, all genotypes of wild relatives of chickpea showed significantly lowest per cent pod damage compared to susceptible checks, ICC 3137 and KAK 2. The genotype IG 69979 (*C. cuneatum*) showed lowest per cent pod damage among all genotypes which was similar to that of resistant check, ICC 506EB.

Oviposition non-preference to *H. armigera* in wild relatives of chickpea revealed that the genotypes IG 70012, PI 599046, IG 70022, PI 599066, IG 70006, IG 70018 (*C. bijugum*), ICC 506EB, ICCL 86111 (resistant checks), IG 72933, IG 72953 (*C. reticulatum*) IG 69979 (*C. cuneatum*) and IG 599076 (*C. chrossanicum*) showed significantly lowest preference for oviposition under multi-choice, dual-choice and no-choice cage conditions and the genotypes PI 599077, PI 568217 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*) were more preferred for oviposition by *H. armigera* compared to susceptible check.

Detached leaf assay revealed that the damage rating and larval weights were significantly low when neonates were fed on the leaves of IG 70012, IG 70022, IG 70018, IG 70006, PI 599046, PI 599066(*C. bijugum*), IG 69979 (*C. cuneatum*), PI 568217, PI 599077 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*) compared to susceptible checks. Larval survival was greater on the wild relatives than on the cultivated chickpea.

Detached pod assay studies revealed that all wild relatives of chickpea exhibited lesser damage rating and pod damage percentage when compared to cultivated chickpea. Percentage of weight gained by larvae was more when fed on cultivated chickpea than wild relatives. The wild relatives of chickpea genotypes, IG 69979 (*C. cuneatum*), IG 72933, IG 72953 (*C. reticulatum*) and PI 5990066, IG 70006, IG 70012 and IG 70018 (*C. bijugum*) showed high levels of resistance compared to cultivated chickpea.

Survival and development of *H. armigera* on artificial diet impregnated with lyophilized leaf powders of different genotypes of wild relatives of chickpea across seasons revealed that antibiosis to *H. armigera* in wild relatives of chickpea was expressed in terms of lower larval survival, per cent pupation and adult emergence, decreased larval and pupal weight, prolonged larval and pupal developmental periods and reduced fecundity. The genotypes IG 70018, IG 70012, IG 70022, PI 599046, PI 599066 and IG 70006 (*C. bijugum*) and ICCW 17148 (*C. microphyllum*) showed high levels of resistance followed by PI 568217, PI 599077 (*C. judaicum*), PI 510663, PI 599109 (*C. pinnatifidum*), IG 69979 (*C. cuneatum*) and IG 599076 (*C. chrossanicum*), while IG 72933 and IG 72953 (*C. reticulatum*) showed moderate levels of antibiosis compared to susceptible check.

Two different types of trichomes *viz.,* glandular and non-glandular trichomes were observed in different genotypes of wild relatives and cultivated chickpea. Highest numbers of glandular trichomes were observed on *C. bijugum* and lowest was observed in *C. chrossanicum*. Among cultivated chickpea genotypes glandular trichome density was less in susceptible check, KAK 2 and ICC 3137, while more was observed in resistant checks, ICCL 86111 and ICC 506EB. Non-glandular trichomes were completely absent in genotypes of *C. pinnatifidum*. Among other species, lowest trichome density was observed in *C. microphyllum* and *C. judaicum* while highest trichome density was observed in *C. reticulatum* and cultivated chickpea. Glandular and non-glandular trichomes showed negative association with oviposition preference by adults of *H. armigera* under multi-choice and no-choice conditions. Glandular trichomes had significant negative association with damage rating, whereas non-glandular trichomes had significant positive association with damage rating and larval weight but negative with larval survival percentage.

There were significant differences in pod wall thickness of different accessions of wild relatives of chickpea. Lowest pod wall thickness was recorded in IG 599076 (*C. chrossanicum*), whereas highest was recorded in IG 72953 (*C. reticulatum*) followed by IG 70006 and IG 70012 (*C. bijugum*). Pod wall thickness showed significant negative association with damage rating and pod damage percentage. Percentage of weight gained by larvae was also negatively associated with pod wall thickness but the relation was not significant.

HPLC finger prints of leaf exudates revealed that, highest amount of oxalic acid was recorded in cultivated species compared to wild relatives of chickpea genotypes. Lowest amount of malic acid was recorded in IG 72933 and IG 72953 (*C. reticulatum*), while highest was recorded in PI 599077, PI 568217 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*) among all the genotypes. Oxalic acid showed negative association with ovipoition preference, while malic acid showed positive and significant association. Oxalic acid and malic acid had significant and negative association with larval survival, which indicates that presence of higher amounts of these acids resulting in reduced larval survival in cultivated chickpea compared to wild relatives in detached leaf assay.

HPLC finger prints of flavonoid content in all genotypes had altogether 39 peaks with varying peak areas and retention times (RT) with a range of 2.15 to 25.70 min. Most of the compounds showed higher peak area in wild relatives compared to cultivated chickpea. Of the 39 peaks, nine compounds *viz.*, chlorogenic acid, ferulic acid, naringin, 3,4-dihydroxy flavones, quercetin, naringenin, genestein, formononetin and biochanin A were identified and quantified by running standards and remaining all were unidentified. These compounds exhibited negative effects on survival and development of *H. armigera* reared on artificial diet impregnated with lyophilized leaf powders by showing a positive correlation with larval and pupal period, and a negative correlation with larval survival, pupation, larval and pupal weight, adult emergence and fecundity, that could be attributed to the presence of flavonoids in wild relatives of chickpea resulted in antibiosis effect on *H. armigera*.

Significant differences were exhibited in proteins, phenols, total soluble sugars and tannin content in wild relatives of chickpea across seasons. Protein content showed a significant negative correlation with larval weight, pupation and adult emergence of *H. armigera* reared on artificial diet impregnated with lyophilized leaf powders of wild relatives of chickpea genotypes. Phenols also exhibited significant negative correlation with larval weight, pupation, pupal weight, adult emergence and fecundity, while significant positive correlation was showed with pupal period. Significant and positive correlation was observed between pupation, pupal weight and total soluble sugars, while with larval period it had shown negative correlation. Tannins showed significant positive association with larval weight, pupation and adult emergence. Proteins and phenols were associated with resistance, while tannins and total soluble sugars were associated with susceptibility against *H. armigera* in wild relatives of chickpea.

Zymogram analysis of trypsin inhibitor (TI) isoforms revealed that the genotypes IG 70018, PI 599066 (*C. bijugum*) and IG 72933 (*C. arietinum*) showed a maximum of seven isoforms, whereas PI 599046 (*C. bijugum*) and PI 599077 (*C. judaicum*) showed six isoforms and IG 70022 (*C. bijugum*), PI 568217 (*C. judaicum*), IG 599076 (*C. chrossanicum*), and IG 69979 (*C. cuneatum*) showed five isoforms. Minimum of two isoforms were observed in PI 510663 (*C. pinnatifidum*). Remaining all genotypes exhibited four isoforms except in PI 599109 (*C. pinnatifidum*), ICCW 17148 (*C. microphyllum*) and susceptible check ICC 3137 (*C. arietinum*) showed three isoforms.

Significant variations were observed in terms of *H. armigera* gut (HG) protease inhibitory potential in wild relatives of chickpea under *in vitro* condition. The genotypes, IG 70018, IG 70012, IG 70006, IG 70022, PI 599066 (*C. bijugum*), IG 72933, IG 72953 (*C. reticulatum*) and IG 69979 (*C. cuneatum*) showed higher inhibitory activity of HG total proteases, HG trypsin and HG chymotrypsin, while PI 510663, PI 599109 (*C. pinnatifidum*), PI 568217 (*C. judaicum*) and ICCW 17148 (*C. microphyllum*) had low protease inhibitory activity compared to cultivated chickpea.

Hemagglutination test involves agglutination of red blood cells or erythrocytes with lectin. The agglutination intensity increased with increase in the concentration of lectin in the plant sample. In some genotypes, ICCW 17148 (*C. microphyllum*), PI 599077, PI 568217 (*C. judaicum*), PI 599046, PI 599066, IG 70006 and IG 70012 (*C. bijugum*) the agglutination was more even at less concentration of plant sample. Among cultivated chickpea, agglutination activity was observed in KAK 2, but the intensity was less when compared to wild relatives of chickpea, whereas in other genotypes the agglutination was not visible in JG 11 and ICC 506EB and very little intensity was observed in ICCL 86111 and ICC 3137 even at higher concentrations.

Schiff's base staining for the detection of lectins in the wild relatives of chickpea indicated that only one type of isoform with a molecular weight of 29 kDa was observed in some of the wild relatives of chickpea genotypes. However, the intensity of band varied among the genotypes.

GC-MS profile peaks identified with hexane extracts at RT of 7.19, 10.30, 11.01, 11.29, 13.98, 18.23, 20.38, 27.56 and 27.85 min associated with resistance to

H. armigera by exhibiting significant negative correlation with damage rating, larval survival and weight indicated that these compounds, *i.e.* peaks at RT of 11.20, 13.38, 14.46, 16.95, 19.94, 21.36, 26.74 and 29.20 min were associated with susceptibility. The peaks at RT of 13.58, 18.23, 19.32, 23.86 and 27.85 min showed significant negative correlation with oviposition, while peaks at RT of 10.39, 11.01, 19.94 and 21.36 min showed significant positive correlation.

Of the 107 GC-MS profile peaks identified with methanol extracts, 18 peaks at different retention times showed significant positive correlation with damage rating, while 14 compounds showed significant negative correlation. Association of larval survival with methanol extracts exhibited 28 peaks with significant positive correlation, whereas 20 peaks showed significant negative correlation at different retention times. Similarly, larval weight showed negative correlation with 21 peaks and positive correlation with 34 peaks. Oviposition preferences revealed that, 28 peaks at different retention times showed significant negative association with oviposition, while 14 peaks showed positive association. The results indicated that methanol extracts of leaf surface chemicals had higher amount of phagostimulants and oviposition repellents than antifeedants and oviposition attractants.

The twenty six SSR markers used for assessing genetic diversity of wild relatives of chickpea detected a total of 186 alleles with an average of 7.15 alleles per marker. PIC values varied from 0.21 (CaM2064) to 0.89 (CaM0958, ICCM0249 and TAA58) with an average of 0.70. Gene diversity varied from 0.24 (CaM2064) to 0.90 (CaM0958, ICCM0249 and TAA58). The observed heterozygosity varied from 0.00 (TA142, CaM0244, GAA47, CaM2064, ICCM0130a and TR42) to 0.62 (CaM1515) with an average of 0.20. The markers CaM0958, ICCM0249 and TAA58 were most informative with most alleles, high gene diversity and highest PIC value.

According to dendrogram of genetic relationships among different species based on unweighted pair group method with arithmetic mean (UPGMA), the cultivated chickpea *C. arietinum* showed a closer genetic relation with the *C. reticulatum*, which is considered to be progenitor of cultivated chickpea. While, the other species *C. microphyllum*, *C. judaicum*, *C. bijugum* and *C. pinnatifidum* which were placed in other cluster showed high genetic distance with the cultivated chickpea. The other species *C. cuneatum* was placed in separate cluster indicated that it is distantly related to species in other two clusters.

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