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Biochemical and biophysical responses against *Helicoverpa armigera* Hub. (Noctuidae: Lepidoptera) in chickpea

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Abstract

In the rabi season of 2021-22, the experiment was conducted at Department of Agricultural Entomology's Experimental Research Farm at VNMKV, Parbhani aiming at investigating the potential of various chickpea traits and biochemical factors to resist the gram pod borer (*Helicoverpa armigera* Hub.). The study sought to establish positive results concerning chickpeas' ability to resist this pest, a significant challenge to chickpea cultivation. The experiment measured chickpea traits such as pod length-width-wall thickness and pod trichome density-length, as well as biochemical factors like malic acid and oxalic acid. The highest pod length was recorded on BDNG 797 (1.79 cm), which was at par with ICC 506 (1.79 cm), ICCV 10 (1.71), KAK 2 (1.70 cm), and ICCL 86111 (1.69). The highest percentage of malic acid content was observed in ICCL 86111 (1.362%), while ICC 3137 had the lowest oxalic acid level, which was substantially lower than all other genotypes examined. The research found an exceptionally substantial correlation with the amount of malic acid in the chickpea genotypes and the mean pod damage ($r = -0.736$) and the mean larval population ($r = -0.680$). Similarly, oxalic acid exudates on the chickpea genotypes showed a potent and negative association with the mean larval population ($r = -0.692$). In summary, the study findings suggest that malic or oxalic acid content in chickpea genotypes could be potential factors in chickpea resistance to the (*H. armigera*) gram pod borer.

Keywords: biophysical, *Helicoverpa armigera*, Lepidoptera, chickpea, *Cicer arietinum* L.

Introduction

The chickpea, commonly referred to as Bengal gram (*Cicer arietinum* L.), is the most significant pulse crop of the Fabaceae family and is colloquially called "chana." The chickpea crop is world's daily need (FAO STAT 2016) [4]. A superior and less expensive source of protein than meat, chickpea is often referred to as "poor man's meat." In Maharashtra, (Indian State) chickpeas were cultivated over an area of 16.94 lac/ha over the 2020–2021 season of cultivation, producing 13.97 lakh tons and 824 kg/ha of production. Production and productivity in the Marathwada region are 10.59 lakh/ha, 7.76 lakh tones, and 707 kg/ha, respectively (Anonymous, 2020) [1]. Early *H. armigera* larvae feed on chickpea leaves, however later instars switch to feeding on flowers and developing pods and can result in production reductions of 80–90% despite repeated use of pesticides (Banu *et al.*, 2005) [2]. This pest has high fertility, several generations, polyphagous feeding behavior, long distance migration, and capacity to acquire resistance to pesticides (Sharma, 2001; Kranthi *et al.*, 2002) [21, 11]. This pest is quite difficult to eliminate. As a result, alternative pest control tactics are required, and host plant resistance is capable of doing so in a way that efficiently decreases crop losses. *Helicoverpa armigera* affects chickpeas from the early vegetative forward through a podding stage, causing 60–80% of crop losses in Maharashtra (Patil *et al.* 2007) [15]. Kanchana *et al.* (2005) [9] discovered that increasing pod length, breadth, The quantity of protein, and larval population all demonstrated beneficial significant connections with pod damage, in contrast to the amount of trichomes on leaves and pods, which indicated a substantially negative correlation with pod damage. Trichomes and trichomal exudates on leaves and pods have been reported to repel *H. armigera* from the plant surface and to considerably impede walking behavior (Romeis and Shanower 1996) [18].

There are biophysical factors that influence chickpea pod borer protection, including chickpea trichome length and density, pod wall thickness, pod length, number of pods per plant as well as pod width, and area. Along with those defences, it has been also interpreted that malic acid and oxalic acid, which have antifeedant consequences for *H. armigera* and cause oviposition non-preference. This study focused on the defence mechanisms of several chickpea cultivars against *Helicoverpa armigera* using their morphological and biochemical characteristics.

Materials and Methods

Details of genotypes

In the screening experiments, ten distinct genotypes of chickpeas were chosen for their responses to *H. armigera*. The genotypes were received from the Badnapur Agriculture Research Station, VNMKV, Parbhani., viz., JG-11, JG 62, ICC 92944, BDNG-797, KAK 2 (Kabuli), ICCL-86111(R), ICC 506, ICCV 3137 (S), BDNGK-798 (Kabuli) and ICCV 10. The investigation was carried out at VNMKV's experimental farm of agricultural entomology department during *Rabi* 2021-22 under randomized block design (RBD) with three replications. Each genotype was sown in a three-row plot, with each row measuring 2 m. A three-row plot with a row length of 3 m was used to sow each genotype. The seeds were scarified at one end with a scalpel to improve water absorption, soaked in water for 24 hours, and treated with thiram (3 g / kg seed) before sown.

Biophysical attributes of chickpea against *H. armigera* infestation

Pod dimensions

A systematic experimental approach was used in this work to analyse the morphological properties of pods from different genotypes (Uday *et al.*, 2013) [23]. To this end, hundreds of pods were randomly selected from each replication, and length and breadth measurements were taken using a screw gauge. The resulting data were then used to compute the means for each genotype (Zaman *et al.*, 2020) [26]. Additionally, pod husk wall thickness was measured using a similar approach, with ten random pods per genotype selected for analysis. (Girija *et al.*, 2008) [5]. In order to ensure accuracy, three measurements were acquired for each pod and averaged to yield a representative value. These findings contribute to the knowledge of the genetic and external factors that affect the morphology of legume pods and may have important implications for crop improvement strategies (Kuzbakova *et al.*, 2022) [13].

Trichome Dimensions

Observations were made from fully developed, green pods to investigate the density and length of trichomes on the chickpea genotypes. According to Bozzola & Russell (1999) [3], the plant material was preserved and examined using a scanning electron microscope (SEM). For primary fixation, fresh pods of every single genotype were picked, immediately placed in separate vials containing a glutaraldehyde (2.5% OCH (CH₂)₃CHO) solution, and then left over night at a temperature of 4°–5°C. After that, the leaf samples were washed three times with distilled water. To aid in secondary fixation, the specimens were immersed in Osmium Tetroxide (4% OsO₄) solution for 3 to 4 hours at 4° to 5 °C. Following post-fixation, the specimens underwent three further washings using distilled water, each

lasting 4 to 8 minutes. For a total of 20 minutes, the specimen was dehydrated using different ethanol concentrations viz., 25, 50, 70, 95, and 100%. Using 100% ethanol, the total dehydration completed over the period of 30 minutes. After the samples had dried to a critical point in CO₂ at 5 °C, they were mounted on aluminium stubs using two-sided carbon tape (Muzira *et al.*, 2018) [14]. The samples were then analysed and captured using a scanning electron microscope with a secondary electron detector operating at a 15 kV increasing voltage. The trichome density was calculated by counting the number of trichomes per millimetre squared. Utilizing the images acquired after length sealing was performed three times using software, the length of the trichomes was also measured. The least significant difference (LSD) was used to compare treatment means, and the F test was employed to establish the significance of treatment differences at P=0.05. ANOVA was used to analyse the data on several biophysical variables and see if there were any significant genetic differences. After this, a correlation analysis was used to link the biophysical parameters with Egg infestation, larval weight and percent pod damage (Hadi *et al.*, 2017) [18].

Biochemical attributes of chickpea against *H. armigera* infestation

Biochemical constituents in chickpea genotypes were studied in order to know any significant difference in chemical Constituents viz., malic acid and oxalic acid in leaf of different chickpea genotypes as per standard method.

Estimation of malic acid

The quantity of malic acid discharged on the leaves was quantified by measuring the titrable acidity of washings of leaf tissues (500 mg in each instance) in accordance with Koundal & Sinha's (1981) [10] recommendations, and using phenolphthalein indicator. The leaf sample for each genotype weighed 500 mg and was macerated before being cleaned with Whatman No. 1 filter paper and rinsed with distilled water. Filtrate collected, and a volume of 25 ml was created. The phenolphthalein an indicator was used to titrate ten ml portion of this washing against 0.01 N NaOH until a pink colour developed (Satoshi *et al.* 1997) [20]. Malic acid is equivalent to 0.67 mg/ml of NaOH. In terms of fresh tissue, the findings were represented as mg malic acid/g. percent Malic acid = $\frac{TV \times E \times N \times 100}{1000 \times W}$. Where, T. V = Titre value E = Equivalent weight of malic acid, N = Normality of NaOH, W = Weight of sample. (Goering & Vansoest, 1975) [6].

Oxalic acid estimation

The quantification of oxalic acid in leaf samples was executed in accordance with the procedure recommended by Yoshida *et al.* (1995) [24]. The leaf tissue was subjected to a hot air oven at a temperature of 80°C until a consistent weight was achieved; following which it was ground into a fine powder. A sample of the dried tissue weighing 500 mg was combined with 1.5 ml of sulfuric acid (4N H₂SO₄) and 1 g of asbestos. Resulting mixture was then transferred to a Whatman filter paper thimble and placed in a Soxhlet extraction apparatus. The extraction process was carried out using diethyl ether as the solvent for a duration of 48 hours. Subsequent to extraction, 5 ml of sodium hydroxide (NaOH 1 N) and distilled water (7 ml) were added to the extract, followed by the rotary evaporation of the ether layer. The

remaining water phase was moved to a centrifuge tube and calcium chloride-acetate (CaCl₂CH₃COOH) buffer (4 ml) was added, allowing the tube to stand overnight for further processing.

For the estimation of oxalic acid, the supernatant was discarded after centrifugation of the centrifuge tube. Before undergoing a second cycle of centrifugation, the pellet was washed in 5 ml of 5% acetic acid that had been saturated with calcium oxalate (CaC₂O₄). The residue that was left over was dissolved in 4-5 ml of sulfuric acid (4N H₂SO₄) and heated in a water bath between 80 and 90 °C. Following filtering, the extract was titrated against a common 0.02N potassium permanganate (KMnO₄) solution. Oxalic acid in the sample was calculated using the conversion factor: 1 ml KMnO₄ (0.02N) is equivalent to 1.265 mg oxalic acid. This calculation allowed the determination of the oxalic acid content in the sample, expressed as milligrams per 100 grams of the original sample weight.

Results

Biophysical attributes of chickpea against *H. armigera*

In this study, we investigated pod morphological characteristics of different chickpea genotypes.

Pod length and width (mm): Our results (Table 1, depicted

in Figure 1) showed that pod length varied from 14.5 to 17.9 mm with the highest pod length was recorded on BDNG 798 (17.9 mm) which found at level par with ICC 506 (17.4 mm), ICCV 10 (17.1 mm), KAK 2 (17.0 mm) and ICCL 86111 (16.9 mm). The shortest pod length was recorded on JG 11 (14.5 mm). The next genotype of shorter pod length were ICC 3137 (14.8 mm) and ICC 92944 (15.3 mm). Pod width of different genotypes varied from 8.00 mm to 10.14 mm. A narrow pod width was recorded in genotype ICC 92944 (8.00 mm). The other genotypes with narrow pod width were BDNG 797 (8.37 mm) followed by JG 11 (8.55 mm), ICCV 10 (8.59 mm) and ICC 3137 (8.89 mm). The broader pod width was recorded in ICC 506 (10.14 mm) which was at par with BDNG 797 (9.65 mm) followed by KAK 2 (9.24 mm), ICCL 3137 (9.01 mm) and JG 62 (9.00 mm).

Pod wall thickness (mm): Pod wall thickness of different genotypes varied from 0.21 to 0.45 mm (Table 1, depicted in Figure 1). The thickest pod wall was recorded in ICC 92944 (0.45 mm) which was at par with BDNG 798 (0.40 mm). The thinnest pod wall was recorded in JG 62, BDNG 797 (0.21 mm) and was followed by ICCL 86111 (0.22 mm), JG 11 (0.26 mm), ICC 3137 (0.27 mm), KAK 2 (0.33 mm) and ICC 506 (0.36 mm) were at par with each other.

Table 1: Biophysical (Gram Pod attributes) character of chickpea genotypes against *H. armigera*

Genotype	Gram Pod attributes		
	Pod length (mm)	Pod width (mm)	Pod wall thickness (mm)
JG-11	14.5	8.55	0.26
ICC 92944	15.3	8.00	0.45
KAK 2	17.0	9.24	0.33
ICC 506	17.4	10.14	0.36
BDNG-798	17.9	9.65	0.4
JG 62	15.8	9.00	0.21
BDNG-797	15.4	8.37	0.21
ICCL-86111	16.9	9.01	0.22
ICC 3137	14.8	8.89	0.27
ICCV 10	17.1	8.59	0.21
SE(M)	0.07	0.42	0.02
CD @ 5%	0.2	1.24	0.07
CV%	6.57	7.96	13.03

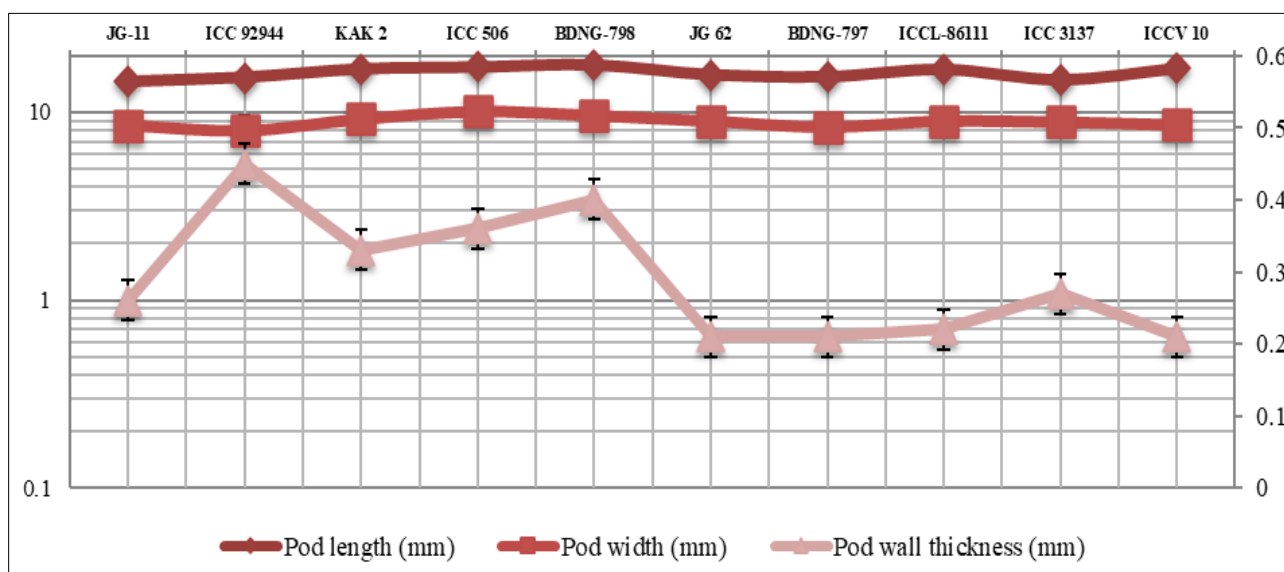


Fig 1: Biophysical (Gram Pod attributes) character differences among genotypes

Trichome density on pods (mm²)

The trichome density on pods of chickpea genotypes varied from 10.19 to 26.03 mm² (Table 2; Figure 2). The highest number of trichomes density were observed on KAK 2 (26.62/ mm²) (Figure 3A) which was at par with ICCL 86111 (25.18 / mm²) and ICC 506 (24.59 / mm²). The genotype JG 11 recorded 2.27 trichomes/ mm² followed by ICC 92944 (19.06 / mm²), JG 62 (18.64/ mm²) and ICCV 10 (16.78 / mm²) and were at par with each other. The lowest number of trichomes was observed on ICC 3137 (10.52/ mm²) (Figure 3B) which was followed by BDNG 798 (11.56 / mm²), BDNG 797 (13.65 / mm²).

Trichome length on pods (µm)

Trichome length on pods of different genotypes ranged from

226.12 to 419.18 µm (Table 2; Figure 2). The highest trichome length on pods was recorded in ICCL 86111 (419.18 µm) (Figure 3C) which was at par with ICC 92944 (413.42 µm) followed by KAK 2 (412.15 µm), ICC 506 (411.02 µm). The lowest trichome length was recorded in JG 11 (226.12 µm) (Figure 3D) which was followed by ICC 3137 (251.22 µm), BDNG 797 (270.34 µm), JG 62 (318.56 µm), ICCV 10 (326.12), BDNG 798 (352.14 µm).

The pod trichome density was suggested to be one of the effective morphological defenses against the pod sucking bug attack (Krisnawati *et al.*, 2022) [12]. Trichome length on pods varied from 226.12 to 425.18 µm, with ICCL 86111, ICC 92944, KAK 2 and ICC 506 having the longest trichomes, and JG 11 having the shortest trichomes.

Table 2: Biophysical character (trichomes) of chickpea genotypes against *H. armigera*

Genotype	Trichome Dimensions	
	Pod Trichome density (mm ²)	Trichome length (µm)
JG-11	20.27	226.12
ICC 92944	19.06	338.42
KAK 2	26.62	399.15
ICC 506	24.59	411.02
BDNG-798	11.56	352.14
JG 62	18.64	318.56
BDNG-797	13.65	270.34
ICCL-86111	25.18	419.18
ICC 3137	10.52	251.62
ICCV 10	16.78	326.12
SE(M)	1.24	1.05
CD @ 5%	2.44	3.15
CV%	1.34	4.25

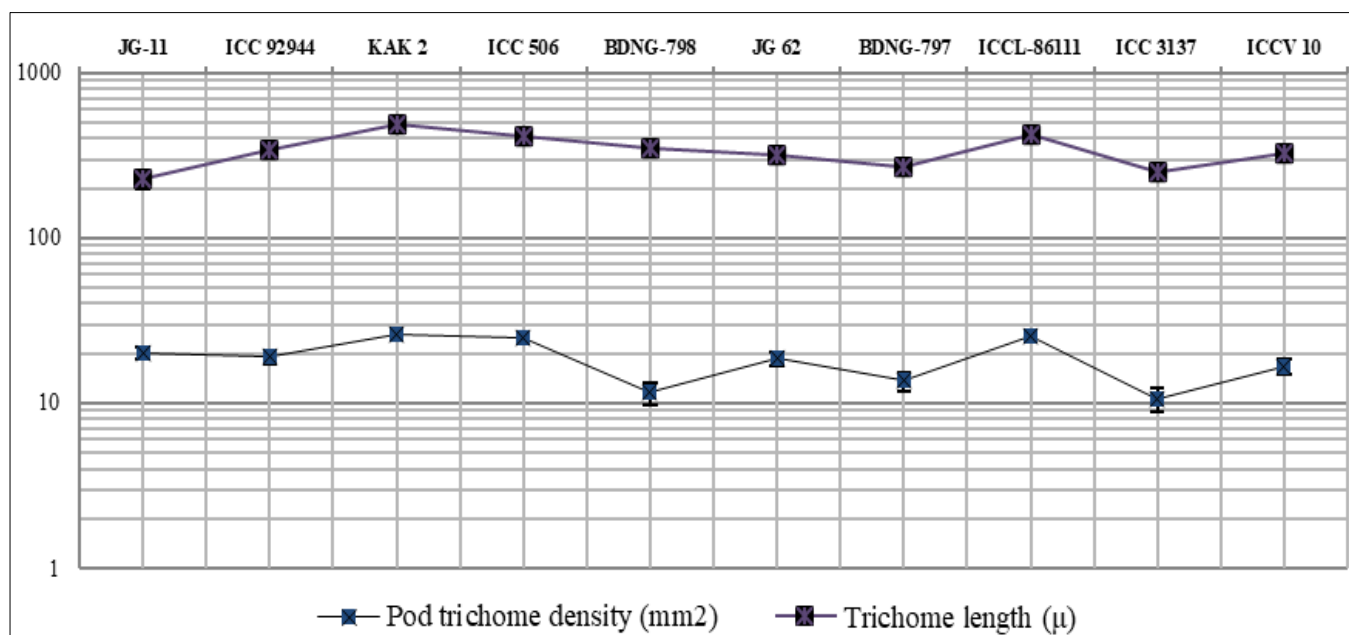
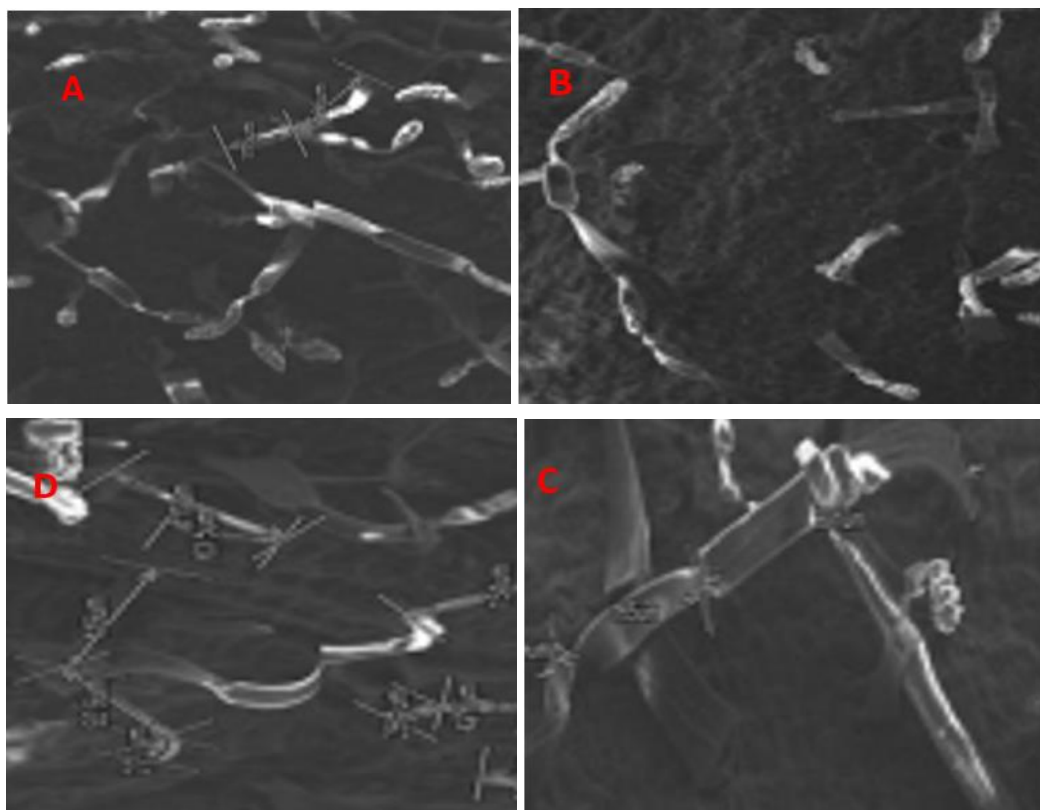


Fig 2: Biophysical (Trichomes) characters differences among chickpea genotypes



A) KAK 2– Highest density (26.62 trichomes / mm² area) B) ICC 3137 – lowest density (10.52 trichomes / mm² area) C) ICCL 86111 – longest length (419.18 μm) D) JG-11 shortest length (226.12 μm)

Fig 3: Trichome density and lengths on pods in chickpea genotypes (Highest and Lowest)

Biochemical attributes of chickpea against *H. armigera*

The data on the biochemical basis of resistance is presented in Table 3 and Figure 4.

Malic acid on leaves

In this study, the malic acid content on leaves of various chickpea genotypes was analyzed to investigate the correlation between malic acid secretion and resistance to insect pests, specifically *H. armigera*. The results revealed significant differences in malic acid content among the tested genotypes. The highest percentage of malic acid was observed in ICCL 86111 (1.362%), followed by ICC 506 (1.293%), JG 11 (1.126%), and ICCV 10 (1.120%), which were significantly superior to all remaining genotypes. The next higher percentage of malic acid content was observed in ICC 92944 (1.101%), which was at par with JG 62 (1.053%), while the lowest percentage was observed in ICC 3137 (0.705%). The Kabuli type genotypes, i.e., BDNG 798 and KAK 2, exhibited 0.761% and 0.789% malic acid, respectively.

Oxalic acid on leaves

The leaves of different chickpea genotypes put forth a bewildering variety in their oxalic acid content. The oxalic acid content spanned a range of 8.05 to 17.20 mg/g across the tested genotypes, as shown in Table 3 and Figure 4. The ICC 506 genotype was a clear winner in the oxalic acid content race, boasting a magnificent 17.20 mg/g, towering over all the other genotypes in the competition. The ICCL 86111 genotype held its own with an impressive 16.95 mg/g, neck to neck with the ICC 506. In contrast, the ICC 3137 genotype was a clear loser with a measly 8.05 mg/g, lagging far behind the rest of the pack. Among the Kabuli

genotypes, BDNG 798 and KAK 2 were found to have 8.67% and 8.53% oxalic acid respectively.

These results (Table 3) are following earlier studies by Chhabra *et al.* (1993), Bhagwat *et al.* (1995), Patnaik & Senapati (1995) [16]; Lateef (1985), which hypothesized that low levels of leaf exudate acidity and malic acid concentration were related to sensitivity to *H. armigera*. Despite the comparatively low frequency of insects on this plant, Koundal & Sinha (1981) [10] suggested that malic acid formation in chickpea be examined in relation to insect pests.

Overall, our findings point to the potential of malic acid as a chemical factor influencing the frequency of insect pests in chickpea plants and imply that malic acid concentration may be a helpful criterion for separating relatively resistant genotypes from vulnerable ones.

These results are following the findings of Surekha Devi *et al.* (2011) [22], who documented oxalic acid content from leaf exudates at the flowering stage. They reported that ICC 506 had 17.70 mg/g of oxalic acid on a dry weight basis, while ICCV 10 had 10.05 mg/g. Yoshida *et al.*, (1997) [25] delved into the both proposed acids content of trichome exudates on chickpea leaves. They discovered that different genotypes had significantly different malic and oxalic acid content. It was also noted that genotypes of *H. armigera* that were resistant accumulated higher levels of oxalic acid on the foliage than susceptible genotypes. The development of *H. armigera* larvae was significantly inhibited when oxalic acid was added to a semi-synthetic diet, suggesting that the substance may be beneficial as a defense against the pest. The suppression of larval development by oxalic acid was most likely related to antibiosis rather than antifeedant effects.

Table 3: Biochemical character of chickpea genotypes against *H. armigera*

Genotype	Malic Acid% on leaves	Oxalic Acid mg/g on leaves
	(Fresh wt. basis)	(Dry wt. basis)
JG-11	1.126	9.54
ICC 92944	1.103	13.95
KAK 2	0.783	8.53
ICC 506	1.293	17.2
BDNG-798	0.761	8.67
JG 62	1.053	10.58
BDNG-797	0.862	9.02
ICCL-86111	1.362	16.95
ICC 3137	0.705	8.05
ICCV 10	1.12	12.51
SE(M)	0.09	0.1
CD @ 5%	0.26	0.3
CV%	1.24	1.58

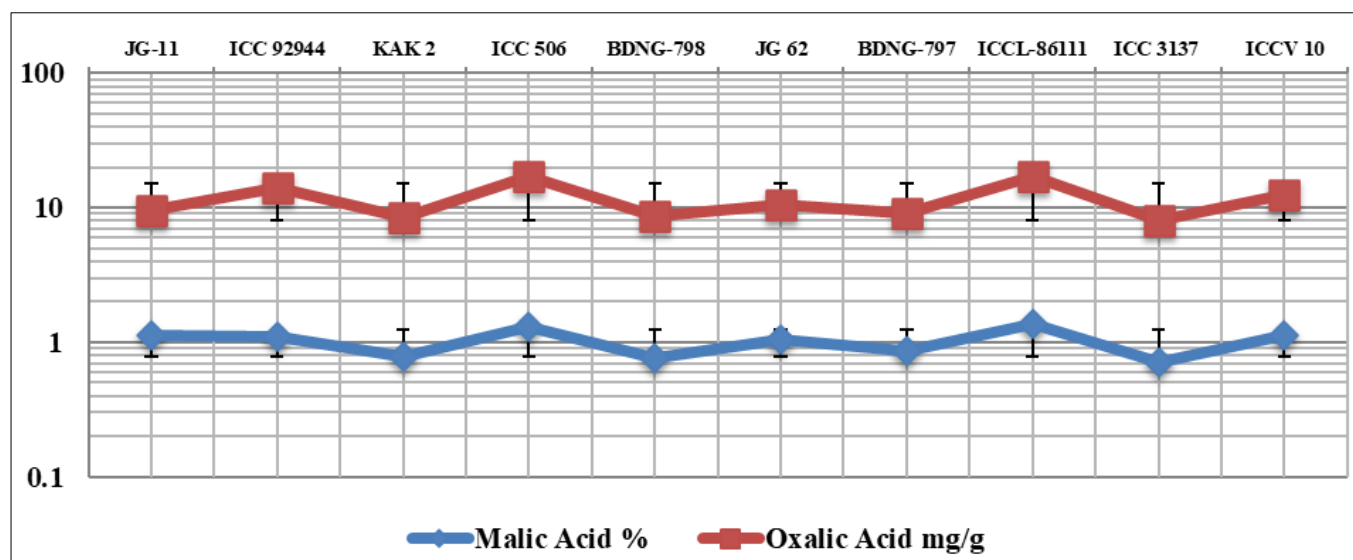


Fig 4: Biochemical character of chickpea genotypes against *H. armigera*

Correlation of biophysical & biochemical attributes with the abundance of *H. armigera* eggs larvae at pod formation stage and percent pod damage: The pertinent information on the relationship between biophysical and biochemical parameters and eggs, larvae, and the percentage of pod damage caused by *H. armigera* is shown in Table 4. The mean eggs population and pod trichome density both showed a negative and significant relation ($r = -0.723$ and $r = -0.693$, respectively). These results suggest that chickpea genotypes exhibiting higher pod trichome density were additional conducive for oviposition, resulting in a higher larval population and ultimately, a higher percent pod damage. This finding highlights the importance of pod trichome density as a determining factor for *H. armigera* infestation in chickpea crops and may aid in the

development of targeted pest management strategies. The present study investigated the relationship between malic acid and oxalic acid content in chickpea genotypes and their impact on the pest population and pod damage caused by *Helicoverpa armigera*. The results revealed a significant and negative relationship between the amount of malic acid and the average pod damage, mean larval population, and average egg population ($r = -0.736$ and -0.680 respectively). In addition, high malic acid content was found to reduce larval weight, pupal weight, larval survival, and adult emergence. Oxalic acid exudates also had a very substantial and negative relationship to the mean larval population and mean pod damage ($r = -0.692$ and 0.782 resp.) showing that high oxalic acid concentration decreased larval weight and pod damage.

Table 4: Correlation between biophysical and biochemical characteristics and mean eggs, larval population, and pod damage

Sr. No.	Biophysical attributes	Eggs population of <i>H. armigera</i> /plant [#]	larval population of <i>H. armigera</i> /plant [#]	Pod damage [§]
1	Pod Length	-0.020	0.035	0.014
2	Pod Width	-0.212	-0.116	-0.116
3	Pod wall thickness (mm)	0.116	0.070	0.060
4	Pod trichome density (mm ²)	-0.723*	-0.693*	-0.533
5	Trichome length (µm)	-0.497	-0.536	-0.359
6	Malic Acid% on leaves (Fresh wt. basis)	-0.690*	-0.680*	-0.736*
7	Oxalic Acid mg/g on leaves (Dry wt. basis)	-0.624	-0.692*	-0.782*

*Significance Level at 0.05% (0.632), [#]Mean population, [§]Mean pod Percent

These results are in line with earlier research that found glandular trichome secretions had phagostimulant and antifeedant effects on *H. armigera* larvae (Green *et al.*, 2003) [7]. Insect herbivores' ovipositional behaviours and host selection process were shown to be significantly influenced by the presence of trichomes and associated exudates on the pod wall surface (Rupakula *et al.*, 2005) [19]. Although trichome density and length had a detrimental effect on larval survival, growth, and development, trichomes were also shown to provide protection against insect pests (Peter & Shanower, 1998) [17].

The findings of the current research were comparable to those of other studies that looked at how key biochemical characteristics of certain chickpea types affected *H. armigera* resistance. While sensitive genotypes with lower trichome density and thinner pod husk thickness demonstrated more pod damage, tolerant genotypes featuring higher trichome density and broader pod husks had less pod damage. (Girija *et al.*, 2008) [5]. As reported by Kanchana *et al.* (2005) [9], increasing protein content showed a substantially negative link with pod damage, while crude fibers had a negative correlation with the pest population. Overall, these results suggest that the content of malic acid and oxalic acid, as well as the presence of trichomes and crude fibers, can significantly reduce the damage caused by *H. armigera* in chickpea crops.

Conclusion

Present investigation has provided valuable insights into the factors influencing the susceptibility or resistance of chickpea genotypes to pod borer infestation. The study has revealed that biochemical and biophysical bases have a significant impact on the susceptibility or resistance of chickpea genotypes, except for pod width. These findings suggest that potential genotypes with desirable biochemical and biophysical traits can be selected for hybridization programs to develop high-yielding, pod borer-resistant or -tolerant chickpea varieties. Such varieties could be used as one of the elements of integrated pest control strategies. Furthermore, the study indicates that the infestation of *H. armigera*, a common pest of legumes, is likely to have a minor to moderate level effect on net yield. This finding is important for farmers and policymakers in developing strategies to manage pest infestations and improve crop productivity. This investigation shows the value of integrated pest management strategies in sustainable agriculture and lays the groundwork for future research into the creation of chickpea cultivars that are resistant to pod borer infestation. Attracting researcher attention to identifying chickpea germplasm that has resistance to pod borers and other natal and corporal challenges to generate high-yielding cultivars with acceptable grain quality as a result of the resistance results.

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