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Screening of maize germplasm through antiobiosis mechanism of resistance against *Chilo partellus* (Swinhoe)

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Abstract
Ten different maize germplasm were evaluated for their relative resistance to maize stem borer *Chilo partellus* (Swinhoe). Several biological parameters of *C. partellus* viz., larval and pupal survival, larval and pupal recovery, larval and pupal weights and percent pupation and plant parameter i.e. leaf injury rating (1-9) were used to study the germplasm susceptibility level. Larval weight (10^{th}, 18^{th} and 25^{th} Days after Artificial Infestation) and pupal weights were significantly lower on the germplasm viz., BML-7 and HKI-323 as compared to the germplasm namely, HKI-1128 and HKI-193-1 indicating the germplasm susceptibility level. The pupae obtained from the relatively resistant germplasm were found deformed with lower weight indicates maximum antibiosis resulted from rearing the larvae on resistant germplasm. The adverse effects on biological parameters were found more pronounced in larval and pupal weight, and larval survival and percent pupation of *C. partellus*. Relatively higher percent pupation and larval survival were observed on highly susceptible germplasm HKI-1128 and HKI-193-1 as compared to resistant ones BML-7 and HKI-323.

Keywords: Screening, leaf injury rating (LIR), antibiosis, *Chilo partellus*, germplasm susceptibility level

1. Introduction
Maize is damaged by more than 139 species of insects during different stages of its growth. The maize stalk borer *Chilo partellus* (Swinhoe) is the key pest which pose a great challenge to increase productivity potential of this crop [3]. The crop losses by *C. partellus* in maize were reported from 26.7 to 80.4% in India [9].

Varietal screening for resistance to stem borer in controlled as well as field conditions can be done by using artificial infestation [12]. Screening of hybrids and varieties of maize against stalk borer, *C. partellus* could be done by comprehending the mechanism of resistance i.e. antixenosis and antibiosis [5]. The term antibiosis encompasses all adverse physiological effects of a temporary or a permanent nature resulting from the feeding by a herbivore on the host plant. The biological parameters related to resistance mechanism were restricted to larval survival/mortality [6, 13], sex-ratio and fecundity [7] in expressing antibiosis. The germplasm is screened to find the sources of resistance which could be used in the breeding programme to develop hybrids. Screening of germplasm is done to identify elite sources of resistance. In the present study different biological parameters like larval survival and larval weight, pupal survival and pupal weight, percent pupation and leaf injury rating (LIR) were selected for studying the germplasm susceptibility level.

2. Materials and methods
2.1. Culture of *Chilo partellus*
The nucleus culture of *C. partellus* was collected from the field of Indian Institute of maize research (IIMR), New Delhi and mass multiplied in the Entomology Laboratory, IIMR under the constant rearing environment at a temperature 26 ± 2.0ºC and relative humidity 65 ± 5%. The field collected larvae were reared on fresh-cut stalks of hybrid HQPM 1, in 2 L glass jars. The culture was multiplied for two successive generations on artificial diet [14]. The third generation neonates were utilized for studying antibiosis mechanism of resistance.

2.2. Experimental procedure

Ten germplasm viz., BML-6, BML-7, CM-139, CM-140, HKI-193, HKI-163, HKI-1128, were selected for the experiment. Five plants of each germplasm were grown in pots of 28 cm height, 18 cm top diameter and 10 cm bottom diameter. Thus 15 plants of one germplasm comprising 3 pots were used for the experiment and five plants in a single pot for each germplasm constitute one treatment. The susceptibility level of each germplasm was determined by taking observations on LIR, larval survival at 10th, 18th and 25th day after artificial infestation (DAI) and their larval weight, pupal recovery, pupal weight and percent pupation. For this, 12-15 day old plants were infested with 5 neonate larvae and the visual grading of leaf injury was done in the scale of 1-9 (Table 1) on the 25th day after artificial infestation (DAI) [1,10]. On 10th and 18th DAI, 15 plants of each germplasm were subjected to destructive sampling and the number of larvae recovered was counted and their weight was taken.

After taking observations for LIR recovered larvae were collected and weighed. The recovered larvae were reared on common natural food and observations i.e., pupal weight, pupal survival and percent pupation were recorded. The number of larvae and pupae recovered were out of five neonates released per plant. The percent pupation was calculated based on a number of pupae formed out of larvae recovered 25 days after artificial infestation. The standard scale used for leaf injury rating is as follows:

Table 1: Scale for Leaf Injury Rating (LIR)

Visual damage	Numerical score	Resistance reaction
Apparently healthy plant	1	Highly resistant
Plants showing slightest damage of leaf or few pin holes on 1-2 leaves	2	Resistant
Plants showing more pin holes or shot holes on 3-4 leaves	3	Resistant
Plants showing injury (pin holes, shot holes and slits) in 1/3 of total number of leaves and mid-rib tunnelling on 1-2 leaves	4	Moderately resistant
Plants showing 50% leaf damage (pin holes, shot holes, slits, streaks) and mid-rib damage	5	Moderately resistant
Plants showing leaf injury in 2/3 of the total number of leaves	6	Susceptible
Plants with every type of leaf injury and almost all leaves damaged	7	Susceptible
Entire plant showing maximum leaf injury and likely to form dead hearts	8	Highly susceptible
Dead hearts (total damage to plant)	9	Extremely susceptible

3. Results

The results of the investigation carried out on the germplasm susceptibility level of ten maize germplasm are presented in Table 1. Data of pupation is given in percentage. The Leaf Injury Rating (LIR) due to larval feeding after 25 DAI was observed and found in the range of 4.2-8.6 on different germplasm. The maximum LIR was recorded on the germplasm HKI-1128 (8.6) and HKI-193-1(8.5) whereas LIR was observed in the intermediate range on the germplasm BML-6(7.3), CM-139(7.46), CM-140(7.06), HKI-163(7.2), HKI-161(6.26) and HKI-1105(6.4). However, LIR was found lower on the germplasm BML-7(4.2) and HKI-323(5.06). Maximum larval weight and larval survival were observed on the germplasm HKI-1128 (13.72, 72.38, 114.64 mg larval weight and 3.66, 3.33 and 2.13 larval survival on 10th, 18th and 25th DAI, respectively). However, minimum larval weight and larval survival was recorded on BML-7 (41.08 mg larval weight 25 DAI and 1.0 larval survival) and HKI-323[(44.69 mg larval weight 25 DAI and 0.86 larval survival)]. Larval weight and larval survival on rest of the germplasm were observed, intermediary. A more or less similar trend for larval survival and larval weight was observed in all the germplasm at different intervals (Figure 1 and 2). The larval survival was found highest on 10 DAI then goes on decreasing on 18 DAI and 25 DAI. The pupal weight was recorded highest from the larvae recovered on the germplasm HKI-1128 (105.28) while, on the germplasm BML-6(70.71), CM-140(70.74), HKI-163(69.08), HKI-193-1(66.94) was found more or less the same and minimum on the germplasm BML-7(44.72), CM-139(56.04) and HKI-323(49.51). The pupal weight was found higher from the larvae obtained on the germplasm BML-7, HKI-323 than the larval weight. The percent pupation was found significantly higher from larvae recovered from the germplasm HKI-1128, HKI-193-1 while larvae the germplasm BML-6, CM-140, HKI-163, and HKI-193-1 was found more or less the same and was less on the germplasm BML-7 and HKI-323.

The results of correlation between the LIR and biological parameetrs of *C. partellus* were presented in Table 2. LIR showed a significant relation with larval weight 25 DAI(r=0.937**), larval survival(r=0.870**), pupal weight(r=757*), pupal survival(r=0.820**). However, LIR showed a non-significant relation with percent pupation(r=0.314). A significant relation was observed for Larval weight 25 DAI with the other parameters LIR (0.937**), larval survival (0.894**), pupal weight (0.840**) and pupal survival (0.835**) except percent pupation(r=0.350) showed a non-significant relation. Larval survival showed a non-significant relation with percent pupation(r=0.391) whereas it showed a significant relation with LIR(r=0.870**), Larval weight 25DAI(r=0.894**), pupal weight (0.852**) and pupal survival(r=0.920**). Pupal weight revealed a significant relation with LIR(r=0.757*), larval weight 25 DAI(r=0.840**), larval survival(r=0.852**) and pupal survival(r=0.948**). However, pupal weight was non-significantly related to percent pupation(r=0.466). Pupal survival was significantly related with all the parameters viz., LIR(r=0.820**), larval weight 25 DAI(r=0.835**), larval survival(r=0.920**), pupal weight(r=0.948**) and percent pupation(r=0.531).

Table 2: Effect of antibiosis in terms of germplasm susceptibility level on *C. partellus*

Germplasm	LIR	Larval weight(mg)			Larval survival			Pupal weight (mg)	Pupal recovery	Percent pupation
		10 DAI	18 DAI	25DAI	10 DAI	18 DAI	25 DAI			
BML-6	7.33±0.11cd	6.8±0.26b	21.33±15.65a	88.12±3.09cd	2.66±0.57a	2.0±0a	1.8±0.2cde	70.71±2.91b	1.06±0.11cd	36.43±2.72a
BML-7	4.2±0.52a	1.1±0.04a	29.34±1.57abc	41.08±5.17a	2.0±0a	1.33±0.57a	1±0.34ab	44.72±1.56a	0.4±0.2a	42.06±8.36a
CM-139	7.46±0.41d	8.7±1.5cd	36.17±4.49bc	85.19±4.52cd	3.0±0b	2.33±0.57a	1.4±0.2bcd	56.04±6.49a	0.73±0.11abc	49.00±35.81a
CM-140	7.06±0.23bcd	7.4±0.25bc	39.51±3.29c	98.44±8.83d	2.33±0.57a	2.0±0a	1.53±0.11cd	70.74±2.48b	0.8±0.2bcd	52.77±24.05a
HKI-161	6.26±0.41b	2.3±0.32a	25.83±1.84ab	73.87±5.94bc	2.0±oa	1.66±0.57a	1.33±0.11abc	45.82±5.91a	0.66±0.30ab	50.59±19.82a
HKI-163	7.2±0.69bcd	9.1±1.20d	32.45±1.30abc	85.18±7.86cd	2.66±0.57a	2.33±0.57a	1.93±0.11de	69.08±5.03ab	1.13±0.11d	64.14±18.42a
HKI-193-1	8.53±0.46e	11.3±1.33e	58.32±2.48d	92.24±11.54d	3.0±1.0ab	2.33±0.57b	1.73±0.41cde	66.94±7.47b	1.06±0.11cd	48.39±19.06a
HKI-323	5.06±0.94a	2.05±0.47a	27.34±6.34ab	44.69±14.30a	2.33±0.57a	1.66±0.57a	0.86±0.41a	49.51±4.73a	0.53±0.11ab	53.32±13.33a
HKI-1105	6.4±0.2bc	2.4±0.59a	35.1±6.68bc	68.87±1.16b	2.0±0a	2.33±0.57a	1.4±0.4bcd	52.12±6.29a	0.8±0.2bcd	61.10±9.61a
HKI-1128	8.6±0.52e	13.79±1.58f	72.38±2.70e	114.63±12.06e	3.66±0.57b	3.33±0.57b	2.13±0.23e	105.28±11.57e	1.8±0.4e	68.26±20.96a
F-value	22.323	64.93	19.65	21.926	3.347	3.389	5.965	26.355	10.657	0.764
CD	0.897	7.40025	27.78975	39.951	1.15575	1.173	0.776	31.48125	0.70725	32.1195

Table 3: Correlation between germplasm susceptibility parameters

Different susceptibility parameters	LIR	Larval weight at 25DAI (mg)	Larval survival	Pupal weight (mg)	Pupal survival	Percent pupation
LIR	1	0.937**	0.870**	0.757*	0.820**	0.314
Larval weight at 25DAI (mg)	0.937**	1	0.894**	0.840**	0.835**	0.350
Larval survival	0.870**	0.894**	1	0.852**	0.920**	0.391
Pupal weight (mg)	0.757*	0.840**	0.852**	1	0.948**	0.466
Pupal survival	0.820**	0.835**	0.920**	0.948**	1	0.531
Percent pupation	0.314	0.350	0.391	0.466	0.531	1
*. Correlation is significant at the 0.05 level (2-tailed).						
**. Correlation is significant at the 0.01 level (2-tailed).						

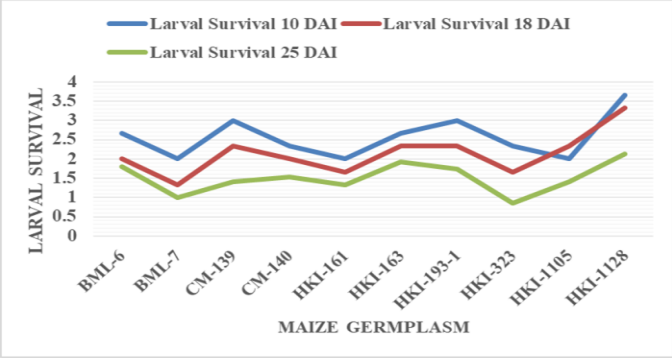

Fig 1: Trend showing Larval Survival at 10 DAI, 18 DAI and 25 DAI

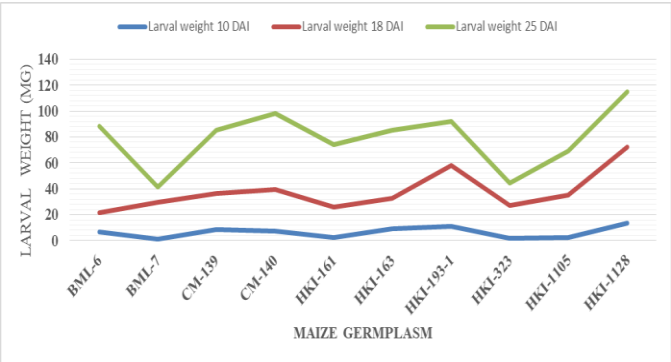

Fig 2: Trend showing larval weight at 10 DAI, 18 DAI and 25 DAI

4. Discussion

The germplasm susceptibility level can be determined by the combination of the different biological parameters such as larval weight, larval recovery, pupal weight, pupal survival and percent pupation. In addition to these biological parameters, Leaf Injury Rating (LIR) indicates the germplasm susceptibility level. Leaf injury, which is the first larval feeding symptoms, has been found to be related to yield losses only under severe infestation [15]. The Leaf Injury Rating (LIR) due to larval feeding in the present investigation after 25 DAI was observed and found in the range of 4.2-8.6 on different germplasm indicating that no germplasm shows the resistant and highly resistant reaction. This may be due to the fact that in glasshouse effect of environmental factors was not present due to controlled conditions so, the effect of natural mortality was also less and sometimes allows the neonates to feed on the resistant germplasm without affecting its biology to a great extent and make the resistant plant nutritious. Present investigation also showed the significant differences in larval weight and larval survival among the different maize germplasm which may give a strong clue about the germplasm susceptibility level rather than the LIR. The adverse effects (antibiosis) measured in biological parameters were more pronounced in larval and pupal weights, and lower larval survival and percent pupation of *C. partellus* [4]. Antibiosis in terms of low larval survival and reduced pupal mass was observed in maize [8]. In controlled conditions, antibiosis and larval antixenosis were evaluated with the artificial infestation. Resistant genotypes may exhibit high levels of antibiosis or larval antixenosis. Larval survival and the ultimate level of damage incurred would be determined by the level of antibiosis present in a given variety [16].

Our findings revealed that the percent pupation, larval survival was more on the germplasm viz., HKI-1128 and HKI-193-1 as compared to BML-7 and HKI-323 indicating the germplasm susceptibility level. All the biological parameters of *C. partellus* were adversely affected on the germplasm such as BML-7; HKI-323 indicating that these germplasm are resistant as compared to others such as HKI-1128, HKI-193-1 which are highly susceptible. The biological parameters were adversely affected on resistant varieties (Antiqua Gr. I and Mex-17) as against susceptible-Basi local and Vijay composite [4]. Larval and pupal weight were recorded higher on susceptible genotypes in comparison to the resistant one. Higher larval mortality was observed in resistant genotypes whereas less larval mortality was recorded on susceptible genotypes [2]. Adverse effects of host plants due to larval feeding on the herbivore were reflected in reduced survival, development and fecundity in later stages of plant colonization [11].

The present investigation showed that the pupal weight was found higher from the larvae obtained on the germplasm BML-7, HKI-323 than the larval weight, this may be due to the fact that the larvae remain undeveloped and underweight due to resistance reaction of the germplasm and on getting suitable natural food it fed for some time and grows well and then undergo for pupation. The LIR and biological parameters showed a differential reaction on different germplasm suggesting that the antibiosis resulted due to the presence of certain biochemical's and secondary metabolites which were produced in the plants due to larval feeding. The parameters such as LIR, larval weight and pupal weight, larval survival and pupal survival were showing high correlation with each other and all these parameters were showing poor correlation with the parameter percent pupation indicating that these parameters contribute more to the germplasm susceptibility level.

5. Conclusion

The differential reaction of *C. partellus* on biological parameters and LIR were giving an indication about the germplasm susceptibility level. Antibiosis in terms of germplasm susceptibility level is due to the production of certain biochemical and secondary metabolites due to insect feeding on the plant which imparts the resistance against the insect. The germplasm showing maximum antibiosis can be further studied for identification of the different biochemical produced in the plant due to insect feeding so that they can be utilized in the breeding programme for imparting resistance against the insects.

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