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Lipophilic profiling of *Sorghum bicolor* (L.) Moench seedlings *vis-à-vis* *Chilo partellus* (Swinhoe) larvae reveals involvement of biomarkers in sorghum-stem borer interactions

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Lipophilic metabolites play important role in the developmental process of insects, however, still there is no clarity on their involvement in plant resistance. Therefore, we carried out the lipophilic profile of host sorghum genotype seedlings and the *Chilo partellus* (Swinhoe) larvae, to understand the role and contribution of certain lipophilic metabolites in sorghum plant defense against the dreaded pest, spotted stem borer, *C. partellus*. There were variations in the form of presence or absence, along with significant differences in lipophilic metabolites across sorghum genotypes and the *C. partellus* larvae. The significantly higher contents of myristic acid, palmitic acid, linoleic acid, linolenic acid, eicosanoic acid and behenic acid in resistant sorghum genotypes; and linolenic acid, methyl 3-methoxytetradecanoate, myristic acid, oleic acid, palmitic acid, palmitoleic acid, lathosterol and squalene in *C. partellus* larvae were significantly lower than those fed on susceptible genotype, indicating their role in insect-plant biochemical disruptions. Myristic acid, methyl 3-methoxy-tetradecanoate, stearic acid, squalene, fucosterol, hexacontane, tetrapentacontane, palmitic acid, l-(+)-ascorbic acid 2,6-dihexa-decanoate, 2-pentadecanone, 6,10,14-trimethyl, lignoceric acid and stigmasterol in sorghum seedlings contributed to 60 to 100% variability in various biological and resistance parameters of *C. partellus*. However, myristic acid, linoleic acid, margaric acid, methyl 14-methylhexadecanoate, methyl 3-methoxytetradecanoate, stearic acid, palmitic acid, palmitoleic acid, eicosanoic acid, gamma-ergostenol, cholesterol, lathosterol, squalene, 1-triacontanol and n-pentadecanol in *C. partellus* larvae contributed to 64 to 100% variability in various biological and resistance parameters of *C. partellus*. The myristic acid, methyl 3-methoxytetradecanoate, palmitic acid, stearic acid and squalene present in both host plant and the test insect, contributed significantly to explain variability in resistance against *C. partellus*, thus could be used as biomarkers for sorghum-stem borer interactions.

Keywords: Antibiosis, Deadhearts, Host-plant resistance, Lipophilic metabolites, Spotted stem borer

There is a complex interplay of signals between insect and plant in response to damage by the herbivore, which determines the resistance/susceptibility reaction of the host plant. The plant defense against herbivores is mainly governed by constitutive and/or induced plant metabolic compounds[1]. A number of secondary plant metabolites such as alkaloids, ketones, tannins, terpenoids, flavonoids, organic acids, etc. have been reported to serve defensive functions against herbivores and pathogens[2-5]. Presence or absence of secondary metabolites[6], and variation in amounts of specific secondary metabolites[7], can impact grain yield and nutritional quality of the host plants[8]. Some dietary components like amino acids, phospholipids, fatty acids, steroids and ascorbic acid also regulate certain physiological and bio-ecological processes in insects[9]. In case the host plant is deficient in particular nutritional constituent, certain herbivore species compensate this requirement by increasing the rate and quantity of food intake, which is reflected in development and survival of the pest and ultimately determine host suitability or plant resistance[8,10-13]. Polyunsaturated fatty acids are one of the most important dietary components of lepidopteran insects[14].

Sorghum [*Sorghum bicolor* (L.) Moench] is an important cereal crop and staple food of millions of people in the semi-arid tropics. Although production has increased over the years, the actual yield potential of this crop has not been fully realized due to several biotic and abiotic constraints[15]. Among the biotic stresses, spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae) is one of the most predominant herbivore causing about 18 to 25%

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yield losses in maize and sorghum under different agro-climatic conditions in Asia and Africa[16]. Although host plant resistance has shown some success in managing several insect pests in sorghum, like any other insect control program, this particular approach is also not free from certain limitations and problems in case of *C. partellus*[13,17]. Several sources of resistance to *C. partellus* have been identified in the germplasm and cultivated gene pool, however the multifarious inheritance and strong influence of environmental factors on the expression of resistance makes it difficult to develop *C. partellus* resistant varieties[18]. Further, limited knowledge on plant-insect biochemical interactions has also been the bottleneck in developing stem borer resistant varieties of sorghum[13,19].

All the three mechanisms of resistance *viz.*, antibiosis, antixenosis and tolerance are although operational in sorghum, antibiosis imparts major contribution in plant defense against *C. partellus*[8]. A number of biochemical factors like protein, amino acids, total sugars, chlorophyll, carotenoids, iron, zinc; and phenolic acids *viz.*, ferulic acid and p-coumaric acid in the host plants have been reported to contribute to resistance/susceptibility to *C. partellus*[20-22,8]. Lipids and their metabolites on the other hand, are required by the herbivores for different physiological and biological functions such as oogenesis, larval growth, metabolism, anti-infection roles, and acts like juvenile hormones and brain hormone. The hydrocarbons and fatty alcohols serve as constituents of insect pheromones and waxes[23,24], and stimulants for plant growth and insect feeding[25]. Fatty acid desaturase derived signal(s) have also been reported to modulate the crosstalk between different defense signalling pathways in response to biotic stress in the host plants[26]. The role and requirement for different lipophilic compounds are highly variable across herbivores[27-29]. Further, the dietary routing of lipophilic metabolites and their assimilation impacts fatty acid profile in insects[30], while some hydrocarbons induce resistance in host plants to various stresses[31,32].

Although some studies have deciphered the role of certain membranous lipophilic metabolites in the developmental process of insects, there is no clarity on their involvement in host-plant interactions. Therefore, aim of the study was to know: (i) The lipophilic profile of *C. partellus* resistant and susceptible genotypes; (ii) influence of sorghum seedlings lipophilic metabolites on lipophilic profile of *C. partellus* larvae; and (iii) association of lipophilic metabolites in sorghum seedlings and the *C. partellus* larvae with biological parameters and resistance indices, and their contribution in describing defense to *C. partellus*.

Materials and Methods

Crop raising and collection of plant samples

The experimental material consisted of two germplasm lines (IS 2123 and IS 2205), two varieties (ICSV 700 and ICSV 708), and one susceptible check, Swarna. Ten seeds of each test sorghum genotype were sown in plastic pots (12 L capacity) having potting mixture of alluvial soil and vermicompost (2:1) added with diammonium phosphate @ 50 g per pot at the Division of Entomology, ICAR-Indian Agricultural Research Institute (Latitude - 28°38'23" N and Longitude - 77° 09'27" E, height above mean sea level is 228.61 m), New Delhi, India. There were 10 pots for each sorghum genotype. The watering, weeding and hoeing were done in the test sorghum pots whenever required. The 21 days old seedlings of each sorghum genotype were harvested from the base separately in the polythene bags and immediately stored at −20°C in the refrigerator. The refrigerated samples were then freeze-dried in a lyophilizer at −50°C (LAB CONCO Free Zone® 6), to avoid changes in biochemical composition of the seedlings. The freeze-dried samples were finely powdered (<80 mesh size) in a mixer-grinder and stored in zip-lock plastic bags at −20ºC in the refrigerator for further biological and biochemical studies.

Damage by *C. partellus* in sorghum genotypes under field conditions

Sorghum genotypes were sown in 2-row plots of 2-m row length with row-row spacing of 60 cm under field conditions at ICAR-Indian Agricultural Research Institute, New Delhi, India, during 2011-2013 *Kharif* (July-October) seasons. The seeds were sown with the dibbling method in three replications in a randomized complete block design (RCBD). Thinning was carried out to maintain the plant-plant spacing of 10 cm after one week of seedling emergence. The watering, weeding and hoeing were done in the test sorghum plots whenever required. Data were recorded on total number of plants and number of plants with deadheart at 45 days after emergence (DAE), and expressed as percentage plants with deadhearts.

Biology of *C. partellus* on different sorghum genotypes

The *C. partellus* culture maintained round the year on artificial diet[33] under laboratory conditions at the Division of Entomology, ICAR-IARI, New Delhi was used in the present studies. The studies on developmental biology of *C. partellus* were carried out on artificial diet impregnated with lyophilized seedling powder of aforesaid sorghum genotypes under laboratory conditions at 27±2°C, 60±5% RH, and 12 h photoperiod. Twenty-five neonate *C. partellus* larvae were released on the artificial diet in each cup (250 mL capacity), and there were three replications in a completely randomized design. Observations were recorded on larval and pupal weights, larval and pupal periods, larval survival and adult emergence of *C. partellus* reared on each test sorghum genotype. The larvae reared on test sorghum genotypes were weighed individually on Precision electronic balance (CB-Series Contech) after 30 days of feeding, and the weights were expressed as mg/larva. The test insects were observed daily for their transformation into different life stages. The day of larval release to pupa formation and pupal formation to adult emergence were considered as larval and pupal periods, respectively, and expressed in days. Pupal weight was recorded on Precision electronic balance (CB-Series, Contech), for each pupa separately one day after pupation, and data were expressed as mg/pupa. Percentage larval survival and adult emergence were calculated based on the total number of larvae released per replication. The data on deadhearts caused by *C. partellus* in test sorghum genotypes under field conditions and above mentioned biological parameters were subjected to calculation of different indices using the method as described in Dhillon *et al.*[34] with appropriate modifications [Deadheart index = deadhearts in test genotype/deadhearts in the susceptible genotype; Antibiosis index = larval weight index + larval period index + larval survival index + pupal period index + pupal weight index + adult emergence index; and Overall resistance index = deadheart index + larval weight index + larval period index + larval survival index + pupal period index + pupal weight index + adult emergence index].

Collection of *C. partellus* larvae samples for lipophilic metabolite analysis

A group of 25 neonate *C. partellus* larvae were released on the artificial diet impregnated with lyophilized seedling powers of aforesaid test sorghum genotypes for obtaining insect samples for lipophilic profiling. The *C. partellus* larvae on attaining the 3rd instar stage (weighing around 100 mg) were collected from each test genotype individually in 2 mL Eppendorf tubes and stored at −20ºC in the refrigerator for lipophilic analysis.

Sample preparation and separation of lipophilic metabolites from test plants and larvae of *C. partellus*

The profiling and estimation of lipophilic metabolites in the seedlings of test sorghum genotypes *vis-à-vis* the *C. partellus* larvae reared on them were carried out on GCMS-QP2010 Ultra system with autosampler AOC-20i, from Shimadzu (Japan) using the standard method given by Kumar & Dhillon[19]. The sorghum seedling (200 mg) and the *C. partellus* larvae samples (the whole body weighing around 100 mg) were weighed and collected separately, and ground in mortar and pestle with 10 mL solvent mixture consisting of chloroform:hexane: methanol (8:5:2 v/v/v). These test samples were kept overnight in the extraction solvent, and filtered the next day. Fatty acids were converted to their respective methyl esters using the modified method of Neff *et al.*[35]. The test samples with volumes of 1.0 µL each were injected with a split ratio of 20:1, and gas chromatography was performed using Rtx®-5MS column (30 mm length, 0.25 mm diameter and 0.25 µm thickness). The injection, interface, and ion source temperatures were set at 250, 270 and 230°C, respectively. Helium gas was used as a carrier with a pressure of 123.5 kPa set at a total flow rate of 28.2 mL/min and column flow rate of 1.2 mL/min. The temperature program was set to 2 min isothermal heating at 180°C, followed by a 5°C min^{-1} oven temperature ramp to 280°C, hold for 5 min and again increased with a ramping rate of 20°C min^{-1} up to 300°C and again hold for 10 min. The oven was equilibrated for 1.0 min prior to injection of the next sample. The mass spectra were recorded between 2.8-30.0 min at two scans per s with an *m*/*z* 50-650 scanning range. The chromatograms and mass spectra were evaluated using the Lab solutions® GCMS solution software version 2.71 (Shimadzu, Japan). Processed data were checked manually and need-based corrections were carried out. All the lipophilic metabolites were identified using MS libraries (NIST08, Wiley8), and the fatty acids were also verified using fatty acid methyl ester (99.9%) standards obtained from SUPELCO Analytical, Bellefonte, PA, USA.

Statistical analysis

Normality test showed non-significant seasonal effects, and thus field data on deadhearts from three seasons were pooled for statistical analysis. Data were subjected to analysis of variance (ANOVA). The significance of differences between treatments were tested by *F*-test, and treatment means were compared by least significant differences (LSD) at $P = 0.05$ using the statistical software SAS® version 9.2. Data on *C. partellus* biological parameters, indices and lipophilic metabolites in plants and insects were subjected to Pearson correlation, multiple linear regression, and stepwise regression analysis to understand the association of host plant and insect lipophilic metabolites on various biological parameters and resistance indices of *C. partellus*.

Results

Developmental biology and damage by *C. partellus* on different sorghum genotypes

The deadhearts caused by *C. partellus* varied significantly across sorghum genotypes ($F_{4,8}$= 3311.37; P <0.001), being significantly lower in all the test sorghum genotypes as compared to susceptible genotype, Swarna. There were significant differences in larval weight ($F_{4,8}$ = 99.7; P <0.001) , larval survival ($F_{4,8}$ = 165.8; P <0.001), larval period ($F_{4,8}$ = 119.8; P <0.001), pupal weight ($F_{4,8}$ = 57.5; P <0.001); pupal period ($F_{4,8}$ = 73.8; P <0.001), and adult emergence ($F_{4,8}$ = 339.8; P <0.001) of *C. partellus* when reared on different sorghum genotypes. The *C. partellus* larvae reared on IS 2123 and IS 2205 had significantly lower larval and pupal weights, larval survival and adult emergence, while longer larval period as compared to other test sorghum genotypes including susceptible genotype, Swarna (Table 1). Furthermore, the *C. partellus* larvae reared on ICSV 700 and ICSV 708 resulted in significantly longer larval and pupal periods, and lower larval and pupal weights, larval survival and adult emergence as compared to those reared on susceptible genotype, Swarna. Altogether, these results indicate that the germplasm lines IS 2123 and IS 2205 have greater resistance to *C. partellus* than sorghum varieties ICSV 700 and ICSV 708 in comparison to susceptible genotype, Swarna (Table 1).

Detection of lipophilic metabolites in sorghum seedlings and larvae of *C. partellus*

The lipophilic profile was composed of fatty acids, fatty alcohols, hydrocarbons, sterols, terpenoids, vitamin derivative and other metabolites, detected across the test sorghum seedlings and the *C. partellus* larvae reared on these genotypes (Table 2). Of the 16 fatty acids, five fatty acids such as palmitoleic acid, margaric acid, methyl 16-methyl-heptadecanoate, oleic acid and erucic acid were absent in the sorghum seedlings, while three fatty acids *viz.*, linolenic acid, behenic acid and lignoceric acid were not detected from *C. partellus* larvae fed on these test sorghum genotypes. Among the other groups of lipophilic metabolites, squalene, cholesterol and l-(+)-ascorbic acid 2,6-dihexadecanoate were found in both sorghum seedlings and *C. partellus* larvae, while none of the fatty alcohols were common in sorghum seedlings and the *C. partellus* larvae (Table 2).

Variability in lipophilic metabolites in the seedlings of different sorghum genotypes

The lipophilic profiling chromatograms revealed variation in metabolite contents in seedlings of different sorghum genotypes (Suppl. Fig. 1. *All supplementary data are available only online along with the respective paper at NOPR repository at http://nopr.res.in*). The results showed significant differences among test sorghum genotypes for contents of lipophilic metabolites *viz.*, myristic acid ($F_{4,8}$ = 12.66; P = 0.002), 2-pentadecanone, 6,10,14-trimethyl ($F_{4,8}$ = 2.19; P <0.001), , palmitic acid ($F_{4,8}$ = 31.87; P <0.001), l-(+)-ascorbic acid 2,6-dihexadecanoate ($F_{4,8}$ = 41.69; P <0.001), margaric acid ($F_{4,8}$ = 3.7; P = 0.05), methyl 3-methoxy-tetradecanoate ($F_{4,8}$ = 14.1; P <0.001), linoleic acid ($F_{4,8}$ = 25.17; P <0.001), linolenic acid ($F_{4,8}$ = 59.12; P <0.001), phytol ($F_{4,8}$ = 42.42; P <0.001), stearic

Table 1 — Damage by and developmental biology of *Chilo partellus* on seedlings of diverse sorghum genotypes

Genotypes	Stem borer deadhearts (%)	Larval weight (mg/larva)	Larval survival (%)	Larval period (days)	Pupal weight (mg/pupa)	Pupal period (days)	Adult emergence (%)
ICSV 700	22.3^{b}	113.7^{b}	65.1^{b}	40.5^{c}	99.0^{b}	10.9^{d}	60.5^{b}
ICSV 708	27.1^{b}	129.2^{c}	68.4^{b}	38.3^{b}	96.6^{b}	9.6^{b}	61.7^{b}
IS 2123	14.2^{a}	69.8^{a}	48.1^{a}	44.4^{d}	82.2^{a}	10.4^{c}	44.8^{a}
IS 2205	13.4^{a}	69.2^{a}	46.7^{a}	43.7^{d}	84.8^{a}	10.3^{c}	43.3^{a}
Swarna	41.7^{c}	145.5^{d}	82.5^{c}	34.5^{a}	110.3^{c}	8.7^{a}	77.8^{c}

[The values within a column following different letters are significantly different at $P = 0.05$]

Table 2 — Categorization of different lipophilic metabolites and their detection in seedlings of sorghum genotypes and the *Chilo partellus* larvae fed on them

Lipophilic compounds	Presence/absence of lipophilic compounds		Lipophilic compounds	Presence/absence of lipophilic compounds	
	Sorghum seedlings	*C. partellus* larvae		Sorghum seedlings	*C. partellus* larvae
Fatty acids			**Hydrocarbons**		
Methyl 3-methoxytetradecanoate	+	+	1-Nonadecene	-	+
Myristic acid	+	+	Tetracosane	+	-
Palmitoleic acid	-	+	9-Hexacosene	+	-
Palmitic acid	+	+	Squalene	+	+
Methyl 14-methylhexadecanoate	-	+	Hexacontane	+	-
Margaric acid	+	+	Tetrapentacontane	+	-
Methyl 16-methyl-heptadecanoate	-	+	**Sterols and terpenoids**		
Linoleic acid	+	+	Cholesterol	+	+
Oleic acid	-	+	.alpha.-Tocopherol	+	-
Linolenic acid	+	-	Campesterol	+	-
Stearic acid	+	+	Stigmasterol	+	-
Methyl 11-eicosenoate	+	+	.gamma.-Ergostenol	-	+
Eicosanoic acid	+	+	Chondrillasterol	-	+
Erucic acid	-	+	.gamma.-Sitosterol	+	-
Behenic acid	+	-	Stigmastanol	+	-
Lignoceric acid	+	-	Fucosterol	+	-
Fatty alcohols			.beta.-Amyrin	+	-
n-Pentadecanol	-	+	Lathosterol	-	+
1-Octadecanol	-	+	Cycloartenol	+	-
Phytol	+	-	.alpha.-Amyrin	+	-
9-Octadecen-1-ol	-	+	**Vitamin derivative**		
1,16-Hexadecanediol	-	+	l-(+)-Ascorbic acid 2,6-dihexadecanoate	+	+
1-Heptacosanol	+	-	**Others**		
1-Triacontanol	-	+	2-Pentadecanone, 6,10,14-trimethyl-	+	-
			(Z)-14-Tricosenyl formate	-	+
			Cinnamic acid	+	-

[The positive (+) and negative (-) sign represents presence and absence of particular lipophilic compound in sorghum seedlings and *Chilo partellus* larvae, respectively]

acid ($F_{4,8}$ = 91.63; P < 0.001), tetracosane ($F_{4,8}$ = 4.96; P = 0.026), methyl 11-eicosenoate ($F_{4,8}$ = 88.07; P <0.001), eicosanoic acid ($F_{4,8}$ = 8.69; P = 0.005), 9-hexacosene ($F_{4,8}$ = 110.69; P <0.001), behenic acid ($F_{4,8}$ = 50.62; P <0.001), 1-heptacosanol ($F_{4,8}$ = 24.47; P <0.001), lignoceric acid ($F_{4,8}$ = 117.44; P <0.001), squalene ($F_{4,8}$ = 25.4; P <0.001), hexacontane ($F_{4,8}$ = 10.11; P = 0.003), tetrapentacontane ($F_{4,8}$ = 6.94; P = 0.01), alpha-tocopherol ($F_{4,8}$ = 11.99; P = 0.002), campesterol ($F_{4,8}$ = 36.05; P <0.001), stigmasterol ($F_{4,8}$ = 29.68; P <0.001), gamma-sitosterol ($F_{4,8}$ = 841.19; P <0.001), stigmastanol ($F_{4,8}$ = 2936.46; P <0.001), fucosterol ($F_{4,8}$ = 78.44; P <0.001), beta-amyrin ($F_{4,8}$ = 75.65; P <0.001), cycloartenol ($F_{4,8}$ = 109.22; P <0.001) and alpha-amyrin ($F_{4,8}$ = 92.4; P <0.001), while no significant differences were found among test sorghum genotypes for cholesterol content ($F_{4,8}$ = 1.86; P = 0.211). The contents of myristic acid, palmitic acid, linoleic acid, linolenic acid, eicosanoic acid and behenic acid were significantly lower, while hexacontane and gamma-sitosterol higher in the seedlings of IS 2123, IS 2205, ICSV 700 and ICSV 708 as compared to susceptible genotype, Swarna (Table 3). Lignoceric acid, squalene, campesterol and stigmasterol were significantly higher in varieties ICSV 700 and ICSV 708 as compared to IS 2205 and Swarna (Table 3). Furthermore, cinnamic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate, phytol, stearic acid, methyl 11-eicosenoate, hexacontane, tetrapentacontane and gamma-sitosterol contents were significantly higher, while margaric acid, methyl 3-methoxytetradecanoate, eicosanoic acid, 9-hexacosene, alpha-tocopherol, stigmastanol and cycloartenol contents lower in the seedlings of resistant genotype, IS 2205 as compared to other test sorghum genotypes including susceptible genotype (Swarna), except in a few cases (Table 3).

Variability in lipophilic metabolites in *C. partellus* larvae fed on various sorghum genotypes

Lipophilic profiling chromatograms revealed variation in metabolite contents in *C. partellus* larvae

Table 3 — Lipophilic contents in the seedlings of different sorghum genotypes

Lipophilic metabolites	Proportion of lipophilic content in seedlings of different sorghum genotypes (%)				
	ICSV 700	ICSV 708	IS 2123	IS 2205	Swarna
Myristic acid	0.14^{b}	0.11^{ab}	0.13^{ab}	0.08^{a}	0.22^{c}
2-Pentadecanone, 6,10,14-trimethyl	0.05^{ab}	0.07^{ab}	0.11^{b}	0.08^{ab}	0.05^{a}
Palmitic acid	9.03^{b}	7.40^{a}	9.20^{b}	7.00^{a}	12.06^{c}
l-(+)-Ascorbic acid 2,6-dihexadecanoate	10.18^{c}	11.10^{cd}	5.40^{a}	12.00^{d}	7.40^{b}
Margaric acid	0.16^{b}	0.11^{ab}	0.10^{ab}	0.09^{a}	0.16^{b}
Methyl 3-methoxytetradecanoate	0.33^{b}	0.21^{a}	0.35^{b}	0.24^{a}	0.35^{b}
Linoleic acid	11.30^{c}	9.31^{a}	10.36^{b}	10.70^{bc}	12.33^{d}
Linolenic acid	28.12^{b}	25.00^{a}	32.88^{c}	26.00^{a}	32.70^{c}
Phytol	17.30^{a}	20.66^{b}	17.30^{a}	20.98^{b}	17.33^{a}
Stearic acid	1.59^{a}	2.70^{d}	1.60^{a}	2.30^{c}	1.80^{b}
Tetracosane	0.17^{b}	0.15^{b}	0.08^{a}	0.13^{ab}	0.12^{ab}
Methyl 11-eicosenoate	0.13^{ab}	0.18^{b}	0.18^{b}	0.43^{c}	0.12^{a}
Eicosanoic acid	0.69^{ab}	0.60^{ab}	0.70^{b}	0.59^{a}	0.82^{c}
9-Hexacosene	0.39^{d}	0.08^{b}	0.03^{ab}	0.00^{a}	0.30^{c}
Behenic acid	0.54^{b}	0.66^{c}	0.43^{a}	0.55^{b}	0.76^{d}
1-Heptacosanol	0.17^{a}	0.34^{c}	0.14^{a}	0.28^{b}	0.24^{b}
Lignoceric acid	0.80^{c}	1.11^{d}	0.62^{a}	0.72^{b}	0.71^{b}
Squalene	0.42^{b}	0.45^{bc}	0.50^{c}	0.30^{a}	0.30^{a}
Hexacontane	0.41^{bc}	0.35^{b}	0.37^{bc}	0.43^{c}	0.28^{a}
Tetrapentacontane	0.19^{ab}	0.23^{bc}	0.16^{a}	0.26^{c}	0.14^{a}
Cholesterol	0.09^{a}	0.04^{a}	0.03^{a}	0.06^{a}	0.06^{a}
alpha-Tocopherol	0.13^{c}	0.04^{a}	0.05^{ab}	0.00^{a}	0.10^{bc}
Campesterol	4.00^{b}	4.53^{c}	2.91^{a}	3.13^{a}	2.73^{a}
Stigmasterol	5.40^{d}	5.40^{d}	4.05^{a}	4.95^{c}	4.60^{b}
gamma-Sitosterol	6.70^{c}	7.49^{d}	5.40^{b}	7.44^{d}	4.59^{a}
Stigmastanol	0.17^{a}	0.29^{a}	5.46^{c}	0.27^{a}	0.47^{b}
Fucosterol	0.18^{a}	0.29^{b}	0.53^{c}	0.33^{b}	0.31^{b}
beta-Amyrin	0.12^{a}	0.34^{c}	0.36^{c}	0.18^{b}	0.10^{a}
Cycloartenol	0.07^{b}	0.43^{d}	0.15^{c}	0.00^{a}	0.13^{c}
alpha-Amyrin	0.06^{ab}	0.43^{d}	0.00^{a}	0.14^{c}	0.09^{bc}

[Values within a row for a particular lipophilic compound following different letters are significantly different at $P = 0.05$]

reared on seedlings of different sorghum genotypes (Suppl. Fig. 1). There were significant differences in lipophilic metabolite contents in the larvae of *C. partellus* fed on different sorghum genotypes *viz.*, (*Z*)-14-tricosenyl formate ($F_{4,8}$ = 673.26; P <0.001), gamma-ergostenol ($F_{4,8}$ = 375.83; P <0.001), 1,16-hexadecanediol ($F_{4,8}$ = 22724.71; P <0.001), 1-nonadecene ($F_{4,8}$ = 1994.69; P <0.001), 1-octadecanol ($F_{4,8}$ = 21131.42; P <0.001), 1-triacontanol ($F_{4,8}$ = 2578.03; P <0.001), 9-octadecen-1-ol ($F_{4,8}$ = 27517.88; P <0.001), cholesterol ($F_{4,8}$ = 1589.29; P <0.001), chondrillasterol ($F_{4,8}$ = 160.86; P <0.001), eicosanoic acid ($F_{4,8}$ = 7085.65; P <0.001), erucic acid ($F_{4,8}$ = 3637.9; P <0.001), l-(+)-ascorbic acid 2,6-dihexadecanoate ($F_{4,8}$ = 11618.63; P <0.001), lathosterol ($F_{4,8}$ = 2906.08; P <0.001), linoleic acid ($F_{4,8}$ = 16145.89; P <0.001), margaric acid ($F_{4,8}$ = 838.12; P <0.001), methyl 11-eicosenoate ($F_{4,8}$ = 1846.21; P <0.001), methyl 14-methylhexadecanoate ($F_{4,8}$ = 804.21; P <0.001), methyl 16-methyl-heptadecanoate ($F_{4,8}$ = 9613.02; P <0.001), methyl 3-methoxytetradecanoate ($F_{4,8}$ = 237.93; P <0.001), myristic acid ($F_{4,8}$ = 286.89; P <0.001), n-pentadecanol ($F_{4,8}$ = 483.53; P <0.001), oleic acid ($F_{4,8}$ = 303.81; P <0.001), palmitic acid ($F_{4,8}$ = 502.66; P <0.001), palmitoleic acid ($F_{4,8}$ = 5007.86; P <0.001), squalene ($F_{4,8}$ = 96.91; P <0.001), and stearic acid ($F_{4,8}$ = 396.41; P <0.001). The larvae of *C. partellus* were found with significantly lower contents of linolenic acid, methyl 3-methoxytetradecanoate, myristic acid, oleic acid, palmitic acid, palmitoleic acid, lathosterol and squalene, when reared on stem borer-resistant germplasm lines (IS 2123 and IS 2205) and varieties (ICSV 700 and ICSV 708) of sorghum as compared to susceptible genotype, Swarna (Table 4). Conversely, the contents of 1,16-hexadecanediol, 1-nonadecene, 1-octadecanol, 1-triacontanol, l-(+)-ascorbic acid 2,6-dihexadecanoate, n-pentadecanol and stearic acid were significantly higher in the *C. partellus* larvae reared on IS 2123, IS 2205, ICSV 700 and ICSV 708 as compared to those fed on susceptible genotype, Swarna (Table 4). However, no consistent trend for increase or decrease in contents

Table 4 — Lipophilic contents in *Chilo partellus* larvae fed on different sorghum genotypes

Lipophilic metabolites	Proportion of lipophilic content in *C. partellus* larvae fed on different sorghum genotypes (%)				
	ICSV 700	ICSV 708	IS 2123	IS 2205	Swarna
(Z)-14-Tricosenyl formate	0.0[a]	0.0[a]	0.05[b]	0.0[a]	0.05[b]
gamma-Ergostenol	0.03[a]	0.10[c]	0.04[b]	0.03[a]	0.08[b]
1,16-Hexadecanediol	0.08[c]	0.66[d]	0.02[b]	0.09[c]	0.0[a]
1-Nonadecene	0.41[b]	1.41[e]	0.99[c]	1.03[d]	0.27[a]
1-Octadecanol	0.35[b]	1.29[e]	0.95[c]	1.06[d]	0.09[a]
1-Triacontanol	0.11[b]	0.15[d]	0.19[e]	0.14[c]	0.05[a]
9-Octadecen-1-ol	0.10[c]	0.86[e]	0.05[b]	0.01[a]	0.21[d]
Cholesterol	1.93[d]	2.35[e]	0.92[a]	1.44[b]	1.72[c]
Chondrillasterol	0.57[a]	0.77[c]	0.77[c]	0.65[b]	0.76[c]
Eicosanoic acid	0.30[b]	1.28[e]	0.57[c]	0.26[a]	1.20[d]
Erucic acid	0.16[b]	0.86[e]	0.29[c]	0.09[a]	0.39[d]
l-(+)-Ascorbic acid 2,6-dihexadecanoate	39.57[d]	20.63[b]	24.40[c]	45.70[e]	18.89[a]
Lathosterol	0.24[d]	0.0[a]	0.06[c]	0.04[b]	0.26[e]
Linoleic acid	5.55[c]	7.77[d]	4.29[b]	4.15[a]	12.81[e]
Margaric acid	0.08[a]	1.07[d]	0.23[b]	0.07[a]	0.65[c]
Methyl 11-eicosenoate	0.12[b]	0.28[e]	0.26[d]	0.04[a]	0.18[c]
Methyl 14-methylhexadecanoate	0.05[a]	0.45[d]	0.16[b]	0.06[a]	0.32[c]
Methyl 16-methyl-heptadecanoate	5.82[d]	7.71[e]	0.28[a]	4.26[c]	0.82[b]
Methyl 3-methoxytetradecanoate	0.04[a]	0.13[b]	0.05[a]	0.06[a]	0.21[c]
Myristic acid	0.22[b]	0.27[c]	0.17[a]	0.26[c]	0.53[d]
n-Pentadecanol	0.23[b]	0.86[d]	0.61[c]	0.86[d]	0.10[a]
Oleic acid	18.74[b]	22.20[c]	25.19[e]	15.23[a]	24.33[d]
Palmitic acid	8.88[bc]	8.07[b]	7.08[a]	9.00[c]	21.72[d]
Palmitoleic acid	1.73[b]	2.45[c]	1.53[a]	2.53[c]	7.25[d]
Squalene	0.35[a]	0.34[a]	0.42[a]	0.40[a]	2.23[b]
Stearic acid	14.40[c]	18.04[d]	30.41[e]	12.77[b]	4.90[a]

[Values within a row for a particular lipophilic metabolite following different letters are significantly different at $P = 0.05$]

of (*Z*)-14-tricosenyl formate, gamma-ergostenol, 9-octadecen-1-ol, cholesterol, chondrillasterol, eicosanoic acid, erucic acid, margaric acid, methyl 11-eicosenoate, methyl 14-methylhexadecanoate and methyl 16-methyl-heptadecanoate was observed among the *C. partellus* larvae reared on resistant or susceptible sorghum genotypes (Table 4).

Association of lipophilic metabolites in sorghum with resistance parameters of *C. partellus*

Sorghum seedling lipophilic metabolites *viz.*, myristic acid, methyl 3-methoxytetradecanoate, stearic acid, squalene, hexacontane and tetrapentacontane showed a significant and positive association (*, ** = *r* significant at $P = 0.05$ and 0.01, respectively) with *C. partellus* deadhearts ($r = 0.54^*$ to 0.89^{**}), larval weight ($r = 0.54^*$ to 0.74^{**}), pupal weight ($r = 0.55^*$ to 0.80^{**}), larval survival ($r = 0.54^*$ to 0.83^{**}), adult emergence ($r = 0.55^*$ to 0.84^{**}), antibiosis index ($r = 0.57^*$ to 0.89^{**}) and overall resistance index ($r = 0.54^*$ to 0.90^{**}), while negative association with larval period ($r = -0.57^*$ to -0.82^{**}). Fucosterol was found significantly and positively associated with larval survival, pupal period, adult emergence, antibiosis index, overall resistance index, while negative association with larval period. Conversely, l-(+)-ascorbic acid 2,6-dihexadecanoate, lignoceric acid and stigmasterol showed a significant and positive association with larval period ($r = 0.60^*$ to 0.86^{**}), and negative association with deadhearts ($r = -0.61^*$ to -0.82^{**}), larval weight ($r = -0.51^*$ to -0.66^{**}), pupal weight ($r = -0.51^*$ to -0.58^*), larval survival ($r = -0.65^*$ to -0.82^{**}), adult emergence ($r = -0.66^*$ to -0.83^{**}), antibiosis index ($r = -0.59^*$ to -0.83^{**}) and overall resistance index ($r = -0.60^*$ to -0.83^{**}), indicating their role in resistance to *C. partellus* in sorghum (Table 5). Furthermore, 2-pentadecanone, 6,10,14-trimethyl, and palmitic acid were also found significantly and negatively associated with larval survival, pupal weight and adult emergence (Table 5).

Contribution of sorghum lipophilic metabolites in resistance to *C. partellus*

Multiple linear regression analysis of lipophilic metabolites in sorghum seedlings with *C. partellus* damage and biological parameters revealed that the myristic acid, 2-pentadecanone, 6,10,14-trimethyl (except deadhearts and larval weight), stearic acid, lignoceric acid (except pupal weight), l-(+)-ascorbic

Table 5 — Association of lipophilic metabolites in sorghum seedlings with different biological parameters and indices of *Chilo partellus*

Lipophilic metabolites in sorghum seedlings	Correlation coefficients (*r*) with biological parameters and indices of *C. partellus*								
	Stem borer deadhearts	Larval weight	Larval survival	Larval period	Pupal weight	Pupal period	Adult emergence	Antibiosis index	Overall resistance index
Myristic acid (X_1)	0.79**	0.59*	0.72**	−0.59*	0.60*	−0.05	0.72**	0.70**	0.73**
2-Pentadecanone, 6,10,14-trimethyl (X_2)	−0.43	−0.47	−0.52*	0.58*	−0.66**	−0.20	−0.51*	−0.55*	−0.52*
Palmitic acid (X_4)	−0.34	−0.43	−0.52*	0.58*	−0.57*	−0.57*	−0.51*	−0.48	−0.45
l-(+)-Ascorbic acid 2,6-dihexadecanoate (X5)	−0.82**	−0.66**	−0.82**	0.86**	−0.58*	0.11	−0.83**	−0.83**	−0.83**
Margaric acid (X_6)	−0.45	−0.30	−0.45	0.34	−0.66**	−0.26	−0.45	−0.42	−0.43
Methyl 3-methoxytetradecanoate (X_7)	0.69**	0.39	0.61*	−0.45	0.48	−0.06	0.62*	0.57*	0.60*
Linoleic acid (X_8)	0.29	0.37	0.35	−0.48	0.02	−0.12	0.34	0.37	0.35
Linolenic acid (X_9)	−0.15	−0.02	−0.06	−0.09	0.15	0.15	−0.06	−0.02	−0.05
Phytol (X_{10})	0.18	0.33	0.25	−0.43	0.13	−0.15	0.24	0.33	0.29
Stearic acid (X_{11})	0.54*	0.54*	0.63*	−0.57*	0.55*	0.45	0.63*	0.57*	0.57*
Tetracosane (X_{12})	0.19	0.08	0.16	0.01	0.12	0.23	0.16	0.08	0.11
Methyl 11-eicosenoate (X_{13})	0.51*	0.26	0.41	−0.25	0.57*	0.07	0.42	0.38	0.42
Eicosanoic acid (X_{14})	0.28	−0.01	0.13	0.04	0.04	−0.16	0.15	0.08	0.13
9-Hexacosene (X_{15})	−0.29	−0.14	−0.29	0.15	−0.13	−0.34	−0.30	−0.19	−0.21
Behenic acid (X_{16})	0.00	0.14	0.00	−0.15	0.06	−0.38	−0.01	0.12	0.09
1-Heptacosanol (X_{17})	0.13	0.37	0.33	−0.42	0.34	0.55*	0.32	0.31	0.27
Lignoceric acid (X_{18})	−0.61*	−0.51*	−0.69**	0.65**	−0.33	−0.36	−0.70**	−0.59*	−0.60*
Squalene (X_{19})	0.63*	0.38	0.54*	−0.38	0.44	−0.04	0.55*	0.50	0.54*
Hexacontane (X_{20})	0.60*	0.54*	0.72**	−0.65**	0.65**	0.63*	0.72**	0.62*	0.62*
Tetrapentacontane (X_{21})	0.89**	0.74**	0.83**	−0.82**	0.80**	−0.25	0.84**	0.89**	0.90**
Cholesterol (X_{22})	0.26	0.33	0.24	−0.36	0.29	−0.36	0.23	0.36	0.34
alpha-Tocopherol (X_{23})	0.21	0.41	0.36	−0.54*	0.18	0.14	0.35	0.39	0.35
Campesterol (X_{24})	−0.36	−0.12	−0.23	0.16	−0.51*	0.34	−0.23	−0.29	−0.31
Stigmasterol (X_{25})	−0.77**	−0.44	−0.65**	0.60*	−0.51*	0.39	−0.66**	−0.65**	−0.68**
gamma-Sitosterol (X_{26})	−0.46	−0.20	−0.40	0.27	−0.20	−0.01	−0.41	−0.33	−0.36
Stigmastanol (X_{27})	0.11	0.25	0.23	−0.22	0.33	0.50	0.22	0.20	0.18
Fucosterol (X_{28})	0.50	0.48	0.62*	−0.56*	0.49	0.62*	0.62*	0.52*	0.52*
beta-Amyrin (X_{29})	−0.04	0.25	0.19	−0.37	0.01	0.49	0.18	0.16	0.11
Cycloartenol (X_{30})	0.11	0.34	0.31	−0.47	0.32	0.47	0.30	0.32	0.27
alpha-Amyrin (X_{31})	−0.51*	−0.21	−0.36	0.18	−0.25	0.22	−0.37	−0.33	−0.37

*, ** = Correlation coefficients significant at $P = 0.05$ and 0.01, respectively.

Multiple linear regression: stem borer deadhearts = $69.9 + 30.1X_1 + 0.25X_5 + 57.8X_7 + 4.75X_{11} - 100X_{13} - 48X_{18} - 100.4X_{19} + 63X_{20} - 15.4X_{21} - 2.96X_{25} + 92X_{31}$ (R^2 = 98.0%); larval weight = $-216 + 634X_1 + 4.02X_5 + 57.4X_{11} + 88.2X_{18} + 455X_{20} - 785X_{21}$ (R^2 = 47.5%); larval survival = $3.67 + 176.25X_1 - 481.4X_2 - 0.64X_4 - 1.83X_5 + 37.96X_7 - 25.2X_{11} + 165.18X_{18} - 127.4X_{19} + 22.66X_{20} + 57.55X_{21} - 2.52X_{25} + 109.95X_{28}$ (R^2 = 100.0%); larval period = $12.3 - 27.3X_1 - 111.4X_2 + 0.15X_4 + 0.59X_5 + 1.97X_{11} - 6.1X_{18} + 91X_{20} - 15.4X_{21} + 5.7X_{23} - 2.49X_{25} + 53.5X_{28}$ ($R2$ = 98.7%); pupal weight = $104.8 + 368X_1 - 777X_2 - 1.71X_4 - 1.74X_5 + 39X_6 + 33.1X_{11} - 49X_{13} + 35X_{20} + 233X_{21} + 7.5X_{24} - 26.35X_{25}$ (R^2 = 90.7%); pupal period = $12.35 + 0.002X_4 - 7.99X_{17} + 6.91X_{20} - 6.85X_{28}$ (R^2 = 62.4%); adult emergence = $-8.2 + 195.7X_1 - 443.6X_2 - 0.46X_4 - 0.42X_5 + 16.1X_7 - 25.34X_{11} + 144.1X_{18} - 102.7X_{19} + 13.5X_{20} + 39X_{21} - 0.91X_{25} + 115X_{28}$ (R^2 = 99.9%); antibiosis index = $-7.67 + 11.52X_1 - 40.6X_2 - 0.06X_5 + 4.39X_7 - 1.35X_{11} + 12.2X_{18} - 11.95X_{19} + 16.4X_{20} - 3.94X_{21} + 0.16X_{25} + 14.88X_{28}$ (R^2 = 98.2%); overall resistance index = $7.05 + 13.9X_1 - 5.7X_2 - 0.17X_5 - 1.65X_7 + 0.97X_{11} + 0.48X_{18} - 2.54X_{20} + 2.45X_{21} - 0.25X_{25} - 5.19X_{28}$ (R^2 = 95.4%).

Stepwise regression: stem borer deadhearts = $51.69 + 93.68X_1 - 48.81X_{13} - 70.48X_{19} - 1.43X_{25} + 30.04X_{31}$ (R^2 = 99.0%); larval weight = $-51.4 + 478X_1 + 114.1X_{18}$ (R^2 = 55.9%); larval survival = $3.67 + 176.25X_1 - 481.4X_2 - 0.64X_4 - 1.83X_5 + 37.96X_7 - 25.2X_{11} + 165.18X_{18} - 127.4X_{19} + 22.66X_{20} + 57.55X_{21} - 2.52X_{25} + 109.95X_{28}$ (R^2 = 100.0%); larval period = $-4.88 - 187.9X_2 + 0.81X_5 + 106.74X_{20} - 2.22X_{25} + 76.57X_{28}$ (R^2 = 99.2%); pupal weight = $59.1 + 406.3X_1 - 629.8X_2 + 28.32X_{11} + 101.3X_{21} + 14.87X_{24} - 23.39X_{25}$ (R^2 = 94.2%); pupal period = $12.38 - 8.01X_{17} + 6.87X_{20} - 6.85X_{28}$ (R^2 = 65.8%); adult emergence = $-1.8 + 207.85X_1 - 431.7X_2 - 0.52X_4 - 0.41X_5 - 26.41X_{11} + 138.1X_{18} - 96.4X_{19} + 49.6X_{21} + 111.7X_{28}$ (R^2 = 99.9%); antibiosis index = $3.62 + 9.25X_1 - 7.65X_2 - 0.08X_5 + 3.17X_{18} - 3.01X_{19}$ (R^2 = 97.1%); overall resistance index = $6.31 + 12.1X_1 - 0.13X_5 + 1.08X_{11} - 4.36X_{20} - 5.55X_{28}$ (R^2 = 97.5%)

acid 2,6-dihexadecanoate, stigmasterol (except larval weight), palmitic acid (except deadhearts and larval weight), hexacontane and tetrapentacontane for deadhearts, larval weight, larval survival, larval period, pupal weight and emergence; including methyl 3-methoxytetradecanoate and squalene for deadhearts, larval survival and adult emergence; fucosterol for larval survival, larval period and adult emergence; methyl 11-eicosenoate for deadhearts and pupal weight; alpha-amyrin for deadhearts; alpha-

tocopherol for larval period; and margaric acid and campesterol for pupal weight explained 47.5 to 100% variability in these the damage and biological parameters of *C. partellus* (Table 5). The multiple linear regression analysis of sorghum seedling lipophilic metabolites with antibiosis and overall resistance indices of *C. partellus* revealed that 94.5 to 98.2% variability in these parameters was due to myristic acid, 2-pentadecanone, 6,10,14-trimethyl, l-(+)-ascorbic acid 2,6-dihexadecanoate, methyl 3-methoxytetradecanoate, stearic acid, lignoceric acid, squalene, hexacontane, tetrapentacontane, stigmasterol and fucosterol (Table 5).

Furthermore, the stepwise regression analysis suggested that myristic acid, 2-pentadecanone, 6,10,14-trimethyl, cinnamic acid, palmitic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate, methyl 3-methoxy-tetradecanoate (except adult emergence), stearic acid, lignoceric acid, squalene, hexacontane (except larval survival), tetrapentacontane and fucosterol in sorghum seedlings explained 99.9 to 100.0% variability in *C. partellus* larval survival and adult emergence (Table 5). The stepwise regression further explained that the 99.0% variability in *C. partellus* deadhearts was due to myristic acid, methyl 11-eicosenoate, squalene, stigmasterol and alpha-amyrin; 55.9% variability in larval weight due to myristic acid and lignoceric acid; 99.2% variability in larval period due to 2-pentadecanone, 6,10,14-trimethyl, l-(+)-ascorbic acid 2,6-dihexadecanoate, squalene, campesterol and stigmastanol; 94.2% variability in pupal weight due to myristic acid, 2-pentadecanone, 6,10,14-trimethyl, stearic acid, tetrapentacontane, campesterol and stigmasterol; and 65.8% variability in pupal period of *C. partellus* due to 1-heptacosanol, hexacontane and fucosterol contents in the seedlings of test sorghum genotypes (Table 5). The stepwise regression analysis further revealed that the sorghum seedling lipophilic metabolites *viz.*, myristic acid, 2-pentadecanone, 6,10,14-trimethyl, l-(+)-ascorbic acid 2,6-dihexadecanoate, lignoceric acid and squalene contributed to 97.1% variability in *C. partellus* antibiosis index, while myristic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate, stearic acid, hexacontane, stigmasterol and fucosterol contributed to 97.5% variability in overall resistance index of *C. partellus* (Table 5).

Association of lipophilic metabolites in *C. partellus* larvae with resistance parameters

Lipophilic metabolites in the *C. partellus* larvae reared on different sorghum genotypes revealed that the contents of gamma-ergostenol, 1-octadecanol, cholesterol, eicosanoic acid, lathosterol, linoleic acid, margaric acid, methyl 14-methylhexadecanoate, methyl 3-methoxytetradecanoate, myristic acid, palmitic acid, palmitoleic acid and squalene were significantly and positively associated (*, ** = r significant at P = 0.05 and 0.01, respectively) with *C. partellus* deadhearts (r = 0.59* to 0.99**), larval weight (r = 0.51* to 0.72**), pupal weight (r = 0.52* to 0.78**), larval survival (r = 0.58* to 0.89**), adult emergence (r = 0.58* to 0.90**), antibiosis index (r = 0.54* to 0.92**) and overall resistance index (r = 0.56* to 0.94**); while negative association with larval period (r = −0.58* to −0.83**), except in a few cases where the correlation coefficients were non-significant (Table 6). However, 1-triacontanol, l-(+)-ascorbic acid 2,6-dihexadecanoate, n-pentadecanol, and stearic acid were significantly and negatively associated with *C. partellus* deadhearts (r = −0.62* to −0.84**), larval weight (r = −0.53* to −0.64**), pupal weight (r = −0.57* to −0.88*), larval survival (r = −0.53* to −0.80**), adult emergence (r = −0.56* to −0.81**), antibiosis index (r = −0.54* to −0.80**) and overall resistance index (r = −0.56* to −0.82**); and positively associated with larval period (r = 0.53* to 0.70**), except in a few cases where the correlation coefficients were non-significant, indicating their deleterious effects on various biological attributes of *C. partellus* leading to resistance in sorghum (Table 6).

Contribution of *C. partellus* larval lipophilic metabolites in resistance parameters

Multiple linear regression analysis of lipophilic metabolites in *C. partellus* larvae with plant damage and biological parameters revealed that the contents of linoleic acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, methyl 3-methoxy-tetradecanoate, squalene (except larval period), gamma-ergostenol (except pupal weight), 1-triacontanol, cholesterol (except deadhearts), eicosanoic acid (except pupal weight), lathosterol (except larval weight and period) and margaric acid (except pupal weight and adult emergence), including methyl 14-methylhexadecanoate for deadhearts, larval weight and larval period; erucic acid for larval period; 1-nonadecene for pupal weight; and n-pentadecanol for pupal weight and adult emergence explained 56.6 to 100% variability in deadhearts, larval weight, larval survival, larval period, pupal weight and adult emergence of *C. partellus* (Table 6). Furthermore, 73.9% and 84.4% variability in antibiosis index and

Table 6 — Association of lipophilic metabolites in *Chilo partellus* larvae with different biological parameters and indices of spotted stem borer

Lipophilic metabolites in *C. partellus* larvae	Correlation coefficients (*r*) with biological parameters and indices of *C. partellus*								
	Stem borer deadhearts	Larval weight	Larval survival	Larval period	Pupal weight	Pupal period	Adult emergence	Antibiosis index	Overall resistance index
(*Z*)-14-Tricosenyl formate (X_1)	0.38	0.05	0.18	−0.02	0.05	−0.43	0.19	0.17	0.22
gamma-Ergostenol (X_2)	0.76**	0.64**	0.71**	−0.77**	0.46	−0.34	0.72**	0.76**	0.77**
1,16-Hexadecanediol (X_3)	0.07	0.26	0.19	−0.37	−0.02	−0.03	0.18	0.23	0.19
1-Nonadecene (X_4)	−0.50	−0.31	−0.49	0.33	−0.57*	−0.33	−0.50	−0.42	−0.44
1-Octadecanol (X_5)	−0.62*	−0.41	−0.60*	0.45	−0.63*	−0.30	−0.61*	−0.54*	−0.56*
1-Triacontanol (X_6)	−0.84**	−0.64**	−0.80**	0.70**	−0.88**	−0.04	−0.81**	−0.80**	−0.82**
9-Octadecen-1-ol (X_7)	0.37	0.46	0.45	−0.60*	0.18	−0.10	0.44	0.49	0.46
Cholesterol (X_8)	0.50	0.61*	0.66**	−0.79**	0.52*	0.30	0.65**	0.66**	0.63*
Chondrillasterol (X_9)	0.35	0.16	0.17	−0.15	−0.02	−0.72**	0.17	0.24	0.27
Eicosanoic acid (X_{10})	0.77**	0.61*	0.70**	−0.73**	0.43	−0.40	0.70**	0.74**	0.76**
Erucic acid (X_{11})	0.43	0.46	0.46	−0.58*	0.14	−0.22	0.46	0.50	0.48
l-(+)-Ascorbic acid 2,6-dihexadecanoate (X12)	−0.64**	−0.46	−0.55*	0.53*	−0.21	0.35	−0.56*	−0.56*	−0.58*
Lathosterol (X_{13})	0.59*	0.42	0.62*	−0.48	0.63*	0.41	0.63*	0.54*	0.56*
Linoleic acid (X_{14})	0.99**	0.72**	0.89**	−0.83**	0.78**	−0.26	0.90**	0.92**	0.94**
Margaric acid (X_{15})	0.61*	0.56*	0.59*	−0.68**	0.32	−0.33	0.59*	0.65**	0.64**
Methyl 11-eicosenoate (X_{16})	0.25	0.22	0.25	−0.30	−0.16	−0.15	0.25	0.23	0.24
Methyl 14-methylhexadecanoate (X_{17})	0.63*	0.53*	0.58*	−0.64**	0.30	−0.41	0.58*	0.63*	0.64**
Methyl 16-methyl-heptadecanoate (X_{18})	−0.10	0.19	0.12	−0.30	0.08	0.42	0.11	0.12	0.07
Methyl 3-methoxytetradecanoate (X_{19})	0.91**	0.63*	0.77**	−0.73**	0.66**	−0.48	0.78**	0.83**	0.85**
Myristic acid (X_{20})	0.89**	0.61*	0.75**	−0.65**	0.80**	−0.36	0.76**	0.80**	0.83**
n-Pentadecanol (X_{21})	−0.63*	−0.41	−0.63*	0.50	−0.57*	−0.35	−0.65**	−0.55*	−0.57*
Oleic acid (X_{22})	0.45	0.27	0.37	−0.30	0.04	−0.23	0.38	0.33	0.37
Palmitic acid (X_{23})	0.86**	0.52*	0.71**	−0.59*	0.74**	−0.31	0.72**	0.74**	0.77**
Palmitoleic acid (X_{24})	0.86**	0.51*	0.69*	−0.58*	0.70**	−0.43	0.70**	0.73**	0.77**
Squalene (X_{25})	0.80**	0.53*	0.65**	−0.50	0.69**	−0.31	0.66**	0.68**	0.72**
Stearic acid (X_{26})	−0.68**	−0.53*	−0.64**	0.60*	−0.82**	0.04	−0.64**	−0.68**	−0.69**

*, ** = Correlation coefficients significant at $P = 0.05$ and 0.01, respectively.

Multiple linear regression: stem borer deadhearts = $162.4 - 165.6X_2 - 1224X_6 - 202X_{10} - 252.7X_{13} + 28.23X_{14} + 15.25X_{15} + 34.4X_{17} + 871X_{19} - 209.3X_{20} - 13.36X_{23} + 13.38X_{24} - 12.78X_{25} + 5.38X_{26}$ (R^2 = 100.0%); larval weight = $8138 - 19521X_2 - 36832X_6 - 1345X_8 - 8254X_{10} + 302X_{14} + 4439X_{15} + 10458X_{17} + 15408X_{19} - 2481X_{20} - 280X_{23} + 311X_{24} - 496X_{25} + 100.7X_{26}$ (R^2 = 56.6%); larval survival = $461 - 1135X_2 - 4208X_6 + 22.4X_8 - 870X_{10} - 1229X_{13} + 139X_{14} + 29X_{15} + 3601X_{19} - 769X_{20} - 55.8X_{23} + 45.7X_{24} - 60.7X_{25} + 22.4X_{26}$ (R^2 = 95.4%); larval period = $157.9 + 12X_2 - 455X_6 + 17.6X_8 + 128.4X_{10} - 445X_{11} - 9.09X_{14} + 181X_{15} + 100.4X_{17} - 165X_{19} - 85X_{20} - 4.54X_{23} + 3.28X_{24} + 1.63X_{26}$ (R^2 = 98.9%); pupal weight = $894 - 171X_4 - 3077X_6 - 27.8X_8 - 846X_{13} - 2.49X_{14} + 702X_{19} - 203X_{20} - 5.3X_{21} - 22.63X_{23} + 23.54X_{24} - 16X_{25} + 5.66X_{26}$ (R^2 = 94.4%); adult emergence = $628.9 - 1187X_2 - 5374X_6 + 28.3X_8 - 1074X_{10} - 1735X_{13} + 171.8X_{14} + 4483X_{19} - 923X_{20} + 48.36X_{21} - 70.64X_{23} + 56.44X_{24} - 75.17X_{25} + 27.91X_{26}$ (R^2 = 99.9%); antibiosis index = $41.2 - 15.1X_2 - 323X_6 - 0.52X_8 - 62.1X_{10} - 78X_{13} + 8.6X_{14} + 16X_{17} + 236X_{19} - 40.9X_{20} - 4.09X_{23} + 3.58X_{24} - 4.89X_{25} + 1.54X_{26}$ (R^2 = 73.9%); overall resistance index = $44.3 - 16.6X_2 - 350X_6 - 0.42X_8 - 66.9X_{10} - 84X_{13} + 9.3X_{14} + 16.5X_{17} + 257X_{19} - 45.6X_{20} - 4.4X_{23} + 3.87X_{24} - 5.2X_{25} + 1.67X_{26}$ (R^2 = 84.6%).

Stepwise regression: stem borer deadhearts = $162.4 - 165.6X_2 - 1224X_6 - 202X_{10} - 252.7X_{13} + 28.23X_{14} + 15.25X_{15} + 34.4X_{17} + 871X_{19} - 209.3X_{20} - 13.36X_{23} + 13.38X_{24} - 12.78X_{25} + 5.38X_{26}$ (R^2 = 100.0%); larval weight = $228 - 918X_6 + 287.8X_{17} - 649X_{19}$ (R^2 = 62.4%); larval survival = $36.28 - 569.1X_2 + 15.78X_{14} - 4.02X_{23} - 10.2X_{25}$ (R^2 = 97.5%); larval period = $159.1 - 459X_6 + 17.82X_8 + 129X_{10} - 450.3X_{11} - 9.04X_{14} + 184.9X_{15} + 101.7X_{17} - 167.3X_{19} - 87.9X_{20} - 4.56X_{23} + 3.27X_{24} + 1.65X_{26}$ (R^2 = 99.5%); pupal weight = $719 - 222.5X_4 - 2189X_6 - 941X_{13} + 382X_{19} - 19.49X_{23} + 20.25X_{24} - 11.88X_{25} + 5.18X_{26}$ (R^2 = 94.7%); adult emergence = $628.9 - 1187X_2 - 5374X_6 + 28.3X_8 - 1074X_{10} - 1735X_{13} + 171.8X_{14} + 4483X_{19} - 923X_{20} + 48.36X_{21} - 70.64X_{23} + 56.44X_{24} - 75.17X_{25} + 27.91X_{26}$ (R^2 = 99.9%); antibiosis index = $5.44 - 9.16X_6 + 0.3X_{14} - 0.13X_{23}$ (R^2 = 94.0%); overall resistance index = $5.53 - 10.32X_6 + 0.4X_{14} - 0.12X_{23} - 0.29X_{25}$ (R^2 = 96.8%).

overall resistance index of *C. partellus* were recorded due to eicosanoic acid, linoleic acid, myristic acid, methyl 14-methylhexadecanoate, methyl 3-methoxy-tetradecanoate, palmitic acid, palmitoleic acid, stearic acid, squalene, gamma-ergostenol, 1-triacontanol, lathosterol and cholesterol (Table 6).

Stepwise regression analysis of *C. partellus* larval lipophilic metabolites revealed 100.0% variability in deadhearts due to gamma-ergostenol, 1-triacontanol, eicosanoic acid, lathosterol, linoleic acid, margaric acid, methyl 14-methylhexadecanoate, methyl 3-methoxy-tetradecanoate, myristic acid, palmitic acid,

palmitoleic acid, squalene and stearic acid (Table 6). Further, the stepwise regression analysis of *C. partellus* larval lipophilic metabolites with different biological parameters revealed that the content of 1-triacontanol, methyl 14-methylhexadecanoate and methyl 3-methoxy-tetradecanoate explained 64.2% variability in larval weight; gamma-ergostenol, linoleic acid, palmitic acid and squalene explained 97.5% variability in larval survival; 1-triacontanol, cholesterol, eicosanoic acid, erucic acid, linoleic acid, margaric acid, methyl 14-methylhexadecanoate, methyl 3-methoxytetradecanoate, myristic acid, palmitic acid, palmitoleic acid and stearic acid explained 99.5% variability in larval period; 1-nonadecene, 1-triacontanol, lathosterol, methyl 3-methoxytetradecanoate, palmitic acid, palmitoleic acid, squalene, and stearic acid explained 94.7% variability in pupal weight; and gamma-ergostenol, 1-triacontanol, cholesterol, eicosanoic acid, lathosterol, linoleic acid, methyl 3-methoxy-tetradecanoate, myristic acid, n-pentadecanol, palmitic acid, palmitoleic acid, squalene and stearic acid explained 99.9% variability in adult emergence (Table 6). The stepwise regression analysis further revealed that the *C. partellus* larval lipophilic metabolites *viz.*, 1-triacontanol, linoleic acid and palmitic acid contributed to 94.0% variability in antibiosis to *C. partellus*, while 1-triacontanol, linoleic acid, palmitic acid and squalene contributed to 96.8% variability in overall plant resistance to *C. partellus* (Table 6).

Discussion

Plant defense against herbivores appears to be complex trait and depends on the interplay of several componential factors including biochemicals[4]. Knowledge on plant-insect biochemical interactions is propelling factor to understand dynamics of plant resistance to herbivores[13,19]. The developmental response of *C. partellus* on different host crops and genotypes, and bio-chemical composition of the host plants have been found to play an important role in plant defense against this pest[8,22,36]. Present studies revealed less plant deadhearts, longer developmental period, reduced weight, and lower larval survival and adult emergence of *C. partellus* in the sorghum germplasm lines IS 2123 and IS 2205 followed by varieties ICSV 700 and ICSV 708 in comparison to susceptible variety, Swarna, indicating variable levels of resistance in test sorghum genotypes against this pest. This differential effect of test genotypes on insect biological attributes could be due to variation in biochemical composition which in turn is a result of genetic makeup of the host plants[8,37].

The allelo-chemicals and nutritional composition determines the host plant quality, and the variation in abundance and performance of herbivorous insects is host plant quality-dependent[38,39]. The knowledge on biochemistry of host plants and the insect pests in response to feeding on diverse food sources better explains insect-plant interactions[9]. Present studies found significant differences in all the lipophilic metabolite components among test sorghum genotypes and in the *C. partellus* larvae reared on these genotypes, except for cholesterol in sorghum seedlings. The contents of myristic acid, palmitic acid, linoleic acid, linolenic acid, eicosanoic acid and behenic acid were significantly lower, while hexacontane and gamma-sitosterol higher, in the seedlings of test sorghum genotypes as compared to susceptible genotype, Swarna. The lipophilic assimilation studies in mosquitoes reared on a range of larval diets revealed greatest impact on fatty acid profiles which exhibited a high degree of dietary routing along with *de-novo* synthesis of a number of important fatty acids[30]. Further, sitosterol has been reported to convert into stigmasterol in plants in response to infections, thus making it more resistant to such infections[40]. Present studies thus indicate that the variation in lipophilic metabolites across sorghum genotypes could be due to different genetic backgrounds and varying response to similar environmental conditions[5,21].

The lower contents of linolenic acid, methyl 3-methoxytetradecanoate, myristic acid, oleic acid, palmitic acid, palmitoleic acid, lathosterol and squalene in *C. partellus* larvae fed on resistant genotypes than on susceptible genotype in the present study indicate that these lipophilic metabolites could be the rate limiting factors for the larval development. The higher content of lipophilic metabolites such as 1,16-hexadecanediol, 1-nonadecene, 1-octadecanol, 1-triacontanol, l-(+)-ascorbic acid 2,6-dihexadecanoate, n-pentadecanol and stearic acid in *C. partellus* larvae fed on resistant genotypes than on susceptible variety, could be to revive the insect larvae from host plant stress and support various physiological and biological functions. Hydrocarbons like nonadecenes and 1-octadecanol have been reported as component of pheromones[23]. Fatty alcohol, 1-triacontanol act as growth stimulant in plants and feeding stimulant in

insects[25], and also constituent of waxes in both plants and insects[24]. The ascorbic acid content is positively associated with larval survival in codling moth, *Carpocapsa pomonella* (L.)[41], while l-(+)-ascorbic acid 2,6-dihexadecanoate considered as a potent inhibitor of hyaluronidase[42]. Since lipids and their metabolites are involved in various physiological and biological functions, present studies suggest that the lipophilic profiling of the herbivores along with their host plants could be helpful in identifying right kind of lipophilic compound to characterize plant defense and insect-plant biochemical interactions.

Nutrient and biochemical components of host plants play greater role in oviposition, feeding, development and survival of phytophagous insects, and express resistance or susceptibility reaction accordingly[4,8,22]. Present studies revealed that the fatty acids *viz.*, palmitoleic acid, margaric acid, methyl 16-methyl-heptadecanoate, oleic acid and erucic acid were not found in test sorghum seedlings but detected in the *C. partellus* larvae; while linolenic acid, behenic acid and lignoceric acid being present in sorghum seedlings were undetectable in *C. partellus* larvae. None of the fatty alcohols profiled were common in sorghum seedlings and the *C. partellus* larvae. The variations in these lipophilic metabolites in sorghum seedlings and the *C. partellus* larvae could be because of their specific requirement and involvement in different metabolic processes/ pathways of the host plant and the test insect. Fatty acids also act as secondary messengers to regulate the activity of transcription factors, and signal to alter lipid composition and adjustment of membrane fluidity[28]. They are also involved in regulatory activities through mediators like oxidatively modified lipids which specifically trigger diverse cellular processes and play a crucial role in various innate immune responses[29]. Of the lipophilic metabolites detected in test samples; myristic acid, palmitic acid, stearic acid and squalene were found present in both sorghum seedlings and the *C. partellus* larvae, and found significantly associated and contributed to variability in damage, development and survival, and resistance indices of *C. partellus* in sorghum. Earlier studies have elaborated that the polyunsaturated fatty acids are one of the most important dietary components of lepidopteran insects[14].

Furthermore, among the lipophilic metabolites present in both host plant and the test insect, methyl 3-methoxytetradecanoate and l-(+)-ascorbic acid 2,6-dihexadecanoate in sorghum seedlings, and margaric acid, linoleic acid, eicosanoic acid and cholesterol in the *C. partellus* larvae, were also found significantly associated and contributed to explain variability in damage, development and survival, and antibiosis and/or overall resistance indices of *C. partellus*, respectively. These findings thus indicate the importance of specified fatty acids and lipophilic metabolites including fatty alcohols in different biochemical processes of sorghum seedlings and the *C. partellus* larvae, pointing towards insect-plant biochemical disruptions and host plant selection by *C. partellus* in sorghum. The positive plant chemistry-herbivore association coincides with general co-evolutionary hypotheses. Assumption that plants maintain diverse mixtures of metabolites to defend from herbivores through action on different physiological or biochemical targets and reduction in herbivore damage, indicate importance in understanding insect-plant biochemical interactions[43]. The plant lipophilic variability having positive association with phytotoxicity could put positive effect on herbivore diversity while negative relationship with herbivory, suggesting that our studies on insect and host plant lipophilic profiling could also be an effective predictor of ecological interactions. Significant positive association of sorghum seedling lipophilic metabolites *viz.*, myristic acid, methyl 3-methoxytetradecanoate, stearic acid, squalene, fucosterol, hexacontane and tetrapentacontane; negative association of cinnamic acid, palmitic acid, l-(+)-ascorbic acid 2,6-dihexadecanoate, 2-pentadecanone, 6,10,14-trimethyl, lignoceric acid and stigmasterol; and involvement of one or the other aforesaid lipophilic metabolites in 60 to 100% variability in damage, biological parameters, and antibiosis and overall resistance indices clearly indicate their magnificent role in some biochemical processes and so in the resistance/susceptibility to *C. partellus* in sorghum. These findings clearly indicate that a large number of biochemical reactions take place in an individual at a particular time governed through intermediary metabolites, and the positive or negative association among metabolites of the individual reflects the interlinking of metabolic pathways depending on the need.

Furthermore, the significant and positive association of lipophilic metabolites in *C. partellus* larvae reared on different sorghum genotypes *viz.*, gamma-ergostenol, cholesterol, eicosanoic acid,

lathosterol, linoleic acid, margaric acid, methyl 14-methylhexadecanoate, methyl 3-methoxytetradecanoate, myristic acid, palmitic acid, palmitoleic acid and squalene; negative association of 1-triacontanol, n-pentadecanol and stearic acid; and involvement of one or more of these lipophilic metabolites in 64 to 100% variability in damage, biological parameters, and antibiosis and overall resistance indices suggest their involvement in insect-plant biochemical interactions and plant defense to *C. partellus* in sorghum. The susceptible and resistant genotypes contain different metabolites, which on utilization as food by the *C. partellus* larvae might have induced different responses in the insect. Earlier studies reported positive association of dietary linoleic acid content with scale condition and adult emergence in red-banded leaf roller, *Argyrotaenia velutinana* (Walker)[44]. The butterfly species, *Morpho peleides* Limpida contains higher amount of linoleic and linolenic acids as compared to other poly-unsaturated fatty acids[27]. The components showing positive association belongs to the pathway required for growth and defense, while those having negative association needs to be suppressed for plant defense. Myristic acid, methyl 3-methoxytetradecanoate and squalene in both sorghum seedlings and the *C. partellus* larvae having significant and positive association with damage, biological parameters and resistance indices indicate their role in different life system metabolic processes of the host plant and the test insect. On the contrary, stearic acid in sorghum seedlings showed significantly positive, while that in spotted stem borer larvae negative association with damage, biological parameters and resistance indices indicate its role in plant defense to *C. partellus*.

Conclusion

Overall, the present studies suggest that the myristic acid, methyl 3-methoxytetradecanoate, palmitic acid, stearic acid and squalene present in both host plant and the test insect, contributed significantly to explain variability in resistance against *Chilo partellus*, thus could be used as biomarkers for sorghum-stem borer interactions. This study will be helpful in understanding the role and contribution of certain lipophilic metabolites in plant-insect interactions and sorghum plant defense from *C. partellus.*

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Conflict of Interest

The authors declare that they have no conflicts of interest.

References

1 Chiozza MV, O'Neal ME & MacIntosh GC, Constitutive and induced differential accumulation of amino acid in leaves of susceptible and resistant soybean plants in response to the soybean aphid (Hemiptera: Aphididae). *Environ Entomol*, 39 (2010) 856.

2 San Francisco M & Cooper-Driver G, Anti-microbial activity of phenolic acids in *Pteridium aquilinum*. *Am Fern J*, 74 (1984) 87.

3 Cipollini D, Stevenson R, Enright S, Eyles A & Bonello P, Phenolic metabolites in leaves of the invasive shrub, *Lonicera maackii*, and their potential phytotoxic and anti-herbivore effects. *J Chem Ecol*, 34 (2008) 144.

4 Sharma HC, *Biotechnological approaches for pest management and ecological sustainability*, (CRC Press, Taylor and Francis Group, LLC, USA), 2009, 526.

5 Kumar S, Singh YP, Singh SP & Singh R, Physical and biochemical aspects of host plant resistance to mustard aphid, *Lipaphis erysimi* (Kaltenbach) in rapeseed-mustard. *Arthro Plant Interact*, 11 (2017) 551.

6 Lieurance D, Chakraborty S, Whitehead SR, Powell JR, Bonello P, Bowers MD & Cipollini D, Comparative herbivory rates and secondary metabolite profiles in the leaves of native and non-native *Lonicera* species. *J Chem Ecol*, 41 (2015) 1069.

7 Howe GA & Jander G, Plant immunity to insect herbivores. *Annu Rev Plant Biol*, 59 (2008) 41.

8 Dhillon MK & Kumar S, Amino acid profiling of *Sorghum bicolor* vis-à-vis *Chilo partellus* (Swinhoe) for biochemical interactions and plant resistance. *Arthro Plant Interact*, 11 (2017) 537.

9 Dadd RH, Nutrition: Organisms. In: *Comparative Insect Physiology, Biochemistry and Pharmacology*, (Eds. GA Kerkut & LI Gilbert; Pergamon Press, Oxford, United Kingdom), 1985, 313.

10 Auclair JL, Aphid feeding and nutrition. *Annu Rev Entomol*, 8 (1963) 439.

11 Weibull J, Glutamic-acid content of phloem sap is not a good predictor of plant resistance to *Rhopalosiphum padi*. *Phytochem*, 35 (1994) 601.

12 Chapman RF, *The Insects: Structure and Function*, 4th edition. (Cambridge University Press, UK), 1998.

13 Dhillon MK, Kumar S & Gujar GT, A common HPLC-PDA method for amino acid analysis in insects and plants. *Indian J Exp Biol*, 52 (2014) 73.

14 Kerkurt GA & Gilbert LI, *Comprehensive Insect Physiology, Biochemistry and Pharmacology*, (Pergamon Press, Oxford, UK), 1985.

15 Huang Y, Sharma HC & Dhillon MK, Bridging conventional and molecular genetics of sorghum insect resistance. In: *Plant Genetics and Genomics: Crops and Models*, (Ed. Paterson AK; Springer, New York, USA), 2013, 367.

16 Dhaliwal GS, Jindal V & Mohindru B, Crop losses due to insect pests: global and Indian scenario. *Indian J Entomol*, 77 (2015) 165.
17 Dhillon MK & Sharma HC, Paradigm shifts in research on host plant resistance to insect pests. *Indian J Plant Prot*, 40 (2012) 1.
18 Sharma HC, Dhillon MK, Pampapathy G & Reddy BVS, Inheritance of resistance to spotted stem borer, *Chilo partellus* in sorghum, *Sorghum bicolor*. *Euphytica*, 156 (2007) 117.
19 Kumar S & Dhillon MK, Lipophilic metabolite profiling of maize and sorghum seeds and seedlings, and their pest spotted stem borer larvae: A standardized GC-MS based approach. *Indian J Exp Biol*, 53 (2015) 170.
20 Torto B, Hassanali A & Saxena KN, Chemical aspects of *Chilo partellus* feeding on certain sorghum cultivars. *Insect Sci Appl*, 11 (1990) 649.
21 Kumar S, Singh D & Dutta M, Quality characteristics in rapeseed-mustard and role of some anti-nutritional factors in plant defense: future strategies. *J Oilseed Brassica*, 5 (2014) 87.
22 Dhillon MK & Chaudhary DP, Biochemical interactions for antibiosis mechanism of resistance to *Chilo partellus* (Swinhoe) in different maize types. *Arthro Plant Interact*, 9 (2015) 373.
23 Gilbert L, *Insect development: morphogenesis, molting and metamorphosis*, (Academic Press, USA) 2009, 730.
24 Szafranek B, Paszkiewicz M, Gołębiowski M & Stepnowski P, Gas chromatographic analysis of plant and insect surface compounds: cuticular waxes and terpenoids. In: *Gas Chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications*, (Ed. S Bekir; InTech Publisher, Croatia), 2012, 346.
25 Joshi UR & Kapadi AH, A Convenient synthesis of 1-triacontanol, a plant growth hormone. *Synth Commun*, 14 (1984) 681.
26 Kachroo P, Shanklin J, Shah J, Whittle EJ, Daniel F & Klessig DF, A fatty acid desaturase modulates the activation of defense signaling pathways in plants. *Proc Nat Acad Sci USA*, 98 (2001) 9448.
27 Wang Y, Lin DS, Bolewicz L & Connor WE, The predominance of polyunsaturated fatty acids in the butterfly *Morpho peleides* before and after metamorphosis. *J Lipid Res*, 47 (2006) 530.
28 Upchurch RG, Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. *Biotechnol Lett*, 30 (2008) 967.
29 Zoeller M, Stingl N, Krischke M, Fekete A, Waller F, Berger S & Mueller MJ, Lipid profiling of the Arabidopsis hypersensitive response reveals specific lipid peroxidation and fragmentation processes: biogenesis of pimelic and azelaic acid. *Plant Physiol*, 160 (2012) 365.
30 Hood-Nowotny R, Schwarzinger B, Schwarzinger C, Soliban S, Madakacherry O, Aigner M, Watzka M & Gilles J, An analysis of diet quality, how it controls fatty acid profiles, isotope signatures and stoichiometry in the malaria mosquito *Anopheles arabiensis*. *PLoS One*, 7 (2012) e45222.
31 Yang J, Kloepper JW & Ryu CM, Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci*,14 (2009) 1.
32 Lee B, Farag MA, Park HB, Kloepper JW, Lee SH & Ryu CM, Induced resistance by a long-chain bacterial volatile: elicitation of plant systemic defense by a C13 volatile produced by *Paenibacillus polymyxa*. *PLoS One*, 7 (2012) e48744.
33 Sharma HC, Taneja SL, Leuschner K & Nwanze KF, *Techniques to screen sorghum for resistance to insect pests*, (Information Bulletin No. 32. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India), 1992, 48.
34 Dhillon MK, Sharma HC, Singh R & Naresh JS, Mechanisms of resistance to shoot fly, *Atherigona soccata* in sorghum. *Euphytica*, 144 (2005) 301.
35 Neff WE, Adlof RO, List GR & EL-Agaimy M, Analysis of vegetable oil triglycerols by silver ion high performance liquid chromatography with flame ionization detector. *J Liq Chromatogr*, 17 (1994) 3951.
36 Dhillon MK & Chaudhary DP, Physicochemical mechanisms of resistance in sorghum to *Chilo partellus* (Swinhoe). *Indian J Exp Biol*, 56 (2018) 29.
37 Kumar VK, Sharma HC & Reddy KD, Antibiosis component of resistance to spotted stem borer, Chilo partellus in sorghum, *Sorghum bicolor*. *Crop Prot*, 25 (2006) 66.
38 Agrawal AA, Mechanisms, ecological consequences and agricultural implications of tri-trophic interactions. *Curr Opin Plant Biol*, 3 (2001) 329.
39 Baldwin IT, Halitschke R, Kessler A & Schittko U, Merging molecular and ecological approaches in plant-insect interactions. *Curr Opin Plant Biol*, 4 (2001) 351.
40 Griebel T & Zeier J, A role for beta-sitosterol to stigmasterol conversion in plant-pathogen interactions. *Plant J*, 63 (2010) 254.
41 Rock GC, Aseptic rearing of the codling moth on synthetic diets: Ascorbic acid and fatty acid requirements. *J Econ Entomol*, 60 (1967) 1002.
42 Botzki A, Rigden DJ, Braun S, Nukui M, Salmen S, Hoechstetter J, Bernhardt G, Dove S, Jedrzejas MJ & Buschauer A, L-ascorbic acid 6-hexadecanoate, a potent hyaluronidase inhibitor: X-ray structure and molecular modelling of enzyme-inhibitor complexes. *J Biol Chem*, 279 (2004) 45990.
43 Richards LA, Dyer LA, Forister ML, Smilanich AM, Dodson CD, Leonard MD & Jeffrey CS, Phytochemical diversity drives plant–insect community diversity. *Proc Nat Acad Sci USA*, 112 (2015) 10973.
44 Rock GC, Patton RL & Glass EH, Studies of the fatty acid requirement of *Argyrotaenia velutinana* (Walker). *J Insect Physiol*, 11 (1965) 91.