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ARTICLE

Research Article

Biochemical basis of resistance in different varieties of maize for their relative susceptibility to *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae)

G. Demissie, R. Swaminathan, O. P. Ameta, H. K. Jain and V. Saharan

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Full Length Research Paper

Biochemical basis of resistance in different varieties of maize for their relative susceptibility to *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae)

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Study on the susceptibility of 20 maize varieties against Angoumois grain moth, *Sitotroga cerealella* (Olivier) was carried out under No-choice test method. The susceptibility was assessed on the basis of moth emergence, developmental period, insect weight, weight of flour, grain weight loss, grain damage and nutritional indices. Besides, biochemical characters of the grains were analyzed. Results showed that Pratap makka-5 was the most resistant variety with longest developmental time of 38.36 days, least adult emergence of 3.5, lowest susceptibility index of 2.88, minimum grain damage of 7.21% and grain weight loss of 1.45% followed by EH-2253 and EH-2101. The most susceptible varieties were PMH-1, Navjot, KH-101 and HQPM-1 with shortest developmental time, highest adult emergence, highest susceptibility index, highest grain damage and highest weight loss percentage. Correlation coefficients between susceptibility parameters and biochemical characteristics were calculated, yielding a clear relationship amongst different chemical/nutraceutical parameters, and susceptibility traits. Ash content positively correlated with the progeny emerged ($r=0.476$) and the susceptibility index ($r=0.559$). Furthermore, ash content negatively correlated with the median developmental time ($r=-0.538$). Amylose and susceptibility index ($r=0.734$) and phenolic and median development time ($r=0.680$) had strong positive relationship. The varieties with low ash content, increased phenolic and reduced amylose concentration had more resistance to *S. cerealella*. This research showed that it is important to breed maize variety considered on the low ash, low amylose and high phenolic content besides other morphological and physical characteristics in order to get maize variety which is resistant to *S. cerealella*.

Key words: *Sitotroga cerealella*, biochemical properties, susceptibility index, maize post-harvest research.

INTRODUCTION

Maize (*Zea mays* L.) is the most widely cultivated and consumed of all cereals occupying an important position

in the world economy and trade as food and feed and industrial raw materials. It is the third important cereal

and cash crop of India. It serves as a vital source of proteins, calories (in the form of carbohydrates and fats), and some of the important vitamins and minerals to billions of people worldwide, particularly in Africa and Asia, and has been considered a 'poor man's nutricereal' (Prasanna et al., 2001). Among other factors, food security is greatly threatened by excessive post-harvest losses caused by stored product insect pests. Stored grain moths represent one of the major factors responsible for the post-harvest losses of maize worldwide.

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) is one of the serious insect pests of stored grains in India (Yadu et al., 2000; Pathak and Jha, 2003). It is cosmopolitan in distribution. Its young larvae bore into grains and feed on the inside contents rendering grains unfit for human consumption. These cereals are vulnerable to this insect attack and can have either one or all deficiencies that include weight loss, reduction in nutritional value, contamination or tanning, rendering the cereal food unfit for human consumption. Anonymous (1979) estimated loss of about 15 to 21% maize in storage due to this pest, but up to 50 to 60% has been reported in the untreated kernel and in tropical countries where summer is hot and storage facilities are improper and inadequate (Ahmad and Ahmad, 2002). Unlike in the developed countries where maize grain is stored in silos with controlled moisture and chemical treatment, maize grain in developing countries like India is often traditionally stored in bags made of hessian and jute fibre. This results in a significant decrease in moisture and humidity especially during summer season, thus leading to conditions highly conducive to infestation by the grain moth.

Although insecticide treatment is recommended for grain moths, control of these insect pests by insecticides give residues and develop insect resistance. Moreover, application of fumigants and pesticides has not been satisfactory to many small scale farmers in the developing countries, as they are expensive and may be applied in inappropriate doses by the farmers (Markham et al., 1994). To reduce grain losses in stores, insect resistant varieties are of particular interest for developing countries. Lot of variation has been reported in grains for resistance to storage insects (Hamed and Khattak, 1997; Shafique and Ahmad, 2003).

Varietal resistance to storage insects is a potential means of reducing post harvest losses of maize crop. Varieties vary in susceptibility and attraction to stored grain insects depending upon their physico-chemical properties. Painter (1968) first elaborated the mechanism of resistance by plant systems against insect pests, and classified resistance into three categories: non-

preference (for oviposition, food or shelter), antibiosis (adverse effect of plant on the biology of insects) and resistance (repair, recover or active ability to withstand infestation). Chemical constituent of the grain play a vital role in determining the relative resistance to *S. cerealella* attack. Effect of chemical composition of grains and host plants on the host plant resistance has been reported by number of workers, (Peters et al., 1960, Pandey and Pandey, 1983; Chatterjee et al., 1977; Ragumoorthy and Gunthilagaraja. 1988).

Keeping in view the food value and economic importance of maize grains, the present investigations have been carried out with the objectives to: i) Evaluate the performance of twenty maize varieties to infestation by *S. cerealella* and, ii) Compare chemical and nutraceutical composition of grain with susceptibility of maize varieties to *S. cerealella* infestation if there is any relationship between these traits.

MATERIALS AND METHODS

Rearing of *S. cerealella*

Pure culture of *S. cerealella* used for this study was obtained from the established stock culture maintained for several years at the storage laboratory of the Division of Entomology, IARI, New Delhi. A stock culture of *S. cerealella* was reared on disinfested and conditioned commercial maize (Pratap Makka) seeds at $28 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ r.h. in 4 L plastic jars covered with muslin cloth. The grain was cleaned and disinfested by keeping at $-20 \pm 2^\circ\text{C}$ in a deep freezer for two weeks and then equilibrated/conditioned before use for additional two weeks to the laboratory conditions ($28 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ RH) at which the culture was reared. To meet the regular supply of insects, the newly emerged moths were released on fresh disinfested and conditioned grain. The culture and the experiments were maintained at a temperature of $28 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ relative humidity in Biological Oxygen Demand (BOD) incubators.

Grain of maize varieties used

The research was conducted from January 2013 to February 2014. The study was undertaken to assess susceptibility and biochemical basis of resistance in twenty maize varieties against Angoumois grain moth, *S. cerealella*. All maize varieties were obtained from all India Co-ordinated Maize Improvement Project, Department of Plant Breeding and Genetics, Rajasthan College of Agriculture, Udaipur (Rajasthan). The maize varieties used and their type, grain colour, grain texture and maturity group are indicated in Table 1.

Biochemical analysis of the grains

The maize kernels were milled using a laboratory mill and stored at 4°C prior to analysis. Proximate composition of the grains (that is, determination of moisture, crude protein, soluble protein, soluble sugar, crude fat, crude fiber, ash, carbohydrates, amylose,

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amylase and phenol) were performed in accordance with the standard method of Association of Official Analytical Chemists Washington, DC, USA632 A.O.A.C. (1990).

Moisture content was determined by Farmex MT-PRO grain moisture meter. Crude protein content was determined using the Kjeldahl procedure. The protein content was estimated by 'N' percent x 6.25 considering that the protein contains 16% nitrogen (Balogun and Fetuga, 1986; Gary, 1986; Amoo, 1998; Adeyeye, 1995). Carbohydrate content was determined by calculating the difference of the total of percentages of protein, crude fat and ash from 100. Carbohydrate content = $100 - \Sigma (\text{Ash \%} + \text{Protein \%} + \text{Fat \%})$. Results from percentages of ash, protein and fat were calculated in the dry material of kernels. Crude fibre was determined by subsequent acid base digestion. Crude fat was determined by ether extract method using Soxhlet apparatus. Ash content was determined using muffle furnace. The value was expressed in percentage. Determination of amylase was carried out by Di-Nitro Salicylic Acid (DNSA) procedure. Total phenols estimation was carried out with Folin-Ciocalteu reagent (FCR). The estimation of amylose content was determined by modified Juliano method (Juliano, 1979; Sadasivam and Manickam, 1992). The amount of total soluble sugars was estimated by following the anthrone reagent method of Hodge and Hofreiter (1962).

Susceptibility test by no-choice method

The F₂ grain, formed through open pollination, was used to evaluate resistance because this represents the generation that is stored by farmers and vulnerable to *S. cerealella*. Previously untreated disinfested and conditioned samples of 200 g of the maize grains were taken from each batch of the selected maize varieties for the experiment and put in a 350 cm³ glass jar. No-choice trials involved placing 50 unsexed adult moths (1-2 days old) in jars to infest the 200 g grains of each variety for ten days to allow oviposition. The jars were covered with muslin close and tightened with rubber band which can permit adequate ventilation and preventing escape of the moths. Each treatment was replicate three times in a completely randomized design (CRD). After 10 days of oviposition, the moths (dead/live) were removed and the jars were then kept at the same experimental condition for F₁ progeny emergence. Based on previous similar research works F₁ progeny emergence, median development time, insect weight, susceptibility index, grain damage, weight losses and nutritional indices were experimented as described here under to categorize the varieties in to different susceptibility groups

Adult progeny emergence

Twenty eight days after moth introduction, the containers were checked every other day for adult emergence and data was recorded for first generation adult emergence. Counting of these adults were done by immobilizing them, using chloroform impregnated cotton plugs. Examination of each jar and collection of emergent moths was continued until no further emergence had been noted.

Median developmental period

The date of moth infestation was recorded for each replicate. Median developmental period that is, number of days from middle of oviposition period to fifty percent F₁ progeny emergence were recorded for each replicate.

Insect weight

Newly emerged moth weights were taken. Total weight of twenty F₁

progeny was calculated as mean adult weight from each entry using a sensitive weighing balance.

Dobie's susceptibility index

Based on the number of moths emerged in each test variety and mean developmental period; index of susceptibility was calculated by the following formula (Dobie, 1978):

$$\text{Susceptibility Index}(SI) = \frac{\text{Natural log } F_1}{D} \times 100$$

Where F₁ is the total number of first generation emerging adults and D is the median developmental period.

The Dobie index, ranging from 0 to 11, was used to classify the maize varieties into susceptibility groups (Dobie, 1974; CIMMYT):

- (i) Dobie index of 0 to 4 classified as resistant;
- (ii) Dobie index of 4.1 to 7.0 moderately resistant;
- (iii) Dobie index of 7.1 to 10.0 susceptible; and
- (iv) Dobie index of ≥ 10.1 classified as highly susceptible

Grain damage and weight loss

At the termination of the experiment, each sample was passed through a 12-mesh sieve for separation of the grains and flour. The flour passed was weighed and discarded while the remaining material will be weighed. The grains containing holes were separated from the sound grains and both damaged and sound grains were weighed and counted. The percent damage and the percent weight loss were calculated according to the following formulae:

$$\text{Grain damage}(\%) = \frac{\text{Number of damaged grain}}{\text{Total number of grain used}} \times 100$$

$$\text{Grain weight loss}(\%) = \frac{(\text{Initial Weight} - \text{Final Weight})}{\text{Initial Weight}} \times 100$$

Nutritional indices

Nutritional indices were calculated as per the method described by Bergvinson (2004) using the following formulas:

$$\text{Approximate Digestibility}(\%) = \frac{(W_i - W_f) - W_{\text{flour}}}{(W_i - W_f)} \times 100$$

$$\text{Efficiency of Conversion}(\%) = \frac{W_{\text{progeny}}}{(W_i - W_f) - W_{\text{flour}}} \times 100$$

$$\text{Efficiency of Conversion of Ingested Food}(\%) = \frac{W_{\text{progeny}}}{(W_i - W_f)} \times 100$$

Where: W_i = Initial weight of grain, W_f = Final weight of grain, W_{flour} = Weight of flour collected and W_{progeny} = Total weight of F₁ progeny

Statistical analysis

All susceptibility traits as well as biochemical parameters were

Table 1. List of maize varieties evaluated against *S. cerealella*.

S/No.	Name of variety	Type	Grain colour	Grain texture	Maturity group
1	PMH-1	Hybrid	Yellow	Dent	Late
2	Seed tech2324	Hybrid	White	Dent	Early
3	Pratap makka-5	Composite	Yellow	Semi-flint	Medium
4	Navjot	Composite	Yellow	Flint	Medium
5	PE HM-2	Composite	Yellow	Flint	Early
6	Arawali makka-1	Composite	White	Flint	Early
7	Pratap chari makka-6	Composite	White	Semi-flint	Late
8	Super 9220	Hybrid	Yellow	Semi-flint	Late
9	KH-101	Hybrid	Yellow	Semi-flint	Late
10	PAC-790	Hybrid	Yellow	Semi-flint	Late
11	NK-30	Hybrid	Yellow	Semi-dent	Early
12	HM – 10	Hybrid	Yellow	Semi-dent	Late
13	GK – 3090	Hybrid	Yellow	Dent	Medium
14	Vivek Hybrid-9	Hybrid	Yellow	Flint	Late
15	HQPM-1	Hybrid	Yellow	Dent	Late
16	EHQ-16	Hybrid	White	Semi-flint	Medium
17	HQPM-7	Hybrid	Yellow	Flint	Late
18	EH-2101	Hybrid	Yellow	Semi-flint	Early
19	EH-2253	Hybrid	Yellow	Dent	Medium
20	EHQ-63	Hybrid	Yellow	Semi-dent	Medium

subjected to Analysis of Variance using the PROC GLM procedure (SAS institute, 2004). Differences among means were compared by Student Neuman Keul (SNK) tests with alpha <0.05. The data of percentage grain damage, weight loss and progeny emerged were analysed after arcsign, square root and log transformation, respectively. Analysis of variance was performed on both transformed and untransformed data but statistical parameters were taken from transformed data. Means of untransformed data are presented in the tables. The correlation between susceptibility traits and biochemical parameters were examined using Pearson's correlation coefficient using PROC CORR procedure of the SAS software (SAS Institute, 2004).

RESULTS

Proximate composition of maize grain

The quantitative estimation of biochemical content in different varieties of fresh maize grain was done and the results were presented in Tables 2 and 3. The correlation coefficient of these characters with susceptibility parameters were determined and summarized in Table 6.

Crude fat/oil content

Fat content was assessed from the dried material of the grains of different maize varieties in both two years. Analysis of pooled data showed that there were no significant differences among the maize varieties. Numerically the lowest fat content (<4%), however, was

observed in entries 2, 11, 15, and 18 while the highest value (>4%) was with the remaining entries. In general percent fats were determined in the range of 3.43% (Seed tech2324 variety) to 4.94% (HQPM-7 variety) (Table 2).

Crude fibre

The crude fibre content of the different maize grain was in a range of 1.39 to 2.60% in the first year of the study while, it was 1.46 to 2.53% in the second year. There were significant differences among the maize varieties as regard to crude fibre content in pooled data. Percent crude fibre was found in the range of 1.44 to 3.00%. In the present study out of twenty different maize varieties eleven were having more than 2% crude fibre while the remaining 9 were having less than 2% (Table 2).

Crude protein

Crude protein was significantly different among kernels. The highest protein content was observed with Arawali makka-1 (19.98%) followed by GK-3090 (19.31%) and it was however, at par with PMH-1, Pratap makka-5, Pratap chari makka-6 and EH-2253, respectively. The lowest protein content was observed in EH-2101 (11.86%) followed by HQPM-1, Seed tech2324 and Vivake hybride-9 (Table 2).

Table 2. Major Nutraceutical composition for the different twenty maize varieties (Mean±S.E).

Entry	Varieties	Crude fat/oil (%)	Crude fibre (%)	Crude protein (%)	Soluble sugar (% mg)	Crude carbohydrates (%)
1	PMH-1	4.55 ±0.19	2.38 ±0.07	18.89 ±1.62	17.14 ±1.76	75.10 ±1.48
2	Seed tech2324	3.43 ±0.28	2.05 ±0.15	12.64 ±3.09	22.16 ±2.48	82.39 ±2.96
3	Pratap makka-5	4.38 ±0.17	1.44 ±0.17	16.73 ±0.61	24.88 ±1.79	77.46 ±0.47
4	Navjot	4.07 ±0.14	2.28 ±0.35	15.57 ±0.30	15.26 ±0.94	78.82 ±0.28
5	PE HM-2	4.23 ±0.14	1.84 ±0.28	15.39 ±0.81	18.24 ±1.19	78.87 ±0.61
6	Arawali makka-1	4.44 ±0.15	1.96 ±0.20	19.98 ±1.05	24.44 ±1.87	74.09 ±0.92
7	Pratap chari makka-6	4.13 ±0.16	2.34 ±0.55	16.63 ±1.17	22.55 ±0.68	77.85 ±1.03
8	Super 9220	4.32 ±0.12	2.06 ±0.14	14.08 ±0.93	25.63 ±1.66	80.14 ±0.98
9	KH-101	4.10 ±0.45	1.78 ±0.20	14.98 ±0.77	24.39 ±0.78	78.98 ±0.88
10	PAC-790	4.12 ±0.42	1.55 ±0.08	14.56 ±0.90	16.88 ±1.38	79.32 ±1.25
11	NK-30	3.72 ±0.53	1.64 ±0.27	15.25 ±1.15	19.15 ±1.68	79.33 ±1.66
12	HM – 10	4.66 ±0.48	1.70 ±0.25	14.21 ±0.59	21.78 ±2.51	79.18 ±1.03
13	GK – 3090	4.07 ±0.40	2.23 ±0.22	19.31 ±1.23	14.61 ±5.37	74.72 ±0.81
14	Vivek Hybrid-9	4.18 ±0.53	1.86 ±0.22	12.91 ±2.75	9.43 ±3.35	81.42 ±3.32
15	HQPM-1	3.86 ±0.44	2.00 ±0.36	12.61 ±1.25	20.47 ±1.18	82.01 ±1.56
16	EHQ-16	4.78 ±0.46	1.69 ±0.19	15.18 ±2.23	24.43 ±2.86	78.68 ±2.68
17	HQPM-7	4.94 ±0.54	3.00 ±1.22	14.64 ±1.32	22.35 ±1.04	78.96 ±1.67
18	EH-2101	3.88 ±0.46	2.35 ±0.43	11.89 ±2.57	27.50 ±1.47	83.13 ±2.85
19	EH-2253	4.63 ±0.42	2.59 ±0.52	16.63 ±0.66	20.72 ±0.42	77.44 ±0.96
20	EHQ-63	4.25 ±0.49	2.19 ±0.35	15.73 ±2.44	16.48 ±1.77	78.86 ±2.33
LSD at 5%		ns	0.79	3.88	5.91	3.93
C.V. (%)		18.56	23.55	22.02	25.26	4.35

ns stands as “non-significant”.

Soluble sugar contents

As regard to the soluble sugar, significant differences were recorded among different varieties. Analysis of pooled data revealed that sugar content was highest in EH-2101 (27.51% mg) but this was at par with entries 8, 3, 6, 16, 9, 7, 2 and 12. The lowest sugar content was observed for Viveke Hybrid-9 (9.44% mg) followed by GK-3090 and Navjot. The remaining entries had sugar content ranging between 16 and 20% mg (Table 2).

Crude carbohydrate

During the studies significant difference was observed for the percent crude carbohydrate in kernels of different maize varieties. The crude carbohydrate was highest in the kernels of EH-2101 (83.13%) followed by Seed tech2324 (82.39%), HQPM-1 (82.02%), Viveke Hybrid-9 (81.42%) and Super 9220 (80.14%) and were statistically at par. The lowest crude carbohydrate content was observed in Arawali makka-1 (74.09%) which was significantly lowest as compared to the rest of the varieties (Table 2). In general carbohydrates are the major chemical component of the maize grains. It was found in the range of 74.09% (Arawali Makka-1 variety) to

83.13% (EH-2101 variety).

Ash content

Significant differences were observed for the ash content in the maize grains. Percent ash content of different maize varieties were found in the range of 1.11 to 2.00%. PAC-790 had the highest ash content of 2.00% whereas EH-2101 had the lowest value of 1.11%. However, most of the varieties have ash content of less than 2%. The mineral or ash contents were also within the typical range (1.1-2.5%) expected for whole kernels (Table 3).

Grain moisture content

Data regarding moisture contents of different maize varieties determined by moisture meter are given in Table 3. Data revealed that the highest grain moisture content (>10.5%) were observed in entries 5, 6, 8, 10 and 15 whereas the lowest (<9.50%) in the entries, 1, 4 and 9.

Amylose content

There were significant differences among kernels of

Table 3. Other proximate composition for the different twenty maize varieties (Mean±S.E).

Entry	Varieties	Ash content (%)	Grain moisture content (%)	Amylose content (%)	Phenolic content (mg/100 g)	Amylase content (mg/g)
1	PMH-1	1.45 ±0.12	9.01 ±0.08	16.11 ±3.49	110.33 ±16.34	12.12 ±0.34
2	Seed tech2324	1.54 ±0.05	9.73 ±0.17	14.77 ±3.93	92.00 ±11.31	10.85 ±0.13
3	Pratap makka-5	1.44 ±0.07	10.41±0.17	20.94 ±2.92	82.33 ±11.84	11.03 ±0.68
4	Navjot	1.54 ±0.06	9.45 ± 0.37	20.01 ±1.54	76.67 ±10.43	12.40 ±1.14
5	PE HM-2	1.50 ±0.11	11.25 ±0.17	23.26 ±2.85	69.63 ±7.14	11.66 ±0.53
6	Arawali makka-1	1.49 ±0.06	10.95 ±0.09	22.72 ±1.36	77.37 ±8.35	14.90 ±1.41
7	Pratap chari makka-6	1.39 ±0.07	10.00 ±0.25	22.57 ±1.96	79.00 ±5.03	11.44 ±0.23
8	Super 9220	1.46 ±0.07	11.04 ±0.16	22.55 ±2.32	75.93 ±2.42	10.94 ±0.24
9	KH-101	1.94 ±0.22	9.35 ±0.09	22.18 ±3.11	83.67 ±7.84	9.19 ±1.02
10	PAC-790	2.00 ±0.26	11.06 ±0.09	23.15 ±1.77	70.67 ±2.86	13.20 ±0.31
11	NK-30	1.70 ±0.16	10.25 ±0.14	22.34 ±0.94	77.43 ±3.22	12.81 ±0.35
12	HM – 10	1.95 ±0.17	10.21 ±0.09	26.06 ±0.93	84.17 ±5.42	9.66 ±0.06
13	GK – 3090	1.90 ±0.23	9.63 ±0.17	21.57 ±1.66	111.13 ±15.44	8.91 ±0.14
14	Vivek Hybrid-9	1.49 ±0.12	9.79 ±0.09	21.91 ±2.21	74.97 ±6.65	8.52 ±0.20
15	HQPM-1	1.51 ±0.08	10.64 ±0.28	23.98 ±2.59	90.97 ±9.59	12.80 ±0.43
16	EHQ-16	1.36 ±0.02	10.50 ±0.08	25.45 ±6.08	77.50 ±6.67	11.55 ±0.09
17	HQPM-7	1.46 ±0.04	10.46 ±0.13	26.29 ±3.97	71.00 ±5.88	10.82 ±0.45
18	EH-2101	1.11 ±0.05	9.66 ±0.06	20.79 ±3.48	78.73 ±8.17	9.63 ±0.08
19	EH-2253	1.31 ±0.05	10.16 ±0.11	20.35 ±1.52	83.40 ±10.43	11.37 ±0.14
20	EHQ-63	1.16 ±0.05	9.90 ±0.03	22.81 ±2.49	81.20 ±9.54	9.65 ±0.19
LSD at 5%		0.33	0.45	3.99	12.82	1.56
C.V. (%)		18.57	9.86	15.81	13.57	12.17

different maize varieties in percent amylose content. The maximum percentage of amylose content (26.29 and 26.06%) was recorded from HQPM-1 and HM-10, respectively; whereas Seed tech2324 and PMH-1 varieties having minimum percent amylose (14.77 and 16.11%, respectively) had non-significant difference among themselves. The rest varieties were having amylose content in the range between 20 and 25% (Table 3).

Phenolic content

The maximum total phenolics (111.13 and 110.33 mg/100 g) in GK-3090 and PMH-1 varieties had significant difference from all other varieties. PEHM-2 and PAC-790 varieties having minimum total phenolics (69.63 and 70.67 mg/100 g, respectively) had non-significant difference among themselves. The resistant variety Pratap makka-5 and moderately resistant EH-2253 had phenolic content of 82.33 and 83.40 mg/100 g, respectively. phenolic content in the rest entries ranged from 71.00 to 92.00 mg/100 g (Table 3).

Amylase content

There were highly significant ($P<0.01$) differences among

maize varieties in amylase content. The highest amylase content was observed for Arawali maka-1 (14.90 mg/g) being significantly different from all the remaining varieties. The lowest amylase content was observed for Vivake hybrid-9 and GK-3090 (8.52 and 8.91 mg/g, respectively) but these were at par with entries 9, 12, 18 and 20 (Table 3).

Insect susceptibility parameters

ANOVA on pooled data of 20 maize varieties revealed significant variations among the varieties for both insect weight, weight of flour, approximate digestibility, efficiency of conversion and efficiency of conversion of ingested food (Table 4). The highest insect weight (37.00 mg) was observed for entry 1 followed by entries 18 and 8 (36.25 and 34.50 mg, respectively) and were at par with each other; whereas, the lowest insect weight (21.25 mg) was observed for entry 3 followed by entries 12, 15, 9, 14, 2 and 7 and were not significantly different from each other. The remaining entries had insect weight ranging from 27 to 31 mg and statistically at par. Furthermore, Table 4 also shows that entry 1 had the highest weight of flour (196 mg) followed by entry 9 (145.75 mg) and entry 7 (109.75 mg), which were significantly different from the other varieties. The lowest weight of flour (41.25 mg) was observed from entries 3 followed by entries 19 (42.50

Table 4. Insect weight, weight of flour and nutritional indices for susceptibility to *S. cerealella* (Mean±S.E).

Entry	Varieties	Insect weight (mg/20)	Weight of flour (mg)	Approximate digestibility	Efficiency of conversion	Efficiency of conversion of ingested food
1	PMH-1	37.00 ±6.11	196.00±79.12	98.40 ± 0.19	2.43 ± 0.26	2.39 ± 0.26
2	Seed tech2324	24.25 ±5.26	66.25±3.59	98.76 ± 0.19	0.97 ± 0.17	0.96 ± 0.17
3	Pratap makka-5	21.25 ±2.44	41.25±5.79	96.33 ± 0.73	0.99 ± 0.38	0.96 ± 0.36
4	Navjot	29.25 ± 3.97	48.75±10.68	98.53 ± 0.31	2.64 ± 0.90	2.59 ± 0.87
5	PE HM-2	29.00 ±4.49	69.25±3.36	98.24 ± 0.10	2.12 ± 0.42	2.09 ± 0.41
6	Arawali makka-1	28.25 ±3.72	55.25±5.61	98.42 ± 0.35	1.75 ± 0.43	1.72 ± 0.43
7	Pratap chari makka-6	25.50 ±2.17	109.75±23.76	98.82 ± 0.18	1.27 ± 0.16	1.25 ± 0.15
8	Super 9220	34.50 ±5.38	58.75±6.17	98.00 ± 0.24	2.66 ± 0.26	2.61 ± 0.25
9	KH-101	22.75 ±2.22	145.75±35.17	96.07 ± 0.79	2.76 ± 0.74	2.66 ± 0.72
10	PAC-790	27.25 ±3.56	84.50±3.74	98.78 ± 0.32	1.78 ± 0.12	1.76 ± 0.12
11	NK-30	27.00 ±4.74	69.75±8.72	98.52 ± 0.22	1.30 ± 0.12	1.28 ± 0.11
12	HM – 10	21.50 ±2.27	65.50±10.53	98.80 ± 0.09	1.36 ± 0.14	1.35 ± 0.14
13	GK – 3090	31.00 ±1.63	82.00±11.07	98.52 ± 0.17	1.64 ± 0.30	1.62 ± 0.29
14	Vivek Hybrid-9	23.25 ±3.28	55.50±2.54	98.39 ± 0.09	1.88 ± 0.25	1.85 ± 0.25
15	HQPM-1	22.25 ±1.53	48.00±0.59	98.74 ± 0.28	1.86 ± 0.48	1.84 ± 0.46
16	EHQ-16	28.25 ±3.48	78.75±23.08	98.39 ± 0.27	1.84 ± 0.59	1.80 ± 0.58
17	HQPM-7	27.00 ±4.31	56.50±1.25	98.53 ± 0.13	2.07 ± 0.49	2.04 ± 0.48
18	EH-2101	36.25 ±4.11	44.75 ± 1.91	98.12 ± 0.22	2.63 ± 0.97	2.58 ± 0.94
19	EH-2253	29.75 ±0.66	42.50 ±2.63	97.89 ± 0.44	2.18 ± 0.82	2.12 ± 0.78
20	EHQ-63	28.50 ±4.72	73.25 ±9.88	97.57 ± 0.65	2.87 ± 0.65	2.79 ± 0.61
LSD at 5%		5.42	0.06	0.96	1.32	1.27
C.V. (%)		17.09	18.30	5.85	17.92	22.12

mg) and 18 (44.75 mg). Analysis of nutritional indices showed that the lowest approximate digestibility (<98) was observed for entries 9, 3, 20 and 19, while all the remaining entries had the highest approximate digestibility of more than 98. On the other hand the lowest efficiency of conversion (0.97 and 0.99) were observed from entries 2 and 3, respectively; whereas, the highest (2.87 and 2.76) were calculated from entries 20 and 9, respectively followed by entries 8, 4, 18, 1, 19, 5 and 17 and were statistically at par with each other. Likewise, a similar trend was observed in the case of efficiency of conversion of ingested food.

There were also significant variations among maize varieties in F_1 progeny emerged, median development time, grain damage, grain weight loss and susceptibility index (Table 5). Minimum progeny emergence was in entry 3 (3.50) and entry 19 (10.67) followed by entry 18 (12.17) and entry 6 (25.17), while the maximum was observed in entry 7 (69.83) and entry 1 (61.00) followed by entry 9 (57.00) and entry 12 (56.00) which were at par with each other. Similarly, entries 18, 3 and 19 recorded lowest grain damage (6.52, 7.21 and 9.01%, respectively) followed by entry 6 (15.28%), while the highest was observed in entry 7 (33.02%), entry 1 (31.12%) and entry 4 (30.49%) followed by entry 20 (28.04%) and entry 9 (25.71%) (Table 5). The weight loss was significantly lower (1.27%) in entry 18 and 19 followed by entry 8

(1.66%) and entry 14 (1.78%), while it was significantly higher in entry 7 (5.49%) followed by entry 16 (3.88%) and entry 2 (3.68%). *S. cerealella* reared on entries 7 (40.00), 3 (38.36), 20 (37.33), 12 (37.33) and 13 (37.17) had relatively longer development period; whereas, those reared on entries 5, 11 and 18 had comparatively shorter development period (31.38, 31.50 and 32.00 days, respectively) (Table 5). Based on the median developmental time and the numbers of F_1 progeny emerged susceptibility index were calculated. The index of susceptibility ranged from 2.88 in entry 3 to 10.81 in entry 15. The higher the index the more susceptible was the variety. The lowest susceptibility index (2.88, 5.84 and 6.88) was shown by the entries 3, 19 and 18, respectively, while the highest one was for the entry 15 (10.81) followed by entries 9, 1 and 4 (Table 5). The same trends were observed for percent grain damage and weight losses. The maximum grain damage and weight losses were recorded for entries 1, 4, 7 and 9, whereas, the minimum for entries 3, 18 and 19 (Table 5).

Correlation between chemical kernel properties and susceptibility parameters

Regarding information depicted in Table 6, the biochemical characteristics (mainly percent crude oil,

Table 5. F₁ insects emerged, median developmental time, susceptibility index, grain damage and weight loss of the maize varieties tested (Mean±S.E).

Entry	Varieties	Total F ₁ progeny emerged (No.)	Median development time (Days)	Susceptibility index	Grain damaged (%)	Weight loss (%)	Resistance category
1	PMH-1	61.00 ± 10.81	35.83 ± 2.85	10.19 ± 0.32	31.12 ± 2.57	3.10 ± 0.48	HS
2	Seed tech2324	34.33 ± 8.38	33.17 ± 1.79	8.00 ± 1.26	20.56 ± 4.73	3.68 ± 1.03	S
3	Pratap makka-5	3.500 ± 0.67	38.36 ± 1.94	2.88 ± 0.67	7.21 ± 1.38	1.45 ± 0.55	R
4	Navjot	50.83 ± 10.39	34.00 ± 0.45	10.02 ± 0.44	30.50 ± 7.22	2.33 ± 0.73	S
5	PE HM-2	35.50 ± 7.47	31.83 ± 1.27	9.61 ± 0.96	15.60 ± 2.81	2.04 ± 0.15	S
6	Arawali makka-1	25.17 ± 3.81	34.17 ± 1.47	8.16 ± 0.35	15.27 ± 0.87	2.31 ± 0.46	S
7	Pratap chari makka-6	69.84 ± 13.94	40.00 ± 1.41	9.43 ± 0.16	33.02 ± 5.81	5.49 ± 1.41	S
8	Super 9220	35.33 ± 9.23	35.83 ± 1.89	8.25 ± 1.28	15.53 ± 4.50	1.65 ± 0.24	S
9	KH-101	57.00 ± 7.49	34.33 ± 1.93	10.43 ± 0.24	25.71 ± 3.42	1.91 ± 0.23	HS
10	PAC-790	41.17 ± 6.92	34.33 ± 1.92	9.56 ± 0.53	18.22 ± 1.34	2.58 ± 0.56	S
11	NK-30	35.33 ± 8.48	31.50 ± 1.56	9.37 ± 0.64	15.41 ± 2.96	2.71 ± 0.45	S
12	HM – 10	56.00 ± 14.85	37.33 ± 2.67	9.15 ± 1.02	22.97 ± 4.48	3.11 ± 0.66	S
13	GK – 3090	48.33 ± 17.57	37.17 ± 2.71	8.27 ± 0.62	22.19 ± 6.16	3.24 ± 0.57	S
14	Vivek Hybrid-9	35.83 ± 1.54	34.50 ± 2.91	9.54 ± 0.69	22.22 ± 2.35	1.78 ± 0.09	S
15	HQPM-1	53.83 ± 5.92	32.67 ± 0.67	10.80 ± 0.42	20.68 ± 1.21	2.98 ± 0.65	HS
16	EHQ-16	41.67 ± 15.03	34.83 ± 3.13	8.46 ± 0.79	20.65 ± 6.48	3.88 ± 1.60	S
17	HQPM-7	37.83 ± 9.38	32.83 ± 0.65	9.39 ± 0.67	21.84 ± 2.82	2.04 ± 0.15	S
18	EH-2101	12.17 ± 2.70	32.00 ± 2.13	6.88 ± 0.98	6.52 ± 1.14	1.27 ± 0.21	MR
19	EH-2253	10.67 ± 2.51	34.17 ± 1.99	5.84 ± 0.29	9.01 ± 2.07	1.27 ± 0.22	MR
20	EHQ-63	54.17 ± 7.38	37.33 ± 3.15	9.81 ± 0.76	28.04 ± 3.56	2.46 ± 0.69	S
LSD at 5%		10.34	5.02	2.04	8.00	0.46	
C.V. (%)		20.49	12.59	20.44	27.13	23.35	

R=resistant; MR=moderately resistant; S=susceptible; HS=highly susceptible.

percent crude fat, percent crude protein, percent amylose content, phenolic content, and percent crude carbohydrate) were correlated to susceptibility parameters. Correlation coefficients revealed a highly significant positive relationship between percent crude oil and insect weight ($r = 0.698^{**}$), percent crude oil and efficiency of conversion ($r = 0.763^{**}$), percent crude fibre and insect weight ($r = 0.749^{**}$), percent moisture content and insect weight ($r = 0.846^{**}$), percent

crude protein and insect weight ($r = 0.896^{**}$), percent crude protein and efficiency of conversion ($r = 0.679^{**}$), amylose and susceptibility index ($r = 0.734^{**}$), amylose and insect weight ($r = 0.609^{**}$) and phenolic content and medial development time ($r = 0.680^{**}$), while a highly significant inverse relationship between percent crude oil and medial development time ($r = -0.696^{**}$), percent moisture content and medial development time ($r = -0.670^{**}$), amylose content and medial

development time ($r = -0.759^{**}$), phenolic content and efficiency of conversion ($r = -0.784^{**}$), crude carbohydrate and insect weight ($r = -0.809^{**}$), and crude carbohydrate and efficiency of conversion ($r = -0.696^{**}$) were observed. There were significant positive relationship between percent crude oil and susceptibility index ($r = 0.493^{*}$), crude fibre and efficiency of conversion ($r = 0.531^{*}$), ash content and progeny emerged ($r=0.476^{*}$), ash content and susceptibility index ($r=0.559^{*}$),

Table 6. Pearson correlations for biochemical and susceptibility parameters of the different maize varieties.

Variables	PCO	PCF	Ash	PMC	PCP	AMY	PC	AC	PCC	TPE	MDT	SI	IWt	WF	PGD	PWL	ADI
PCO	1.00																
PCF	0.698**	1.00															
Ash	ns	ns	1.00														
PMC	0.542*	0.859**	ns	1.00													
PCP	0.672**	ns	ns	ns	1.00												
AMY	0.684**	0.743**	ns	0.788**	0.708**	1.00											
PC	-0.514*	-0.611**	ns	-0.671**	ns	-0.659**	1.00										
AC	ns	ns	ns	ns	ns	ns	ns	1.00									
PCC	-0.759**	-0.495*	ns	ns	-0.976**	-0.767**	ns	ns	1.00								
TPE	ns	ns	0.476*	-0.535*	ns	ns	0.487*	ns	ns	1.00							
MDT	-0.696**	-0.524*	-0.583*	-0.670**	ns	-0.759**	0.680**	ns	ns	0.727**	1.00						
SI	0.493*	ns	0.559*	0.511*	ns	0.734**	ns	ns	ns	0.885**	-0.699**	1.00					
IWt	0.698**	0.749**	ns	0.846**	0.896**	0.609**	-0.529*	ns	-0.809**	ns	-0.709**	0.503*	1.00				
WF	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	1.00			
PGD	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.898**	0.718**	0.671**	ns	0.514*	1.00		
PWL	ns	ns	ns	ns	0.579*	0.525*	ns	ns	ns	0.897**	ns	0.517*	ns	0.679**	0.645**	1.00	
ADI	ns	ns	ns	ns	ns	0.510*	ns	0.494*	ns	0.521*	ns	0.479*	ns	-0.872**	0.512*	0.676**	1.00
EOC	0.763**	0.531*	-0.477*	ns	0.679**	0.522*	-0.784**	ns	-0.696**	ns	ns	0.505*	0.730**	-0.543*	ns	-0.807**	-0.709**

PCO= Percent crude oil; PCF=Percent crude fibre; PMC=Percent moisture content; PCP=Percent crude protein; AMY=Amylose content; PC=Phenolic content; AC=Amylase content; PCC=Percent crude carbohydrate; TPE=Total progeny emerged; MDT=Median development time; SI=Susceptibility index; IWt=Insect weight; WF=Weight of flour; PGD=Percent grain damage; PWL=Percent weight loss; ADI=Approximate digestibility; EOC=Efficiency of conversion.^a Correlation coefficients with an asterisk (*) represent P values < 0.05 and with two (**) <0.01; ns stands as “non-significant”.

amylose content and grain weight loss ($r = 0.525^*$), amylose content and approximate digestibility ($r = 0.510^*$), amylose content and efficiency of conversion ($r = 0.522^*$), phenolic content and progeny emerged ($r = 0.487^*$) and amylase content and approximate digestibility ($r = 0.494^*$). Crude fibre and median development time ($r = -0.524^*$), ash content and efficiency of conversion ($r = -0.477^*$), ash content and median development time ($r = -0.583^*$), percent moisture content and progeny emerged ($r = -0.535^*$) and phenolic content and insect weight ($r = -0.529^*$) had a significant inverse relationship. No significant correlations were observed between

the remaining biochemical and susceptibility parameters.

The number of progeny emerged, median development time, susceptibility index, percent grain damage and grain weight loss were highly correlated (Table 6). The correlation between progeny emerged and median development time ($r=0.727^{**}$), progeny emerged and susceptibility index ($r=0.885^{**}$), progeny emerged and grain damage ($r=0.898^{**}$), progeny emerged and grain weight loss ($r=0.897^{**}$), median development time and grain damage ($r=0.718^{**}$), susceptibility index and grain damage ($r=0.671^{**}$), insect weight and efficiency of conversion ($r=0.730^{**}$), weight of flour

and grain weight loss ($r=0.679^{**}$), grain damage and grain weight loss ($r=0.645^{**}$) and grain weight loss and approximate digestibility ($r=0.676^{**}$) were positive and highly significant. However, median development time and susceptibility index ($r=-0.699^{**}$), median development time and insect weight ($r=-0.709^{**}$), grain weight loss and efficiency of conversion ($r=-0.807^{**}$) and approximate digestibility and efficiency of conversion ($r=-0.709^{**}$) showed a highly significant negative relationship. Correlation between progeny emerged and approximate digestibility ($r=0.521^*$), susceptibility index and insect weight ($r=0.503^*$), grain weight loss ($r=0.517^*$), approximate

digestibility ($r=0.479^*$) and efficiency of conversion ($r=0.505^*$), weight of flour and grain damage ($r=0.514^*$) and grain damage and approximate digestibility ($r=0.512^*$) were positive and significant; whereas, weight of flour and efficiency of conversion was negatively correlated ($r=0.543^*$). Besides, significant positive as well as negative relationships among biochemical parameters were obtained (Table 6).

DISCUSSION

The characteristics of grain resistance to stored insects, is still debatable (Shafique and Chaudry, 2007; Abebe et al., 2009; Nadeem et al., 2011). This study demonstrated considerable variation among the maize varieties with respect to *S. cerealella* resistance. Relatively longer developmental time was required on the resistant variety Pratap makka-5 and lower number of F_1 insects emerged, while the reverse is true for the susceptible varieties. In this study, we used F_2 grains resulting from cross fertilization. The embryo contributes 11%, and the endosperm 83% of the dry matter of the kernel (Tollenar and Dwyer, 1999), pointing to a potential positive effect of xenia (direct cross fertilization effect on the grain traits of the female component) on grain yield. The effect of xenia on grain resistance to the *S. cerealella*, however, needs further investigation. Shafique and Chaudry (2007) suggested that the low insect population and low weight loss of grain can be used as one of the attribute of the grain resistance to insects. In addition to weight loss and number of F_1 progeny, Abebe et al. (2009) used median developmental time and percentage of seed damage as indicators of the susceptibility of maize varieties to the attack of *S. zeamais*. Our result on median development time was in agreement with Fouad et al. (2013), who found that the median developmental period from egg to adult reared on maize was between 34.03 and 39.33 days. If the number of F_1 progeny emerged could be regarded as the infestation intensity, then it could be suggested that the weight loss due to the infestation of *S. cerealella* corresponds with the infestation intensity. This suggestion is supported by the data presented in Table 4 which show that the lowest weight loss (1.27%) occurred on entries 18 and 19, although it did not significantly differ with entries 8 and 14, but was significantly different with the other entries.

Our results in biochemical analysis of different maize grains were in close agreement with previous findings by different authors. Ijabadeniyi and Adebolu (2005) determined the percent fat content of three maize varieties grown in Nigeria in the range of 4.77 to 5.00% for the maize grains, which is in an agreement with the present study. The same authors also reported similar results (2.07 – 2.77%) of the fibre content for the maize varieties grown in Nigeria. Peplinski et al. (1989) reported values of ash between 1.3 and 1.5%. This notion is in agreement with the results of the present study.

Maziya-Dixon et al. (2000) found results in the range of 1.4 to 3.3%, which are a bit higher than the values determined in the present study. Aisha and El-Tinay (2004) investigated the ash value in the range of 1.0 to 2.0% which is matching with present results of our study. The same authors found the moisture value in 12 corn genotypes in the range of 4.3 – 6.7 per cent which is a bit lower than results in the present study. Samir et al. (1998) measured the moisture content in the range of 9 to 19%, which is in agreement with our results obtained in the current study. Ijabadeniyi and Adebolu (2005) reported slightly lower values (65.63 to 70.23%) of the carbohydrate content for the maize varieties grown in Nigeria. The values of protein content obtained in the current study are considered typical because most of them lie within the range of 7.3 to 15.6% reported by Waniska and Rooney (2000).

The result of correlation analysis between biochemical and susceptibility parameters showed that variables of crude protein, fat, fibre and carbohydrate content are negatively but not significantly correlated with variables of F_1 progeny emerged. The findings in this study are in agreement with Dobie (1976) who reported a negative and non-significant correlation between protein and beetle attack. He therefore, concluded that crude protein was independent of maize kernel hardness or softness. This means that resistance is not dependent on the nutritional content of the maize varieties studied. However, both Singh and McCain (1963) and Dobie (1977) reported the factors contributing to grain resistance to weevils to include increased sugar content. Peters et al. (1972) also reported negative correlation between the fat content and moth weight. Low protein content has been observed to contribute resistance to rough rice against *S. cerealella*; but relationship was not distinct (Chatterjee et al., 1977). However, Yadu et al. (2000) reported a positive relationship between protein content and damage by *S. cerealella* to maize. Rao and Sharma (2003) further reported that protein content in wheat had little role in resistance.

On the other hand, the maize kernel with high ash content would favour the high number of F_1 progeny emerged and faster development and consequently would be susceptible to the *S. cerealella* attacks. Phenolic content of maize kernels had significantly negative correlation with the number of F_1 progeny emerged, the susceptibility index and insect weight. Furthermore, this variable had positively correlated with the median developmental time. This result revealed that high phenolic content attributed low number of F_1 progeny emerged and the slow development of *S. cerealella*. As a result, the maize varieties with the high phenolic content appear to be resistant to the *S. cerealella* attacks. Phenolic compounds have also been named as contributing significantly to maize resistance to maize weevil (Classen et al., 1990). Ranason et al. (1992) reported that resistance in maize grain to the weevil was also contributed by the anti-feedant effect of

phenolic compounds and weight loss of grains was negatively correlated to total phenolics in the grain. Cogburn et al. (1983) and Ahmad et al. (1998) stated that varieties resistance to stored rice insects is related to grain hardness, amylase content, non-chalkiness and phenolic content in the rice kernels. Noris and Kogan (1980) said that plant defence to the insect attack by its physical characteristics and chemical content such as the phenolic compounds. Serna -Saldivar (2010) indicated that phenolics have an important role in protecting kernels against biotic and abiotic stresses. In maize, phenolics are related to kernel resistance through physical and toxicological mechanisms (García-Lara et al., 2004) and in sorghum, a positive relationship between phenolic content and *Sitophilus oryzae* resistance has been reported (Ramputh et al., 1999). Our results in the present studies were in close agreement with the above mentioned findings.

Peters et al. (1972) reported higher amylose content in resistant varieties of corn than the susceptible varieties against weevils and beetles. Similarly in paddy higher amylose content was recorded in resistant varieties in comparison to susceptible ones against *S. oryzae* (Ragumoorthy and Gunthilagaraja, 1988). In contrast our finding revealed that amylose content was negatively correlated with median development time but positively correlated with susceptibility index, insect weight and grain weight loss which means higher amylose content was found in susceptible variety than resistant ones. These results showed a preference of *S. cerealella* to high amylose kernels. Flores (1970) observed that there was no significant correlation between quantities of amylose, sugars and protein in corn varieties and damage by *S. cerealella* but negative correlation was observed between lipid content and mean development period of the insect. These indicated that different insect species have different preference to different biochemical contents of the grain. In general our findings were similar to that of Fouad et al. (2013), who studied relationship between physico-chemical characteristics of corn kernels and susceptibility to *S. cerealella*.

Conclusion

In the present research work significant differences in susceptibility to *S. cerealella* were observed amongst maize varieties evaluated. The study reported here show that the most susceptible varieties were PMH-1, KH-101 and HQPM-1 whereas the least susceptible was Pratap Makka-5. Besides, EH-2253 and EH-2101 were categorized as moderately resistant. The remaining fourteen varieties were categorized as susceptible. Chemical parameters associated to susceptibility were: ash, amylose and phenolic contents. The most resistant maize can be described as a variety with low ash, increased phenolic content and reduced amylose concentration. This research showed that it was important

to breed maize variety considered on the low ash, low amylose and high phenolic contents besides other morphological and physical characteristics in order to get maize variety which is resistant to *S. cerealella* infestation. The effect of xenia on grain resistance to the *S. cerealella*, however, needs further investigation. It is also suggested that breeders should consider developing varieties which, in addition to high potential yield, have moderate resistance to stored product insects. So, it is recommended that studies on the reaction of grains to storage insects are made a part of variety release proposal. Therefore, the resistant and least susceptible or moderately resistant varieties can be utilized as an eco-friendly way to reduce damage by *S. cerealella* under traditional storage conditions.

Conflict of Interest

The authors have not declared any conflict of interest.

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