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Role of Oxalic Acid in Expression of Resistance against the Pod Borer *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Chickpea Varieties

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ABSTRACT

Background: Pod borer is most serious problem to cultivation of chickpea across the world. In India, Madhya Pradesh rank first in both area and production of chickpea. This region is considered most affected yield of chickpea due to *H. armigera*. Host plant resistance is an important component for managing this pest. To develop cultivars with resistance to insects, it is important to understand the role of oxalic acid associated with resistance to this pest. The current study aimed to study the role of oxalic acid in expression of resistance against the pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae) in chickpea varieties under climate change.

Methods: This investigation was carried out at Research Farm of Soybean Seed Production-Unit, College of Agriculture, JNKVV (MP) during *Rabi* season 2019-20 and 2020-21. The field experiment was laid out in split split plot design with 24 treatments and three replications. The treatments included three date of sowing *i.e.* 15th November, 30th November and 15th December as a main plots, two irrigation levels *i.e.* I_0-no irrigation and I_{1-} irrigation at 35 DAS as a sub plots and four chickpea varieties *i.e.* JG 12, JG 36, JG 14 and JG 24 as sub sub plots. The observation on number of eggs and larval population of *H. armigera* were recorded from one-meter row length (mrl). At five randomly selected places were averaged separately for each plot and made in to number of eggs and larval population per meter row length (mrl). The amount of oxalic acid in chickpea leaves was determined by UFLC method. The correlation was worked out with population of pod borer and amount of oxalic acid in chickpea varieties.

Result: In this investigation, the ultra-fast liquid chromatography (UFLC) profiles of the leaf exudates of chickpea varieties exhibited amounts of oxalic acid significantly negative correlation with egg count and larvae incidence of *H. armigera*.

Key words: Chickpea varieties, Date of sowings, Eggs larvae of *H. armigea,* Irrigation levels, Oxalic acid, UFLC method.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important pulse crops of the *rabi* season, cultivated mainly in semi-arid and warm temperature regions of the world. In India chickpea was cultivated in about 10.17 million hectares, with a production of about 11.35 million tonnes with average productivity of 1116 kg/ha (Anonymous, 2020). Chickpea was cultivated in about 1.93 million hectares with a production of 2.48 million tonnes with a productivity of 1288 kg/ha in Madhya Pradesh and occupied first position in country (Anonymous, 2020).

Chickpea is a source of high-quality protein for the poor people in many developing countries, including India. Chickpea yields are quite low and have remained almost stagnant for the past 2 to 3 decades. It is damaged by over 50 insect species in different parts of the world, of which the pod borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera) is the most damaging pest worldwide (Sharma, 2005). It causes an estimated loss of US $328 million in chickpea in the semi-arid tropics. Its control is largely based on insecticides. However, with the development of resistance to insecticides in *H. armigera* populations (Kranthi *et al*., 2002), there has been a renewed interest in developing alternative methods of pest control, of which plant resistance to *H. armigera* is an important component (Sharma *et al*. 2005).

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Host plant resistance, as one of the important components of integrated pest management, can play a major role in management of *H. armigera* (Sarwar *et al*. 2011). Use of resistant or tolerant varieties is economically

viable, ecologically safe and compatible with other IPM strategies (Nadeem *et al*. 2010). Resistant chickpea plants were reported to show non-preference for oviposition and larval feeding by *H. armigera* (Lateef, 1985). Oxalic acids in cultivated chickpea exert antifeedant and antibiotic effects on *H. armigera* (Narayanamma *et al*., 2013). The concentration of oxalic acid is higher on the leaf surface of resistant genotypes than on susceptible ones and this acid retards the growth of *H. armigera* larvae (Yoshida *et al*., 1995).

However, the aim of this study was to evaluate the effects of oxalic acid on oviposition preference and larvae density of *H. armigera* to investigate in a field experiment. A basic understanding of the interactions between the secondary metabolites in genotypes of chickpea and *H. armigera* is important to develop appropriate strategies to develop chickpea cultivars with high levels of resistance to *H. armigera*.

MATERIALS AND METHODS

Experimental site and layout

The field experiment was conducted at Research Farm, Breeder Seed Production-unit (Soybean), College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) during *rabi* season of 2019-20 and 2020-21. The experimental site lies between 23°10′N latitude and 79°56′E longitude and 411.78 m above mean sea level. The field experiment was laid out in split split plot design with 24 treatments and three replications. The treatments included three date of sowing *i.e.* 15th November, 30th November and 15th December as a main plots, two irrigation levels *i.e.* I_0-no irrigation and I_{1-} irrigation at 35 DAS as a sub plots and four chickpea varieties *i.e.* JG 12, JG 36, JG 14 and JG 24 as sub sub plots. The treatments were randomly allocated in each replication.

Observations on eggs and larval population of *H. armigera*

During study period, the data on number of eggs and larval population of *H. armigera* were recorded from one-meter row length (mrl) at five randomly selected places at 30, 45, 60, 75 and 90 DAS from each plot. The counts recorded from five randomly selected places were averaged separately for each plot and made in to number of eggs and larval population per meter row length (mrl).

Estimation of oxalic acid in leaf exudates

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

Preparation of chemicals

Oxalic acid in chickpea leaves was determined by UFLC. Standards were prepared with oxalic acid technical grade (obtained from Sigma- Aldrich pvt.ltd) by mixing 50 mg in 50 ml of water to get concentration of 1000 ppm. The mobile phase of 25 mM KH_2PO_4 of pH 2.5 with H_3PO_4 was prepared; for this 6.805 g of KH_2PO_4 was weighed and taken in a 2 litre conical flask and mixed with 1 litre of Millipore water until KH_2PO_4 was completely dissolved. Then 4 ml of H_3PO_4 was added and made up the volume to 1.8 L. The pH was adjusted to 2.5 by adding H_3PO_4 drop by drop and finally made up the volume to 2 litres.

Extraction of leaf organic acids

The chickpea leaf samples were collected early in the morning (before 9 AM) at 30, 45, 60, 75 and 90 DAS of chickpea. Three replications were maintained for each sample. About 2 g of fresh leaves sample was homogenized and extracted in 16 ml of 1 M HCl for 15 min at 100°C in a water bath and then kept overnight at room temperature. Then the crude extract was filtered and diluted with H_2O in a ratio of 1:1. Before injection, 4 ml of the diluted filtrate were passed through a Sep-Pak cartridge with a 10 ml syringe. The first 2 ml discarded and the rest were filtered with 0.45 µm filter disk and kept in 2.5 ml vial for determination. and quantification of oxalic acids in the leaf exudates.

UFLC procedure

The purified samples of leaf exudates of different chickpea varieties were arranged in an auto sampler. The instrument was connected to a photodiode array detector (PDA) having software able to compute detector response in the form of peak area. Chromatographic separation was done using a C-18 column (3 µm particle size and 5 cm length) using mobile phase (25 mM KH_2PO_4 of pH 2.5) with a flow rate of 0.35 ml min^{-1}. The injected volume of each sample was 20 µl. Retention time of oxalic acid in the leaf sample was found to be 1.37 minutes.

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

Data analysis

The data were subjected to analysis of variance and the amounts of oxalic acid were correlated with oviposition preference and larvae population of *H. armigera* at 30, 45, 60, 75 and 90 DAS of the crop. Diversity among the chickpea varieties were assessed using similarity matrix analysis.

RESULTS AND DISCUSSION

The present investigation involved three date of sowing, two irrigation level and four chickpea varieties. The dates of sowing significantly affected on ovipositional preference and larval population of *H. armigera* 30 45, 60, 75 and 90 DAS during both years of experimentation and average value (Table 1). It indicated that early sowing environment was congenial for avoidance of oviposition preference and incidence by *H. armigera*. It gradually increased with the

Table 1: Oviposition, larval population of *H. armigera* and oxalic acids in different chickpea varieties at as influenced by sowing dates and irrigation levels (mean of 2019-20 and 2020-21).

Factors	Oxalic acid (µg g^{-1})					No. of eggs/mrl					No. of larvae/mrl				
	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS
D_1 (15^{Th} Nov.)	47.2	39.87	52.37	53.45	54.39	0.14	0.20	0.56	0.68	0.19	0.15	0.38	1.22	1.50	0.38
						(0.80)	(0.84)	(1.01)	(1.06)	(0.82)	(0.80)	(0.93)	(1.29)	(1.39)	(0.93)
D_2 (30^{Th} Nov.)	45.17	29.49	46.82	47.69	48.66	0.34	0.86	1.16	1.55	0.35	0.5	1.19	2.09	2.58	0.56
						(0.91)	(1.15)	(1.26)	(1.40)	(0.90)	(1.00)	(1.29)	(1.60)	(1.74)	(1.01)
D_3 (15^{Th} Dec.)	42.51	26.74	42.35	43.14	44.79	0.43	1.26	2.41	2.88	0.66	0.69	1.65	3.40	4.18	0.86
						(0.96)	(1.31)	(1.63)	(1.88)	(1.01)	(1.08)	(1.45)	(1.93)	(2.12)	(1.10)
Sem±	0.81	.071	0.59	0.53	0.54	0.02	0.02	0.03	0.04	0.02	0.03	0.02	0.04	0.03	0.04
C.D. (P=0.05)	3.11	2.77	2.31	2.05	2.11	0.08	0.07	0.12	0.14	0.09	0.1	0.09	0.17	0.1	0.13
I_0 (no irrigation)	44.96	31.36	46.55	47.46	48.73	0.31	0.83	1.44	1.82	0.44	0.44	1.13	2.30	2.87	0.63
						(0.89)	(1.12)	(1.33)	(1.44)	(0.93)	(0.96)	(1.24)	(1.63)	(1.78)	(1.02)
I_1 (irrigation at 35 DAS)	44.96	32.71	47.81	48.73	49.84	0.30	0.72	1.32	1.60	0.36	0.46	1.01	2.17	2.64	0.57
						(0.89)	(1.07)	(1.28)	(1.37)	(0.89)	(0.97)	(1.20)	(1.59)	(1.72)	(1.00)
Sem±	0.55	0.27	0.44	0.45	0.44	0.01	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.01
C.D. (P=0.05)	NS	0.77	NS	1.25	NS	NS	0.05	NS	0.05	0.03	NS	0.05	NS	0.05	0.03
JG12	25.12	25.08	41.11	42.05	43.31	0.31	1.20	2.42	2.82	1.12	0.71	1.49	3.32	3.84	1.40
						(0.89)	(1.24)	(1.65)	(1.78)	(1.24)	(1.08)	(1.68)	(1.89)	(2.05)	(1.34)
JG36	43.14	25.98	43.59	44.47	45.65	0.27	0.79	1.07	1.77	0.27	0.47	1.08	1.98	2.98	0.47
						(0.87)	(1.11)	(1.23)	(1.47)	(0.87)	(0.97)	(1.31)	(1.56)	(1.84)	(0.97)
JG14	52.63	35.17	48.12	49.03	50.24	0.37	0.47	0.57	0.72	0.06	0.32	0.74	1.42	1.67	0.21
						(0.92)	(0.97)	(1.01)	(1.08)	(0.75)	(0.90)	(1.22)	(1.37)	(1.45)	(0.84)
JG24	58.94	41.91	55.9	56.82	57.93	0.26	0.64	1.44	1.51	0.15	0.31	0.97	2.22	5.23	0.32
						(0.87)	(1.104)	(1.32)	(1.29)	(0.80)	(0.89)	(1.57)	(1.59)	(1.65)	(0.89)
Sem±	0.96	0.47	0.77	0.77	0.77	0.03	0.03	0.04	0.03	0.02	0.02	0.03	0.03	0.03	0.02
C.D. (P=0.05)	NS	NS	NS	NS	NS	0.07	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Mean of five samples and three replications and *Figures in parenthesis are the transformed data $\sqrt{X + 0.5}$.

advancement in the growth stages till 75 DAS and then increment was decreased or even almost stopped at maturity under all treatments. At 30 days after sowing, crop sown on 15th November was recorded significantly lower mean oviposition and larval population of *H. armigera* (0.14 eggs/mrl and 0.15 larvae/mrl respectively) as compare to 30th November (0.34 eggs/ mrl and 0.51 larvae/mrl respectively) and 15th December (0.43 eggs/ mrl and 0.69 larvae/mrl respectively) sowing. Similar trends were also observed at 45, 60 75 and 90 days after sowing. This might be due to the fact that during 15th November sowing, temperature, humidity and other climatological parameters were unfavourable for oviposition on chickpea. Similarly, Ambulkar *et al.* (2011) studied the effect of date of sowing and irrigation levels on the incidence of *Helicoverpa armigera* (Hubner) on chickpea crop. In the October 28 and November 20 sown crop harbored least larval population whereas December 11 sown crop showed highest larval population. Parmar *et al.* (2015) also reported that the minimum eggs and larval population was observed on November 07 sown crop which was significantly superior over the other six sowing dates. Variation in irrigation levels significantly influence ovipositional preference and larval population of *H. armigera* at all the growth stages expect 30 and 60 DAS during both the year of experimentation and average of the years. At 45 DAS, the oviposition and larval population of *H. armigera* influenced by irrigation levels, significantly lowest mean oviposition and larval population of *H. armigera* were observed with irrigation at 35 DAS (0.72 eggs/mrl and 1.01 larvae/ mrl respectively), followed by no irrigation (0.83 eggs/ mrl and 1.13 larvae/ mrl respectively). Similar trends were also observed at 75 and 90 days after sowing. This might be due to effect of ecological variation on chickpea varieties. The ovipositional preference by *H. armigera* was significantly affected due to chickpea varieties at 30 DAS but at 45, 60, 75 and 90 DAS was found non significant differences. Among varieties, the lowest mean oviposition by *H. armigera* was observed in JG 24 chickpea variety (0.26 eggs/mrl) over JG 14 (0.37 eggs/mrl) and at par with JG 36 (0.27 eggs/mrl) and JG 12 (0.31 eggs/mrl). Similar variations among genotypes were also observed by Pavani *et al.* (2019) who reported that ICC 3137 had the highest number of eggs across the seasons. Across seasons, ICC 3137 was most preferred for egg-laying followed by KAK 2. ICCV 10 and JG 11 were relatively non-preferred for egg-laying. Contrasting results were reported by Shankar *et al.* (2014) who evaluated chickpea genotypes in the two sowings at 30 days interval (early sown crop in November and late sown crop in December) for resistance to pod borers, *Helicoverpa armigera* under field conditions and observed non-significant differences in numbers of *H. armigera* eggs among the test genotypes. Variation in oviposition preference for *H. armigera* is present which is explained by Brar (2014) who reported that genotype 5282 recorded the lowest number of eggs and was statistically at par with genotype ICCL 86111, RSG 963 GL 25016 respectively. In case of chickpea varieties larval population of *H. armigera* during all growth stages (30 45, 60, 75 and 90 DAS) of crop were found non significant differences. In contrast with the present finding Kumar *et al* (2013) screened 50 genotypes of chickpea against *H. armigera* under field conditions. The lowest larval population and lowest pod damage were recorded in resistant genotype C 235. Deshmukh *et al.* (2010) conducted field screening of chickpea germplasms against pod borer, *H. armigera* and found that BG-372, HC-1, SAKI-9516, Vijay and Avrodhi were comparatively less susceptible as these harbored lower larval population.

The date of sowing significantly influenced the oxalic acid content in leaves of different chickpea varieties at all the time intervals during both years of experimentation and pooled value (Table 1 and graphically presented in Fig 1). It indicated that early sowing was congenial for production of high amount of oxalic acid content in chickpea leaves which was non preference for oviposition and larval incidence by *H. armigera*. At 30 DAS, Crop sown on 15th November recorded significantly higher oxalic acid content in leaves (47.20 $\mu g/g^{-1}$) which was at par with 30th November (45.17 $\mu g/g^{-1}$), followed by 15th December (42.51 $\mu g/g^{-1}$) sowing. At 45 DAS, Crop sown on 15th November recorded significantly higher Oxalic acid content in leaves (39.87 $\mu g/g^{-1}$), followed by 30th November (29.49 $\mu g/g^{-1}$) and 15th December (26.74 $\mu g/g^{-1}$) Sowing. Similar trends were also observed at 60, 75 and 90 days after sowing. Irrigation levels has not shown any significant differences for oxalic acid content at 30,60 and 90 days after sowing (DAS). At 45 days after sowing, one irrigation recorded significantly higher oxalic acid content (32.71 $\mu g/g^{-1}$) as compared to no irrigation (31.36 $\mu g/g^{-1}$). Similar trend was observed for different irrigation treatments on oxalic acid content at 75 days after sowing. Varietal variations revealed non significant differences on oxalic acid content. At 30 DAS, JG 24 variety exhibited higher oxalic acid content (58.88 $\mu g/g^{-1}$), followed by JG 14 (52.62 $\mu g/g^{-1}$), JG 36 (43.14 $\mu g/g^{-1}$) and JG 12 (25.12 $\mu g/g^{-1}$) similar trend was observed during 45, 60, 75 and 90 DAS. Rembold *et al* (1989) reported that chickpea leaf exudates contain malate and oxalate as the main components. The varieties with a high amount of malic and oxalic acid content were found resistant to *H. armigera* and *Liriomyza cicerina*. Comparable results were reported by Srivastava and Srivastava (1989) that ICC 3137, K 850 and ICC 1403 were susceptible to *H. armigera* with more eggs and larvae than the resistant chickpea genotypes. These further concluded that low amount of acidity in the leaf extracts was associated with susceptibility to *H. armigera*. The accumulation of oxalic acid in leaves promote/ enhance resistance mechanisms in chickpea against *H. armigera* reported by Yoshida *et al* (1995) who investigated mechanisms of resistance to *H. armigera* in chickpea and analyzed acid components of the leaf exudates by high-performance liquid chromatography and reported that oxalic acid and malic acid were detected as major components in genotypes resistant to *H. armigera* than susceptible genotypes.

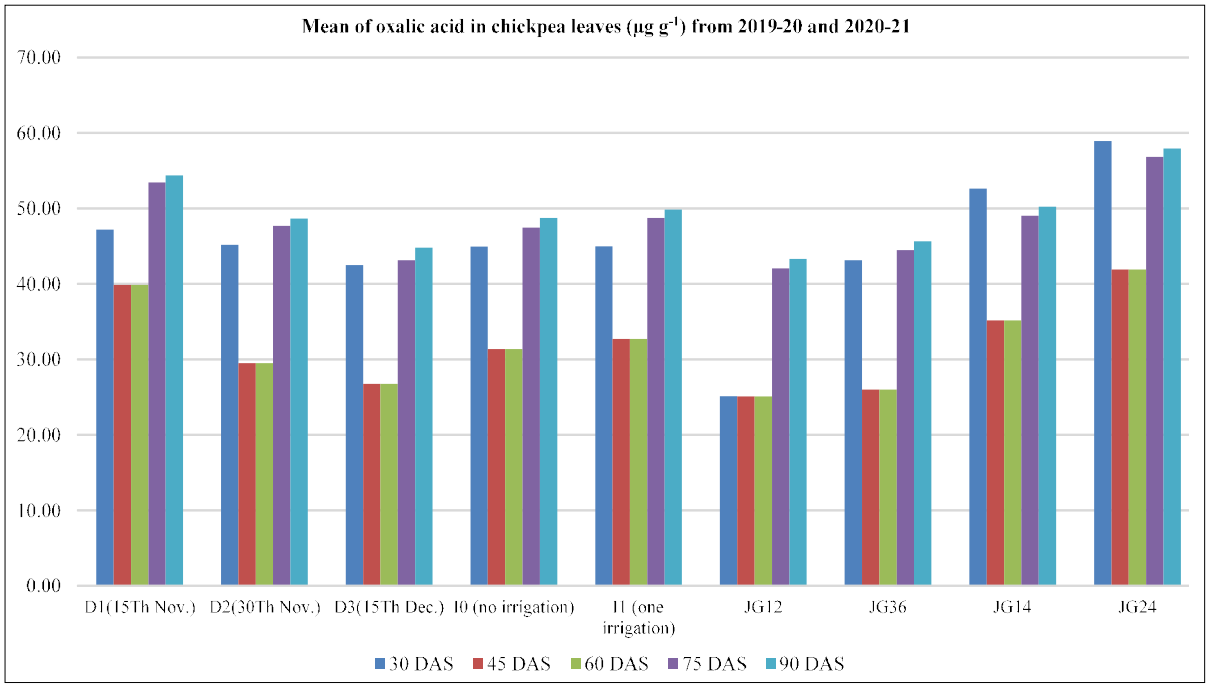

Fig 1: Oxalic acid content in chickpea leaves influenced by different treatments.

Table 2: Association of leaf oxalic acid content with oviposition preference and resistance to *H. armigera* in chickpea varieties in field condition (mean of 2019-20 and 2020-21).

Days	Oviposition preference	Number of larvae
Oxalic acid (µg/g) at 30 DAS	-0.149 (0.488)	-0.627** (0.001)
Oxalic acid (µg/g) at 45 DAS	-0.730** (0.00)	-0.696** (0.00)
Oxalic acid (µg/g) at 60 DAS	-0.433* (0.035)	-0.500* (0.013)
Oxalic acid (µg/g) at 75 DAS	-0.573** (0.003)	-0.630** (0.001)
Oxalic acid (µg/g) at 90 DAS	-0.410* (0.047)	-0.416* (0.043)

Note: (i) *Significant at 5% and **Significant at the 1%.
(ii) Figures in parenthesis are the 'P' Value.

The oxalic acid content recorded in the leaves of chickpea varieties during different growth stages. Results revealed significant negative correlation with oviposition preference at 45 DAS (-0.730**), 60 DAS (-0.433*), 75 DAS (-0.573**) and 90 DAS (-0.410*). Oxalic acid content in the leaves exhibited significant negative correlation with number of larvae at 30 DAS (-0.627**), 45 DAS (-0.696**), 60 DAS (-0.500*), 75 DAS (-0.630**) and 90 DAS (-0.416*), (Table 2). Similarly, Patnaik and Senapati (1995) reported that egg and larval counts of pod borer, *H. armigera* were negatively correlated with increasing concentration of acid exudates of chickpea. Present finding is in accordance with those of Yoshida *et al.* (1997) who reported that oxalic acid showed neither stimulation nor inhibition of oviposition at 0.25-1.7 µmol/cm^2. Correlations between the amount of oxalic acid in trichome exudate on leaf, *H. armigera* populations and pod damage were investigated in a field experiment using 14 chickpea genotypes. They were found significant negative correlation between pod damage and oxalic acid levels. Oxalic acid, which had been reported to have an antibiotic effect on *H. armigera* larvae, has an important role in resistance to this pest in chickpea. Similar results were observed by Peter and Shanower (1998) who reported that chickpea trichome exudates contain acidic chemicals such as malic acid, oxalic acid and succinic acid. Oxalic acid has an antibiosis effect on the larvae of pod borer, *H. armigera*, which results in reduced pod damage. Narayanamma *et al.* (2013) reported that the amounts oxalic acid showed a negative association with leaf damage by *H. armigera*. kumar *et al.* (2017) and Bangar *et al.* (2018) also reported that negatively correlated of oxalic acid with oviposition and larval incidence and pod damage by *H.armigera*.

CONCLUSION

Resistance/tolerance pod borer is a complex character and it is controlled by many factors. For effective selection to improve resistance, it is necessary to have an understanding of various associated traits and nature of their association with host plant resistance. Association analysis employed in this study provides such required information. The present studies indicated that oxalic acid play an important role on resistance to pod borer *H. armigera*. Monitoring the amounts of oxalic acid through UFLC can be used to select chickpea variety for resistance to *H. armigera*.

Conflict of interest: None.

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