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Biological activity of pod borer, *Helicoverpa armigera* (Hubner) influenced by chickpea genotypes

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ABSTRACT

Investigations on biological activities of pod borer**,** *Helicoverpa armigera* (Hubner) on 20 promising chickpea genotypes using detached leaf assay method at Agriculture Research Station Kalaburagi, UAS, Raichur, Karnataka was carried out during *rabi* 2016-17. The results revealed that at vegetative and flowering stage there was significantly lesser larval survival (70 to 76.67 %), larval weight (8.37 to 8.90 mg) and damage rate (3.67 to 4.67 of visual Rating in 1-9 Scale) on resistant check ICCL 86111, HC-1 and DBGV-3104 genotypes where as maximum per cent larval survival (86.67 to 90 %), larval weight (13.60 to 14.10 mg) and damage rate (7.67 to 8.00 of visual Rating in 1-9 Scale) was recorded in susceptible checks (ICC-3137, A-1 and JG-11). During pod formation stage, highest weight gain by larvae was noticed on susceptible check ICC-3137 (409.18 mg), on the contrary lowest weight gain by larvae was found on resistant check ICCL-86111 (275.43 mg) also these genotypes recorded more amount of malic acid and trichomes indicating that biological activity of insect was affected through antibiosis mechanism which is one of the component of resistance to *H. armigera* in chickpea.

Key words: Antibiosis, Chickpea, Larvae, *Helicoverpa armigera* (Hubner).

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the most important legume crop of India and is considered as "king of pulses". The gram pod borer (*Helicoverpa armigera*, Hubner) is the major constraints in production of chickpea crop world wide (Sharma *et al*., 2005), which causes losses upto 95 per cent (Prakash *et al*., 2007).

Despite of spending millions of rupees by farmers for pesticides spray to control this pest, farmers are unable to manage the pest to desired level. It has developed high level of resistance to conventional insecticides (McCaffery *et al.*, 1991) and polluting the environment. To avoid these problems, non-chemical pest management strategies need to be identified and promoted. Among them host plant resistance (HPR) is one of the cheapest and easy adoptable method under rainfed farming situations. The wild and resistant gene pools are the potential source of beneficial gene that offers considerable resistance to the insect pests. Insecticidal proteins *viz.*, lectins, a-amylase inhibitor, urease, protease inhibitor, arcelins and cyclotides present in wild and resistance germplasms have been suggested to play a major role in insect resistance which are considered as most promising weapons that confer resistance against insects and which will be eco-friendly alternative to synthetic pesticides (Sagar and Heena, 2018). There is a continuous effort to explore such wild source to evolve some promising genotypes by All India Co-ordinated Crop Improvement Research Projects (AICRP chickpea) and ICRISAT in India. With these points in mind, the present investigations were carried out to identify the good stable resistant genotypes.

MATERIALS AND METHODS

Antibiosis study on *H. armigera* in 20 chickpea genotypes developed under AICRP and ICRISAT in resistance breeding programme mentioned in (Table 1 to 2) using detached leaf assay method given by Sharma *et al.* (2005) was carried out in the laboratory at Agriculture Research Station Kalaburagi, University of Agricultural Sciences, Raichur, during *rabi* 2016-17. To carry out this investigation, pure culture was maintained as given below.

Gram pod borer larvae were used in bio-assays experiments in the laboratory, for these field-collected larvae of *H. armigera* were reared in the laboratory on an artificial diet (Armes *et al.*, 1993). In a multi-cavity tray to avoid cannibalism, each cavity well had sufficient amount of diet (7 ml) to support larval development until pupation. The pupae were removed from cavity, and kept in groups of 25 in plastic jars containing vermiculite. Upon emergence of adults were released inside an oviposition cage (30 x 30 x 30 cm). Covered with black cloth inside the cage, adults were provided with 10 per cent sucrose or honey solution on a cotton swab for feeding. Fresh chickpea leaflet twigs containing flower buds were kept inside oviposition cage for the females to lay eggs, twigs were removed every two days and then placed inside the plastic box with diet. After

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egg hatching, the larvae were moved to the artificial diet, neonate larvae were used for bioassays studies under laboratory conditions using detached leaf assay method.

The chickpea genotypes were grown in earthen pots, containing a potting mixture of black soil (Vertisols), sand, and farmyard manure (2:1:1). The bioassays conducted in the laboratory at 27 ± 2°C, 65-75 % RH and a photoperiod of 12: 12 [L:D] h. Plastic cups (4.5 x 11.5 cm diameter) were used in this experiment, had a moistened filter paper attached to the lid to keep the chickpea leaves in turgid condition. Further the procedure for detached leaf assay method given by Sharma *et al* (2005) was followed. Ten neonate larvae of *H. armigera* per replication were released on the chickpea leaves.

The experiment was conducted in completely randomized design with five replications at three stages of the crop. For vegetative (30 days after germination) and flowering (nearly 60 days after germination) stages, ten neonate larvae per replication were released per cup, whereas at the podding stage (90 days after germination), used little bigger plastic cups of 9 x 6.5 cm. Twigs with pods were collected from the potted plant culture and placed in agar-agar substratum and a third instar pre-weighed larva was released in each cup as explained above. The experiment was terminated 5 days after releasing the larvae.

The test genotypes were rated for leaf feeding by the larvae visually on 1 to 9 scale (1=, < 10 %, 2= 11-20 %, 3= 21-30 %, 4= 31-40 %, 5= 41-50 %, 6= 51-60 %, 7= 61-70 %, 8= 71-80 % and 9= > 80 % leaf area damaged). The number of larvae survived and larval weight gain was assessed 4 hours after the feeding period. During podding stage, data was also subjected to work out weight gain by the larvae using the following formula.

$$\text{Weight gain (\%)} = \frac{\text{Final weight of the larva - Initial weight of the larva}}{\text{Initial weight of the larva}} \text{ X100}$$

Malic Acid content in leaves was estimated by determining the titrable acidity of extract of 1 gm of leaves of 3rd, 4th and 5th leaves from top of the shoot collected at 09:00 hr on 70 days old plants. A single fully opened leaf was removed from plant (Plant age: 70 days) to determine the number trichomes per unit area . The numbers of trichomes were recorded from an area of one mm^2 by using ocular microscope at 25x magnification.

RESULTS AND DISCUSSION

Neonate *H. armigera* larvae when fed on chickpea branches during vegetative stage using detached leaf assay, the larval survival ranged between 70 to 90 per cent. Significantly lesser larval survival (70.00%) was noticed in ICCL 86111, HC-1 and DBGV-3104 genotypes and was found to be on par with each other and further the genotypes Phule G-08108 and ICCV-08108 though recorded 76.67 per cent larval survival rate but did not differ statistically with the above mentioned genotypes. Maximum of 90 per cent larvae survived on susceptible checks ICC-3137, A-1 and JG-11 and

Table 1: Leaf damage rating, larval survival and larval weight of *H. armigera* in 20 chickpea genotypes by using detached leaf assay during vegetative stage.

Genotypes	At Vegetative stage (30 days aged crop)			At Flowering stage (60 days aged crop)		
	Damage rating	Larval survival (%)	Larval weight (mg)	Damage rating	Larval survival (%)	Larval weight (mg)
GJG-1320	6.00	86.67(68.86)*	12.83	6.00	83.33 (66.14)	11.17
Phule G-13103	6.00	86.67 (68.86)	10.43	5.67	90.00 (71.57)	13.23
NBe G-806	6.67	90.00 (71.57)	11.13	6.67	90.00 (71.57)	12.40
CSJ-855	6.33	80.00 (63.43)	10.80	6.00	86.67 (68.86)	9.63
Phule G-0616	6.00	80.00 (63.43)	12.77	6.33	90.00 (71.57)	11.10
Phule G-13107	6.33	90.00 (71.57)	12.27	6.00	86.67 (68.86)	11.83
NBe G-740	5.67	86.67 (68.86)	12.53	6.67	80.00 (63.43)	10.97
GJG-1307	5.67	83.33 (66.14)	11.93	5.67	80.00 (63.43)	12.23
DBGV-3104	4.67	70.00 (56.79)	8.90	4.67	76.67 (61.22)	10.30
Phule G-08108	5.00	76.67 (61.22)	9.57	5.00	70.00 (56.79)	10.87
ICC-14872	6.67	80.00 (63.43)	12.13	5.33	80.00 (63.43)	10.53
ICCV-07104	6.33	80.00 (63.43)	11.43	7.33	86.67 (68.86)	10.43
ICCV-08108	5.67	76.67 (61.22)	11.23	5.67	80.00 (63.43)	10.53
ICCV-09118	6.00	80.00 (63.43)	9.63	6.00	83.33 (66.14)	9.70
HC-1	4.00	70.00 (56.79)	8.47	4.00	73.33 (59.00)	8.23
ICCV-92944	6.67	83.33 (66.14)	12.23	6.00	80.00 (63.43)	11.30
ICCL-86111	3.67	70.00 (56.79)	8.37	4.33	70.00 (56.79)	8.50
A-1	8.00	90.00 (71.57)	13.67	8.00	90.00 (71.57)	13.27
ICC-3137	8.00	90.00 (71.57)	14.03	7.67	86.67 (68.86)	13.60
JG-11	7.67	90.00 (71.57)	14.10	8.00	90.00 (71.57)	13.60
S.Em±	0.63	1.52	0.26	0.52	1.64	0.32
CD at 1%	2.42	5.83	0.99	2.01	6.28	1.21
CV (%)	18.10	4.05	3.95	15.08	4.31	4.90

Figures in parentheses are arc sin transformed values

larval survival on GJG-1320, Phule G-13103 and NBeG-740 genotypes was 86.67 per cent and were at par with susceptible check (ICC-3137) and differed significantly from other genotypes (Table 1). The weight gain by larvae reared on resistant check ICCL 86111 was significantly lower (8.37 mg) in weight and was on par with HC-1 (8.47 mg), DBGV-3104 (8.90 mg) followed by Phule G-08108 and ICCV-09118 with 9.57 mg and 9.63 mg body weight, respectively. The weight gain by larvae was maximum and on par with each other when reared on JG-11, ICC3137 and A-1 (Table 1).

The damage rate among tested genotypes ranged between 3.67 (ICCL 86111) to 8.00 (ICC 3137). The leaf feeding rate was observed significantly lower on resistant check ICCL 86111 (3.67) and was on par with HC-1 (4.00), DBGV-3104 (4.67) and Phule G-08108 (5.00). While it was significantly higher (8.00 to 6.67) in susceptible check ICC 3137 and other test entries (A-1, JG-11, ICC-14872, ICCV-92944 and NBeG-806) and were at par each other (Table 1). At flowering stage the pest damage more and trend was same as that noticed at vegetative stage of crop (Table 1).

During the podding stage, when a single third-instar larva was released on chickpea branches with young pods, the weight gain by the larvae weight ranged between 275.43 mg (ICCL 86111) to 418.19 mg (JG-11). Weight gain by larvae was significantly lesser when reared on resistant check ICCL 86111 (275.43 mg) and was on par with HC-1(276.48 mg), GJG-1320 (279.93 mg), ICC-14872 (290.96 mg), Phule G-13103 (302.40 mg), CSJ-855 (303.83 mg) and DBGV-3104 (304.19 mg) genotypes. The larval weight gain was significantly higher on JG-11 and susceptible check ICC 3137 (409.18 mg) and were on par with each other, followed by ICCV-09118, A-1, ICCV-08108 and Phule G-13107 with larval weight of 365.95 mg, 363.67 mg, 357.63 mg and 351.68 mg, respectively (Table 2).

Antibiosis is the adverse effect of a plant on some aspects of the insect's biology. The effects of antibiosis may be reduction in size and weight, fecundity, abnormal length of life and increased mortality of the insects (Yoshida *et al.*, 1995). Variation in biochemical composition of chickpea genotypes which have direct effect on insect metabolism (Singh and Sharma 1970). During vegetative and flowering stage leaf damage rating was ranged between 4.2 (resistant check, ICC 12475) to 8.2 (susceptible check, ICCC 17), larval survival was lower on resistant check ICC 12475 (68 %), the unit larval weight was ranged between 5.45 mg (ICC 12475) to 8.55 mg (ICC 4918). During the podding stage, significantly more weight was gained by the larva on ICCC 37 (387.5 mg**)** as reported by Narayanamma and her co-workers in the year 2007.

High malic acid content has been reported by several workers as one of the mechanism of pod borer resistance in chickpea. In the present investigation resistant genotypes showed high malic acid than the susceptible ones (ICC-3137 and A-1) which had high per cent pod damage

Table 2: Larval weight (mg), weight gain by the larva (%) of *H. armigera* in 20 chickpea genotypes by using detached leaf assay during 8podding stage

Genotypes	larval weight (mg)		Weight gain by the larva in		Malic acid (%) content	Trichome density on leaves (per mm^2)
	Initial	Final	mg	%		
GJG-1320	29.47	309.40	279.93	950.00	0.491^{b}	18.33 (4.40)^{b}
Phule G-13103	32.26	334.67	302.40	937.30	0.469^{a}	20.00 (4.58)^{c}
NBeG-806	30.64	338.20	307.56	1003.79	0.536^{c}	19.00 (4.47)^{bc}
CSJ-855	30.97	334.80	303.83	981.16	0.715^{g}	28.67 (5.45)^{j}
Phule G-0616	30.41	342.23	311.82	1025.27	0.581^{d}	23.00 (4.90)^{de}
Phule G-13107	30.89	382.57	351.68	1138.48	0.491^{b}	18.00 (4.36)^{b}
NBeG-740	33.39	343.90	310.51	930.05	0.625^{e}	22.00 (4.80)^{d}
GJG-1307	30.96	364.07	333.10	1075.80	0.625^{e}	24.67 (5.07)^{fg}
DBGV-3104	30.21	334.40	304.19	1006.92	0.759^{h}	27.00 (5.29)^{i}
Phule G-08108	33.93	372.90	338.97	999.14	0.715^{g}	26.33 (5.23)^{hi}
ICC-14872	32.80	323.77	290.96	886.99	0.625^{e}	26.00 (5.20)^{ghi}
ICCV-07104	31.06	375.57	344.51	1109.29	0.670^{f}	26.67 (5.26)^{i}
ICCV-08108	31.60	389.23	357.63	1131.62	0.670^{f}	28.67 (5.45)^{j}
ICCV-09118	30.12	396.07	365.95	1214.96	0.625^{e}	24.00 (5.00)^{ef}
HC-1	28.59	305.07	276.48	967.04	0.804^{i}	29.00 (5.48)^{j}
ICCV-92944	30.39	381.83	351.44	1156.44	0.491^{b}	15.67 (4.08)^{j}
ICCL-86111	30.50	305.93	275.43	903.06	0.581^{d}	16.33 (4.16)^{j}
ICC-3137	30.22	439.40	409.18	1353.84	0.536^{c}	16.67 (4.20)^{a}
A-1	33.13	396.80	363.67	1097.59	0.849^{j}	28.67 (5.45)^{j}
JG-11	31.15	449.33	418.19	1342.64	0.670^{f}	25.00 (5.10)^{fgh}
S.Em±	1.04	8.09	7.99		0.007	0.047
CD at 1%	NS	30.96	30.59		0.029	0.180
CV (%)		4.08	4.20		2.130	1.653

NS- Non significant

showed low malic acid content of 0.491 and 0.581 per cent, respectively. Genotypes with lower pod damage exhibited higher malic acid content. ICCL-86111 (8.77) which had low per cent pod damage showed high malic acid content of 0.804 (Table 2). However, there were not always in linear relationship were few exceptions for example the genotype Phuleg-0616 with lower per cent pod damage of 16.83 had comparatively lower malic acid content (0.581). It implies that, the malic acid may not be the only factor responsible for the resistance behavior. Similar results were also observed by Girija *et al.* (2008). On the contrary Rembold (2001) reported that, the main components of chickpea exudates, malic and oxalix acid did not affect the larvae and adult behavior of *H. armigera* unlike in monophagous leaf miner, *Liriomyza cicerina*.

High amounts of malic and oxalic acids in leaves affected the growth of *H. armigera*, and contributed to plant resistance to *H. armigera* (Simmonds and Stevenson, 2001). The relationship between oxalic acid and incidence of *H. armigera* was also documented by Yoshida *et al*. (1995). The amount of oxalic acid and chickpea resistance to *H. armigera* observed in the present investigations could be ascribed to the antixenosis (non-preference) to feeding.

Acid exudates from leaf hairs contribute to plant resistance to *H. armigera* in chickpea (Yoshida *et al*., 1995). Malic acid and oxalic acid on the leaves were responsible for chickpea resistance to pod borer (Cowgill and Lateef, 1996). Sharma *et al*. (2006) reported that ICC 12475 suffered more damage by *H. armigera*, which was correlated with less oxalic acid in chickpea leaves due to heavy rainfall received during experimentation.

Bhaghwat *et al.* (1995) reported that the highest amounts of malic acid were observed in ICC 506 EB at 60 days after sowing, which harboured lowest numbers of *H. armigera* larvae. A significant and negative association was observed between the amounts of the malic acid and leaf feeding (r = - 0.83), larval survival (r = - 0.93) and larval weight (r = - 0.95) (Shaila *et al.*, 2017) HPLC profile studies (Narayanamma *et al*., 2007) of leaf exudates showed that amount of malic acid were negatively correlated with leaf feeding by *H. armigera* larvae. Malic acid, crude fiber, hemi cellulose, cellulose, lignin and non reducing sugars were negatively correlated with the pest population (Kaur *et al*., 1999; Chabra *et al*., 1993). Further, there are also reports to support that the chickpea plants have protein that inhibits trypsin in *H. armigera* (Kansal *et al*., 2008).

The mean trichome density on leaves ranged from 15.67 to 29 trichomes per mm^2 and varied significantly among genotypes. Highest number of trichomes (29 trichomes/ mm^2) were observed in resistant chickpea genotype ICCL 86111, However, minimum number of trichomes (15.67 trichomes/ mm^2) were seen in susceptible check ICC-3137 which was on par with the A-1 and JG-11which were recorded 16.33 and 16.67 trichomes per mm^2, respectively (Table 2). Similar observations were made by Husandeep *et al*, (2014) and found that mean trichome density on leaves ranged from 16 to 33.66 trichomes per mm^2 and varied significantly among different genotypes. Tolerant genotypes, *viz*., ICCL 87315, ICC 506 and ICC 12479 with higher number of trichomes exhibited less percent pod damage, while susceptible genotypes, viz., Annigeri and ICCV 2 with lesser number of trichomes showed higher pod damage is reported by Johnpeter *et al.* (1995). Density of trichomes on leaves showed significant and negative correlation with number of eggs, larval population, larval survival and per cent pod damage (r = -0.76, -0.77*, -0.78* and -0.67*, respectively) indicating that more the trichome density or leaf pubescence, lesser would be the number of *H. armigera* eggs, larval population, larval survival and pod damage. Thus, pubescent chickpea genotypes were less prefered for feeding and oviposition by *H. armigera* as compared to glabrous ones. Ascensao *et al* (1995) reported that higher densities of non glandular trichomes may also act as a physical barrier to feeding by *H.armigera* larvae. Girija *et al.* (2008) observed that the tolerant genotypes had higher number of trichomes and thicker pod husks and exhibited less per cent pod damage.

Kanchana *et al.* (2005) observed the effect of certain morphological and biochemical parameters on selected chickpea varieties against *H. armigera* and indicated number of trichomes on leaves and pods per unit area showed a significant negative effect on pod damage.

The variation in bio chemical composition of susceptible and resistant genotypes which have bearing on insect food preference and its physiology. The less preferred and physiological supportive biochemical composition having plants (Resistant genotypes) got damaged less. Further insects feeding on such plants was less and which in turn influenced its biological parameters e.i., less body weight, more larval duration. Leaf exudates played an important role in *H. armigera* resistance in chickpea (Rembold, 1981; Srivastava and Srivastava, 1990; Rembold *et al.*, 1989 and Yoshida, *et al.*, 1995; Narayanamma *et al.*, 2007, Shankar *et al*., 2014) and might be responsible for antibiosis to this pest.

CONCLUSION

From present study is that the both bio-physical and biochemical characters associated with genotypes have bearing on pod borer feeding and its growth and developmentin chickpea. From present study found that ICCL 86111, HC-1 and DBGV-3104 genotypes were resistant to pod borer damage.

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