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Direct effect of CryIIa transgenic chickpea on coccinellid, *Cheilomenes sexmaculatus* (Fabricius)

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Abstract
The experiments were conducted during 2012-2014 at ICRISAT, Hyderabad to study the direct effects of transgenic chickpea lines on coccinellid beetle, *Cheilomenes sexmaculatus* (Fabricius). The direct effects on coccinellids were greater when fed on 0.1% *Bt* intoxicated diet, followed by diets with 0.05% and 0.02% *Bt*. The survival and development of coccinellid grubs were slightly affected when reared on aphids fed on diets with different concentrations (0.02%, 0.05% and 0.1%) of transgenic chickpea leaf powder. The coccinellids fed on diets with 0.05% BS5A.2(T2) 19-3P1 leaf powder showed a marginal reduction in survival and development as compared to that on other transgenic lines.

Keywords: CryIIa, *Cheilomenus sexmaculatus,* transgenic chickpea, direct effect

1. Introduction
Chickpea (*Cicer arietinum* L.) is the third most important pulse crop, grown in an area of 8.21 m ha, with a total production of 7.48 m tonnes globally [1]. The crop is largely grown by subsistence farmers in rain-fed areas (>70 per cent), which are less fertile and poor in moisture retention capacity. Genetically modified plants expressing *Bt* δ-endotoxin genes have been developed for resistance to insect pests, and some of them have been deployed successfully on a commercial scale for pest management [2]. Transgenic cotton and maize with resistance to lepidopteran insects have been released for cultivation in several countries, and were grown on more than 100 m ha worldwide in 2012. India ranks first in the world having 11.1 m ha area under *Bt*-cotton in 2011 (>90% of total cotton area in India), followed by China and USA [3]. The impact of genetically engineered insect-resistant crops on non-target organisms including biological control agents is one of the most widely discussed ecological effects. Natural enemies are of major concern as they often play an important role in regulation of pest populations, and are therefore of economic value. There is a concern that the insecticidal proteins expressed in transgenic plants may either effect the natural enemies directly (toxic effect) or indirectly (change in the prey or host-quality or abundance) [4].
Several studies have reported the direct and indirect effects of transgene products and the transgenic plants on the beneficial insects [5, 6, 7, 8, 9]. The *Bt* toxins are not transported to the phloem in some crops, and therefore, insect pests such as corn leaf aphid, *Rhopalosiphum maidis* (Fitch.) and the natural enemies feeding on it are not directly affected by the *Bt* toxins [10, 11]. The cotton aphid, *Aphis gossypii* Glover, is insensitive to *Bt* toxins, but trace amounts of *Bt* toxins were detected in the aphids when fed on *Bt* cotton [12]. Presence of Cry IAc toxin in phloem sap from *Bt*-oilseed rape and in *Myzus persicae* Sulzer has indicated the importance of having an estimate of the effects of expected amounts of *Bt* toxins in the diets of non-target organisms predating on aphids fed on the transgenic crops [13]. Moreover, some *Bt* isolates such as INS 2.13, HFZ 24.8 and GU 9.1 exhibit different levels of toxicity (LC_{50} values of 62, 328 and 114 ng/ml, respectively to the cotton aphid, *A. gossypii* [14]. The main objective of the study is direct effects of transgenic chickpea lines to generalist predator, *Cheilomenes sexmaculatus*.

2. Materials and Methods
Studies were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during 2012-14. The six transgenic chickpea lines, BS5A.1(T2) 18-1P1, BS5A.1(T2) 18-2P1, BS5A.2(T2) 19-1P2, BS5A.2(T2) 19-2P1, BS5A.2(T2) 19-3P1, BS5A.2(T2) 19-3P2 and two non transgenic chickpea lines, ICC506 EB (Resistant check) and Semsen (Control) were sown in greenhouse during the post rainy seasons of 2012-2014. The plants were used for the bioassays in the laboratory under uniform environmental conditions (27 ± 2 ^{0}C, 65-75% RH, and a photoperiod of 12:12 h. (Light: Dark).

Bioassays were conducted at the vegetative [30 days after emergence (DAE)] and flowering stages (45 DAE).

2.1 Insect culture

Cultures of the aphid, *Aphis craccivora* (Koch); and the predatory coccinellid, *C. sexmaulatus* were maintained on cowpea, *Vigna unguiculata* (L.) Walp. plants in a nylon net-house under ambient conditions. The aphids and coccinellids were obtained from *Glaricidia maculata* (Kunth.) Walp. growing at the ICRISAT farm. The *C. sexmaulatus* eggs were obtained from the net house-reared coccinellids as and when needed**.** The coccinellid eggs were removed from the oviposition substrate (to avoid fungus development and resultant larval mortality), and transferred on to a carbon paper in a plastic cup. The neonate *C. sexmaulatus* larvae from these plastic cups were used in the experiments.

2.2 Feeding *C. sexmaulatus* larvae on sucrose solution

The neonate *C. sexmaulatus* larvae were fed on one of the following food sources: (i) Pure 2M sucrose solution, (ii) 2M sucrose solution containing *Cry1Ia* transgenic leaf powder (0.02%, 0.05% and 0.1%), (iii) water, and (iv) no food. The survival of *C. sexmaulatus* larvae was recorded daily to assess whether the predator larvae had actually fed on sucrose solution or sucrose solution containing *Cry1Ia* transgenic leaf powder. The experiment was conducted twice with 15 replications each, thus forming a total of 30 replicates for each treatment in a CRD. Ingestion of *Cry1Ia* protein by the coccinellid grubs was confirmed by ELISA (EnviroLogic Inc., Portland, ME, USA). ELISA test was carried out to detect the *Cry1Ia* in 2M sucrose mixed *Cry1Ia* transgenic leaf powder (0.02%, 0.05% and 0.1%) as described earlier.

2.3 Direct effects of *CryIIa* transgenic chickpea lines on survival and development of *C. sexmaculatus*

CryIIa transgenic leaf powder was dissolved in a 2M sucrose solution at the concentrations of 0.02%, 0.05% and 0.1% to assess the direct effects. Neonate *C. sexmaculatus* larvae were fed on: (i) pure 2M sucrose solution (sucrose and aphids), (ii) 2M sucrose solution containing *CryIIa* (*CryIIa* aphids) or *CryIIa* (*CryIIa* aphids) at 0.1% on alternate days. The *C. sexmaculatus* larvae were provided ad libitum *A. craccivora* (mixed stages) after every 24 h of feeding on one of the above foods till pupation. One set of *C. sexmaculatus* larvae were fed on *A. craccivora* only. The neonate *C. sexmaculatus* larvae were kept individually in bioassay cups (3.3 cm in diameter, 3.5 cm in depth), and fed on above mentioned foods in the insectary at 26 ± 8 ^{0}C, 80-95% RH, and a 12-h photoperiod. The experiment was conducted twice with 15 replications each, thus, forming a total of 30 replicates for each treatment in a CRD. Observations were recorded on larval and pupal periods, larval survival, weights of male and female larvae, adult emergence, and weights of male and female adults of *C. sexmaculatus*.

2.4 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using GenStat, version 14.1. The treatment means were compared by least significant differences (LSD) at $P \leq 0.05$. The figures presented in the tables are means across replications with F-probability and LSD values.

3. Result and Discussion

There were significant differences in survival and development of coccinellid grubs when fed on diets intoxicated with 0.02% transgenic chickpea leaf powder as compared to those fed on diets with leaf powder of non-transgenic chickpeas. The larval survival was reduced on diets with BS5A.2(T2) 19-2P1 leaf powder (50.0%) as compared to that on Semsen and ICC5O6 EB (80.0 and 78.3%, respectively) (Fig. 1). There was a slight prolongation in the larval period when fed on diets intoxicated with transgenic chickpea leaf powder (6.0 to 7.5 days) as compared to Semsen and ICC 506EB (6.6 and 6.1 days, respectively) (Fig 4). The pupal period was significantly prolonged on BS5A.2(T2) 19-2P1 (9.0 days) as compared to that on Semsen and ICC 506EB (3.1 days) (Table 1, Fig 5).

Pupation and adult emergence were significantly reduced on BS5A.2(T2) 19-2P1 (33.3 and 21.6%, respectively) and BS5A.1(T2) 18-2P1 (35.0 and 21.6%, respectively) as compared to that on ICC 506EB (65.0 and 55.0%, respectively) and Semsen (61.6 and 48.3%, respectively) (Fig 2). Among the transgenic lines, pupation and adult emergence were highest when fed on diets with BS5A.2(T2) 19-1P2 leaf powder (45.0 and 40.0%, respectively) (Table 1; Fig 3).

At 0.05% concentration, the survival of grubs was significantly lower on diets with BS5A.2(T2) 19-1P2 (43.3%) leaf powder as compared to that on non-transgenic chickpeas (80.0 to 81.6%) (Fig 1). The larval period was prolonged in grubs fed on diets with BS5A.2(T2) 19-1P2 leaf powder (9.3 days) as compared to that on Semsen and ICC 506EB (6.6 and 5.8 days, respectively) (Fig 4). The pupal period was prolonged (4.0-4.6 days) when coccinellids were fed on diets with transgenic leaf powder as compared to that on non-transgenic Semsen and ICC 506EB (3.0 and 3.1 days, respectively) (Table 1; Fig 4).

Pupation was significantly lower when the grubs were fed on diets with BS5A.2(T2) 19-2P1 leaf powder (18.3%) as compared to that on Semsen and ICC5O6 EB (66.6%) (Fig 2). Adult emergence was also significantly reduced in *C. sexmaculatus* grubs fed on diets with BS5A.2(T2) 19-2P1 leaf powder (11.6%) as compared to that on Semsen and ICC 506EB (50.0 and 48.3%, respectively) (Fig 3). There were significant differences in the survival and development of coccinellid grubs fed on diets with 0.1% transgenic and non-transgenic chickpea leaf powder. The Larval survival was significantly reduced when fed on diets intoxicated with BS5A.2(T2) 19-3P2 (45.0%), BS5A.2(T2) 19-3P1 (50.0%), BS5A.1(T2) 18-1P1 (53.3%), BS5A.2(T2) 19-1P2 (53.3%), BS5A.1(T2) 18-2P1 (60.0%), BS5A.2(T2) 19-2P1 (70.0%) as compared to that on Semsen and ICC506 EB (71.6 and 75.0%, respectively). There was no significant effect on larval period. There were no significant differences in pupal period (3.3 to 4.0 days). Pupation and adult emergence were significantly reduced on BS5A.2(T2) 19-3P2 (33.3 and 11.6%, respectively) as compared to that on Semsen (60.0 and 45.0%, respectively) and ICC 506EB (61.6 and 48.3%, respectively). The survival and development of coccinellids was reduced when fed on diets with 0.1% of BS5A.2 (T2) 19-3P1 and BS5A.2 (T2) 19-3P2 leaf powder, but not on diets with BS5A.1(T2) 18-2P1 leaf powder (Table 1; Fig 1).

Based on the earlier studies, the CryIAb has been detected in the phloem sap of *Bt*- oilseed rape and the aphids, *Myzus persicae* feeding on the *Bt*-oil seed rape plants [13]. *Bt* toxins have also been detected in the coccinellid, *Propylaea japonica* larvae and the prey, *A. gossypii* when reared on *Bt* cottons [15]. There was a significant and positive correlation between *Bt* detection in aphids, and survival of coccinellids larvae and adults. The amounts of *Bt* toxins detected in coccinellid grubs were higher as compared to the aphids, suggesting that coccinellid larvae accumulated *Bt* toxins in their gut [16].

Table 1: Direct effect of *Cry IIa* transgenic chickpea lines on *Cheilomenes sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%) (2012-2014)

Genotype	0.02%					0.05%					0.1%				
	Larval survival (%)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Larval survival (%)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Larval survival (%)	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)
BS5A.1(T2) 18-1 P1	58.3^{a} (49.8)	7.1^{cd}	4.0^{a}	43.3^{bc} (41.1)	31.6^{ab} (34.1)	61.6^{b} (50.8)	8.0^{bc}	4.3^{b}	41.6^{c} (40.1)	28.3^{b} (34.1)	53.3^{ab} (46.9)	5.8^{a}	3.3^{ab}	40.0^{a} (38.9)	30.0^{bc} (32.5)
BS5A.1(T2) 18-2 P1	51.6^{a} (46.0)	7.5^{d}	4.1^{a}	35.0^{ab} (36.2)	21.6^{a} (27.6)	50.0^{a} (45.0)	8.1^{bc}	4.5^{b}	35.0^{bc} (36.2)	21.6^{ab} (27.6)	60.0^{bc} (51.0)	5.6^{a}	3.3^{ab}	43.3^{ab} (40.9)	35.0^{bcd} (35.8)
BS5A.2(T2) 19-1 P2	61.6^{a} (51.9)	7.0^{bcd}	3.5^{a}	45.0^{c} (42.1)	40.0^{bc} (39.1)	43.3^{a} (41.0)	9.3^{c}	4.5^{b}	26.6^{ab} (31.0)	13.3^{a} (39.1)	53.3^{ab} (46.9)	5.6^{a}	3.5^{ab}	38.3^{ab} (38.0)	23.3^{ab} (28.5)
BS5A.2(T2) 19-2 P1	50.0^{a} (45.0)	7.5^{d}	4.0^{a}	33.3^{a} (35.0)	21.6^{a} (25.3)	45.0^{a} (42.0)	8.8^{c}	4.6^{b}	18.3^{a} (20.9)	11.6^{a} (25.3)	70.0^{cd} (57.0)	5.5^{a}	4.0^{b}	43.3^{a} (41.0)	30.0^{bc} (32.6)
BS5A.2(T2) 19-3 P1	61.6^{a} (51.8)	6.5^{abc}	4.0^{a}	35.0^{ab} (36.1)	28.3^{ab} (32.0)	45.0^{a} (42.0)	8.0^{bc}	4.3^{b}	28.3^{ab} (31.5)	16.6^{ab} (32.0)	50.0^{ab} (45.0)	5.8^{a}	3.8^{ab}	35.0^{a} (35.7)	20.0^{ab} (18.0)
BS5A.2(T2) 19-3 P2	61.6^{a} (51.9)	6.0^{a}	4.8^{a}	35.0^{ab} (36.2)	30.0^{ab} (33.0)	46.6^{a} (43.0)	8.0^{bc}	4.0^{b}	28.3^{ab} (32.1)	13.3^{a} (33.0)	45.0^{a} (42.0)	6.1^{a}	3.6^{ab}	33.3^{a} (34.5)	11.6^{a} (26.0)
Semsen (Control)	80.0^{b} (63.6)	6.6^{abcd}	3.1^{a}	61.6^{d} (51.8)	48.3^{cd} (44.0)	81.6^{c} (65.2)	6.6^{ab}	3.0^{a}	66.6^{d} (54.8)	50.0^{c} (44.0)	71.6^{cd} (58.0)	5.5^{a}	3.5^{ab}	60.0^{b} (50.9)	45.0^{cd} (42.1)
ICC 506 EB (Resistant check)	78.3^{b} (62.5)	6.1^{ab}	3.1^{a}	65.0^{d} (53.7)	55.0^{d} (47.8)	80.0^{c} (63.6)	5.8^{a}	3.1^{a}	66.6^{d} (54.7)	48.3^{c} (47.8)	75.0^{d} (60.4)	5.3^{a}	3.1^{a}	61.6^{b} (51.9)	48.3^{d} (43.8)
SE $\pm$	4.1	0.2	0.4	3.0	3.9	3.7	0.5	0.2	3.8	4.2	4.3	0.3	5.2	2.3	0.5
Fp	<0.001	0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.75	<0.001	0.001	0.27
Vr	6.9	3.9	1.0	17.2	9.4	17.8	4.6	5.3	22.5	13.2	6.5	0.6	5.4	3.9	1.3
LSD (P 0.05)	11.9*	0.8*	NS	8.6*	11.3*	10.8*	1.5*	0.7*	11.0*	12.2*	12.5*	NS	15.1*	6.8*	NS

*Figures followed by the same letter within a column are not significantly different at $P \leq 0.05$
Figures in parenthesis are Angular transformed values

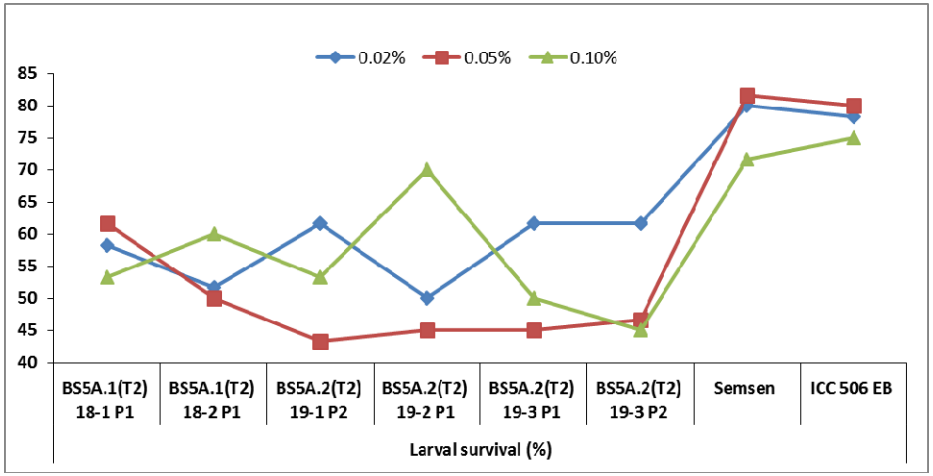

Fig 1: Direct effect of *Cry IIa* transgenic chickpea lines on larval survival (%) of *C. sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%, 2012-14)

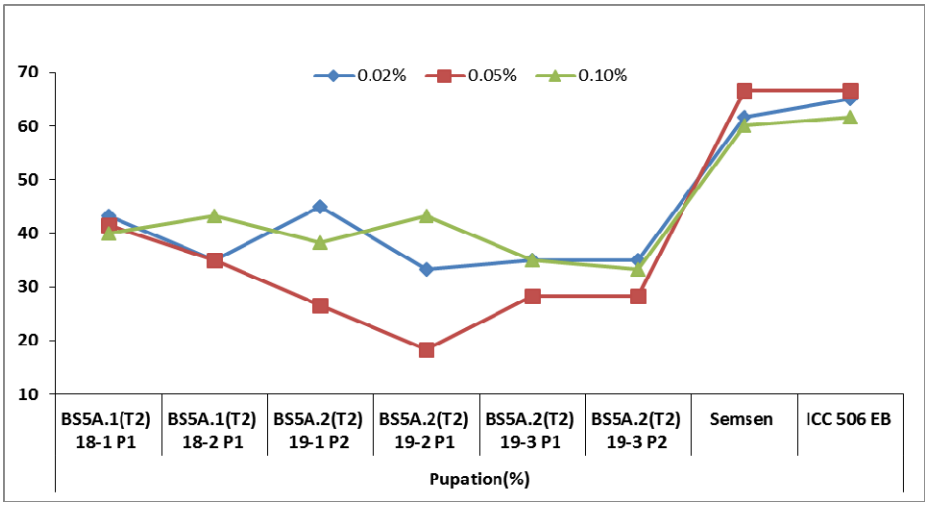

Fig 2: Direct effect of *Cry IIa* transgenic chickpea lines on Pupation (%) of *C. sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%, 2012-14)

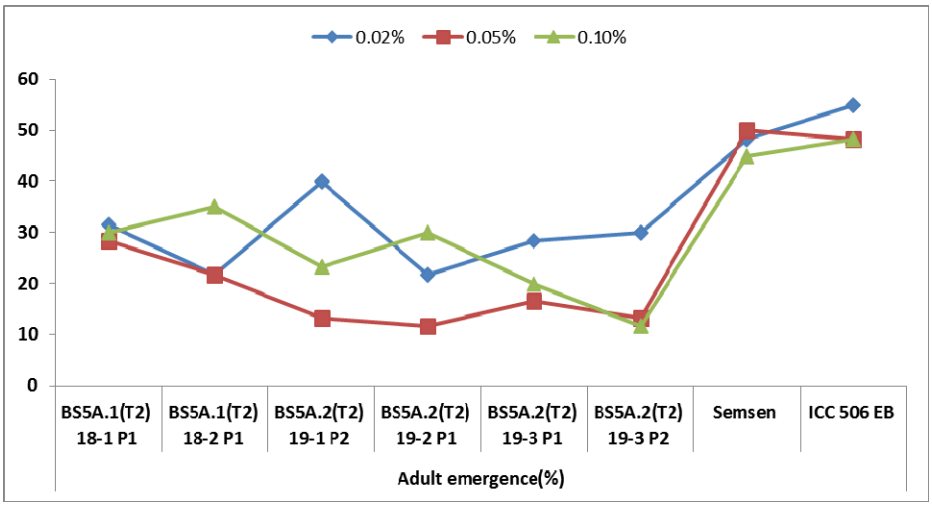

Fig 3: Direct effect of *Cry IIa* transgenic chickpea lines on Adult emergence (%) of *C. sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%, 2012-14)

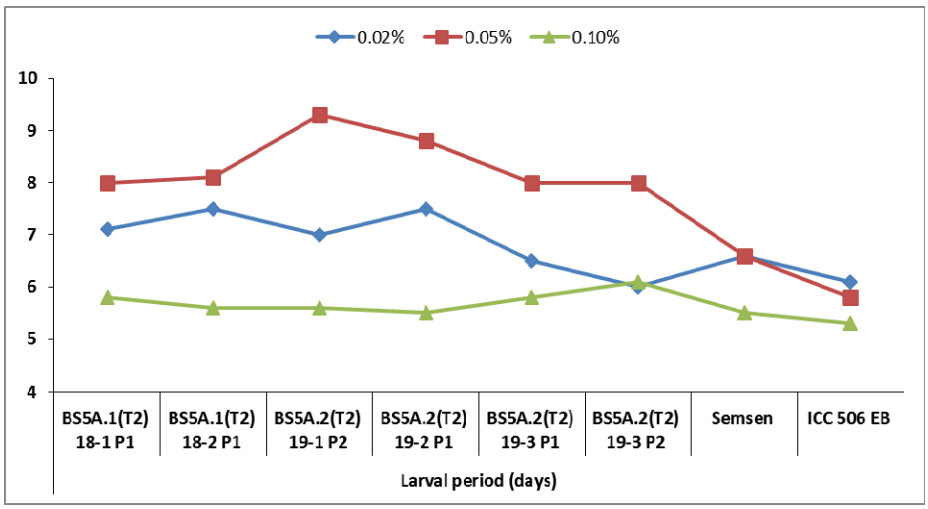

Fig 4: Direct effect of *Cry IIa* transgenic chickpea lines on Larval period (days) of *C. sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%, 2012-14)

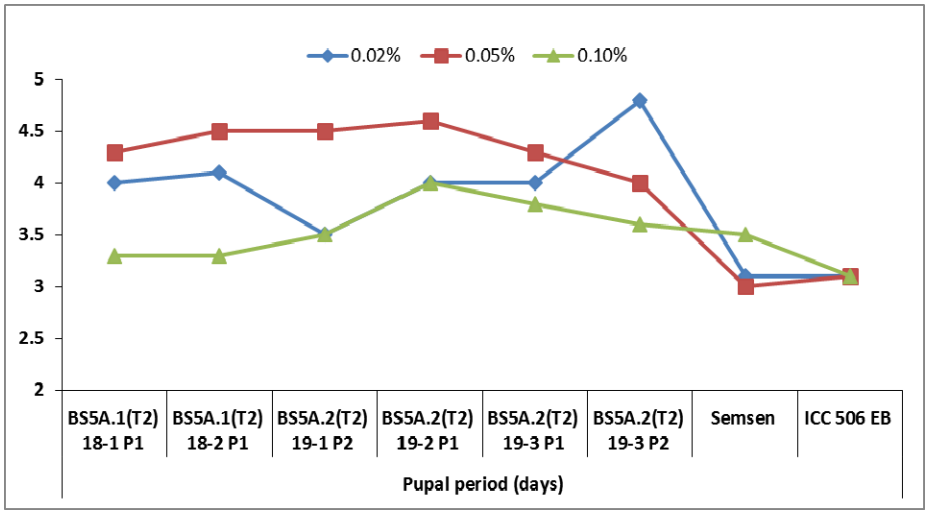

Fig 5: Direct effect of *Cry IIa* transgenic chickpea lines on Pupal period (days) of *C. sexmaculatus* at different concentrations (0.02%, 0.05% and 0.1%, 2012-14)

4. Conclusions

The direct effects on coccinellids were greater when fed on 0.1% *Bt* intoxicated diet, followed by diets with 0.05% and 0.02% *Bt*. The survival and development of coccinellid grubs were slightly affected when reared on aphids fed on diets with different concentrations (0.02%, 0.05% and 0.1%) of transgenic chickpea leaf powder. The coccinellids fed on diets with 0.05% BS5A.2(T2) 19-3P1 leaf powder showed a marginal reduction in survival and development as compared to that on other transgenic lines.

5. Acknowledgement

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