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RELATIVE RESISTANCE OF MAIZE VARIETIES AGAINST MAIZE WEEVIL, *Sitophilus zeamais* (MOTSCHULSKY), (COLEOPTERA: CURCOLIONIDAE)

Muhammad Mamoon-ur-Rashid[1,*], Riaz-ud-Din[1], Muhammad Naeem[1], Muhammad Ahsan Khan[2] and Muhammad Ashfaq[2]

[1]Department of Entomology, Gomal University, Dera Ismail Khan, Pakistan; [2]Department of Entomology, University of Agriculture, Faisalabad, Pakistan.
*Corresponding author's e-mail: mamoondik@yahoo.com

The maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), is one of the most damaging pests of stored cereals causing severe damage to stored grains. In current investigation, six open pollinated maize varieties *viz.* JALAL (white), AZAM (white), SADAF (yellow), ZARD LOCAL (yellow), KASHMIRI (yellow) and PAHARI (white) were screened out in the laboratory of Entomology Department, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Pakistan for their relative susceptibility/resistance to maize weevil. The experiment was laid out following completely randomized design (CRD) with 5 replicates. Results revealed that minimum (23.8) days to adult emergence of maize weevil were recorded when it was reared on variety Azam; whereas; maximum (34.2) days were recorded on variety Sadaf. Total number of F_1 adults emerged were maximum (91) in Azam while minimum (62) in variety Sadaf. Percent infestation of maize kernels was maximum (39.01%) in Azam while minimum (25.80%) was recorded in variety Sadaf. Percent weight loss was maximum in Azam *i.e.* 26.12% while minimum percent weight loss (16.88%) was recorded in Sadaf. Maximum longevity (68.4 days) of adult weevils was recorded on Azam while minimum (53.4 days) was recorded on variety Sadaf. All the tested varieties had no significant effect on the sex ratio of emerged weevils. High germination percentage was recorded in Sadaf (90%) whereas; low germination was found in Azam (57%). The grain hardness test indicated that yellow varieties including Sadaf, Zard local and Kashmiri had maximum hardness having 317, 302.33 and 296 N grain hardness, whereas; white varieties *i.e.* Pahari, Jalal and Azam had minimum values of grain hardness *i.e.* 264.33, 251.66 and 238.33 Newton. The present work has shown that Azam is the most susceptible variety and should not be stored for longer periods whereas; Sadaf is the resistant variety against maize weevil.

Keywords: Stored Grain, maize weevil, maize varieties, susceptibility, resistance and weight loss.

INTRODUCTION

Maize (*Zea mays* L.) is the third most important cereal crop grown in Pakistan. It was grown on an area of 1229 thousand hectare with total production of 5.702 million tones during 2017-2018 (Anonymous, 2018). Maize crop is one of the significant crops for its diverse utilization as a human food and livestock feed and is the most important component of industrial raw material (Gupta *et al*., 2015). Maize crop occupies third position among cereal crops after wheat and rice globally (Siwale *et al.*, 2009). Maize is the important component of the world's food security and provides major source of diet to millions of people. It is the only food grain that is consumed from flower to flour (Boutard, 2012). In developing countries; it is grown as a source of food as well as feed. Its production is crucial to raise rural status and increase economic growth (Byerlee *et al*., 1997). Maize grains are stored mainly for maintaining its availability round the year for food and seed purposes for planting during coming season (Adetunji, 2007).

Stored grains face many problems during storage *i.e.* attack of pathogens (bacteria and fungi) and insect pests due to improper storage conditions. In developing countries, despite other constraints, insect pests cause losses ranging from 20 to 50% to maize grains under storage conditions (Nukenine *et al*., 2002; Dhliwayo and Pixley, 2003). It is evident from earlier studies that during storage conditions of maize in tropical and sub-tropical regions of the world maize weevil is the most damaging insect (Rees, 2004; Akob and Ewete, 2007). The pest is capable to multiply to large populations and causing severe damage to stored grains ranging from 10 to 40% world-wide (Mathews, 1993; Parwada *et al*., 2012). Under favorable circumstances, the maize weevil can cause grain losses ranging from 12 to 20% and the degree of damage may go up to 80 % (Keba and Sori, 2013).

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Chemical insecticides are generally used for the protection of stored grains against the ravages of insect pests which are not an economically viable option for the poor growers. Moreover, the indiscriminate use of chemical insecticides is a great threat causing pollution, development of insecticide resistance, residual effects in food products causing health issues, causing population imbalance of non-targeted organisms and high input costs (Cherry *et al*., 2005).

In contrast to synthetic chemicals planting of weevil resistant varieties is a sustainable and economically viable option for minimizing losses caused by maize weevil (Derera *et al*., 2014; Zakka *et al*., 2015). Many varieties of stored grain possess qualities which make them less attractive to insect attack compared to others and are regarded as resistance or less susceptible varieties. In maize, different factors including grain hardness and pericarp traits are considered as resistance source against maize weevil (Gudrups *et al*., 2001). Hence, the most promising alternative to minimize reliance on chemical insecticides is the use of crop varieties which are resistance to insect pests (Nwankwo *et al*., 2014).

Keeping in view the economic significance of maize crop in the country and devastating nature of maize weevil to stored maize grains, it was aimed to investigate the resistance of six approved maize varieties which are widely grown in the country against maize weevil infestation.

MATERIALS AND METHODS

***Experimental Site*:** The experiment was carried out in the Laboratory of the Department of Entomology, Faculty of Agriculture Gomal University Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

***Maize varieties*:**

The six maize varieties viz. Jalal, Azam, Sadaf, Zard local, Kashmiri and Pahari widely grown in Khyber Pakhtoonkhwa and Punjab provinces were screened out against maize weevil. These varieties were obtained from the Federal Seed Certification and Registration Department Regional Directorate, Peshawar, Pakistan.

***Insect cultures*:** Maize seeds, plastic jars (5-L), muslin cloth, funnel and mesh sieves used to culture corn weevils were thoroughly cleaned. Maize grains having 12-14% moister content (MC) were used to culture the weevils. Five hundred grams maize grains were placed in each plastic jar. Initial culture of *S. zeamais* was obtained from the laboratory of Entomology section, Agricultural Research Institute, Dera Ismail Khan. The maize grains were sterilized by keeping them in a deep freezer maintained at -20 ± 2°C for two weeks to remove the chances of previous infestation in the grains (Isah *et al*., 2012). The weevils were cultured in an incubator at controlled temperature of 27 ± 2°C and 65 ± 5% relative humidity under 12:12 hour day length (L:D). Mixture of two hundred, one week old male and female adult maize weevils were introduced in each jar. After introduction of the insects, the top of the jars were covered with muslin cloth and tighten by rubber band in order to prevent the insects from escaping and to allow exchange of gases in and out of the jars. The jars were then placed in an incubator at controlled temperature for ten days. After ten days the parent insects were removed through sieving and introduced to other jars in order to multiply the culture of the insects. The jars containing infested maize grains were left undisturbed for twenty days. After twenty days merging adult insects were collected and were kept in separate jars according to their age. Adults that emerged on same day were considered of the same age and were used for the experimental purpose.

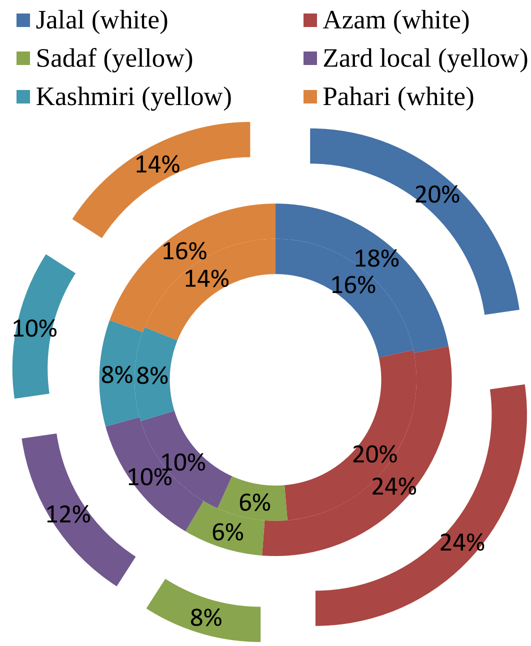

Figure 1. Distribution of adult maize weevil on different varieties of maize crop under laboratory conditions.

***Experimental protocols*:** Six aforementioned cultivars of maize were screened out against maize weevil. The experimental conditions were maintained at 27 ± 2^0C and 65 ± 5% R.H. and a photoperiod of 12:12 hours (L: D). The experiment was laid out in completely randomized design (CRD) with 5 replicates and six treatments (varieties). 20 g seeds of each maize variety was kept in 250 ml transparent plastic jars and then10 pairs of newly emerged and 24 hours starved adult weevils were released in each plastic jar. The plastic jars were then covered with a piece of muslin cloth, fastened with rubber band to prevent escape or entry of new weevils. The weevils were allowed 7 days for mating and oviposition purposes. After 7 days of the release of weevils, the adult weevils were removed from all jars. The data on F_1 adult emergence was recorded 20 days after the release of adult weevils and continued up to 45 days at 5 days interval. The data were recorded on the following parameters:

***Days to F_1 adult emergence*:** The adult weevils were removed on seventh day after their release, by emptying the contents of each jar on a piece of white paper by using a camel hair brush. Subsequently, the maize grains of each jar were carefully returned into the jar, leaving the contents undisturbed for 20 days to enable maize weevils to complete their developmental process. After 20 days, each jar was visually monitored on daily basis to record data on the emergence of F_1 adults.

***Total number of F_1 adults emerged*:** The F_1 progenies were counted and removed from the jars on daily basis till 30 days and afterward with interval of five days up to 45 days for calculating total F_1 progenies emerged.

***Percent infestation*:** After 45 days of the start of experiment the total number of seeds (20g) were counted, represented by T_n, whereas; number of seeds having holes were represented by N_b. The following formula was used to calculate percent infestation of maize grains (Enbakhare and Law-Ogbomo, 2002):

$$\text{Infestation (\%)} = \frac{N_b}{Tn} \times 100$$

Where, N*b* = Number of bored seeds, T*n* Total number of seeds

***Percent weight loss*:** The grain weight loss (GWL) was calculated using the formula (Zunjare *et al*., 2015):

$$\text{\% weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Sex ratio: Adult sex ratio was recorded as the number of males per 50 females during 45 days experimental duration (Zunjare *et al.*, 2014).

***Adult longevity*:** Newly emerged adult maize weevil (Male and female) from each variety were sieved out and were cultured in a separate plastic jar to find out the adult longevity. For this purpose, 40 insects (20 pairs) were selected from each variety. The adult weevils were also provided maize grains for feeding and oviposition purposes. The maize grains were changed in each jar after 15 days to prevent the emergence of F_2 generation. The insects were checked on daily basis to record the mortality if any. The dead insects were removed on daily basis till the 100% mortality of F_1 generation.

***Effect of different varieties of maize on the distribution of maize weevil*:** The effect of different maize varieties on the distribution of maize weevil was evaluated under choice experimental conditions. For this purpose, newly emerged adult weevils were collected and presented with a choice of dispersing on to grains of different varieties of maize in the tested arena. The arena was made of transparent cages of 20.5 cm × 20.5 cm × 20.5 cm. White filter paper was placed on the bottom of the cages to facilitate movement and visibility of the adult weevils. 50 grains of each variety were placed at equal distance from each other in the arena. Fifty newly emerged adult weevils were released in the middle of the arena and later the arena was closed to prevent the escape of weevils.

After 24, 48 and 72 hours the numbers of weevils settled on grains of different varieties were recorded. Each treatment was replicated three times. While recording the distribution data, those weevils that did not respond *i.e.* they settled on different parts of the arena rather than on maize grains, were excluded from the data.

***Germinations of maize seed after 45 days exposure to maize weevil*:** Maize kernels of six varieties exposed to maize weevils after 45 days were placed in an incubator for germination test at constant temperature of 28^0C in Petri dishes (6cm diameter). Soaked filter papers with distilled water were placed in Petri dishes. Twenty seeds (damaged and undamaged) were selected randomly from each variety after 45 days exposure to maize weevil and were imbibed in distilled water for six hours to enhance the germination of the seeds, according to the ISTA rule (2006). The Soaked maize grains were placed on the moist filter paper in a Petri dish and were then stored in an incubator. Each treatment was replicated for five times. Germinated seeds were counted after 11 days and data were calculated by following the below formula (Zibokere, 1994):

$$\text{Germination percentage} = G_1/G_2 \times 100$$

Where G_1 = Total Germinated grain, G_2 = Total grains in Petri dish

***Grain Hardness (N)*:** Five maize grains of each variety were randomly selected and were placed under hardness tester machine in vertical position (ERWEDA Apparatebau, Type T-B24, Nr. 45857, 220 volt (50/60) Hz, 500 Newton), for measuring grain hardness. Maize grains of selected varieties having 12-14% moisture contents, were placed in vertical position on a cylindrical probe of (70mm in diameter) and were crushed. The probe was fixed at 18mm/sec measuring 400 Newton adjusted 48mm/sec of testing speed. 500kg capacity was applied for load cell. Total Newton force used in compressed machine till the breakage of seeds divided by 9.8 digit. It indicated the force applied in Kg. (Zakka *et al*., 2015; Zunjare *et al*., 2015).

***Statistical analysis*:** The data recorded during the experiments were analyzed statistically using one-way analysis of variance technique and subsequently Least Significance Difference (LSD) Test was applied for comparing the treatment means at 5% probability level. The statistical analysis was carried out using computer software Statistix (version 8.1).

RESULTS

***Days to F_1 adult emergence*:** The developmental duration of maize weevil differed significantly ($P<0.05$) when it was reared on different varieties of maize. Maximum number of 34.20 days to F_1 adult emergence was recorded when the weevils were reared on Sadaf (yellow) followed by Zard local (yellow) having 31.00 days whereas; minimum number of days were recorded on Azam (white) having 23.80 days to F_1 adult emergence.

***Total number of F_1 adults emerged*:** The obtained data demonstrated that all the tested six varieties had significant effect (P<0.05) on the progeny production of maize weevil (Table 1). The maximum number of F_1 progeny was recorded in Cv. Azam (white) *i.e.* (91.40) followed by Jalal (white) *i.e.* (86.20) respectively. Among the tested varieties, the variety Sadaf (yellow) was found most resistant variety against the tested insect leading to least number of F_1 progeny productions *i.e*. (62.40).

***Percent infestation of maize kernels*:** Significant differences (P<0.05) were observed among the varieties with regard to the percentages of seed damage. The highest seed damage was recorded in variety Azam (white) and Jallal (white) *i.e*., 39.01% and 36.22% in terms of percent infestation by maize weevil whereas; minimum percent infestation was recorded in Sadaf (yellow) *i.e*., 25.80%. The varieties Pahari (white), Khasmir (yellow) and Zard local (yellow) were regarded as moderately resistant to weevil with 33.18, 26.87 and 26.72% infestation of maize grains.

***Weight loss*:** Differential response of maize varieties was observed with respect to weight loss of maize grains caused by maize weevil. Among the tested varieties, minimum weight loss of 16.88 % was observed in Sadaf (yellow) while; maximum weight loss was noted in Azam (white) followed by Jalal (white) *i.e.,* 26.12 and 25.20% respectively. Hence among the tested varieties the Azam (white) and Jalal (white) were found more susceptible in terms of weight loss caused by maize weevil while Sadaf (yellow) showed resistance against the test insect.

***Adult longevity*:** All the tested maize varieties had significant (P<0.05) effect on the adult longevity of maize weevil when the test insects were reared on maize grains. Among the tested varieties, adult longevity was significantly shortest (62.40 days) when weevils were reared on grains of variety Sadaf (yellow). The maximum adult longevity of 91.49 and 86.20 days were observed when the weevils were reared on Azam (white) and Jalal (white) varieties followed by Pahari (white), Kashmiri (yellow) and Zard local varieties having 72.20, 67.00 and 66.00 days, respectively, adult longevities.

***Adult Sex ratio*:** The sex ratios of maize weevil did not differ significantly (P>0.05) when weevils were reared on grains of different varieties of maize. The mean sex ratios show that always less males emerged as compared to females in all the treatments, however, no significant (P>0.05) differences were observed among the different varieties.

***Distribution of maize weevil on different varieties of maize*:** The response of maize weevil differed on tested varieties of maize when they were given a choice of distribution on different varieties of corn. Maximum number of (10) adult weevils settled on variety, Azam (white) followed by Jalal (white) (8) and Pahari (white) (7), when data was recorded 24 hours after the start of experiment. Minimum number of weevils settled on the variety Sadaf (yellow) (3), Kashimir (yellow) (4) and Zard local (yellow) (5). Similar trend of

Table 1. Mean (± SE) days to F_1 adult emergence, total number of F_1 adult progeny and grain weight (g) loss on different maize varieties by maize weevil.

Maize varieties	Days to F_1 adult emergence	Total number of F_1 adults emerged	Percent infestation of maize kernels
Jalal (white)	25.20 ± 0.83 e	86.20 ± 0.57 b	36.22 ± 0.64 b
Azam (white)	23.80 ± 0.83 f	91.40 ± 1.25 a	39.01 ± 0.44 a
Sadaf (yellow)	34.20 ± 0.83 a	62.40 ± 1.82 e	25.80 ± 0.28 e
Zard local (yellow)	31.00 ± 0.70 b	66.00 ± 0.95 d	26.87 ± 0.27 d
Kashmiri (yellow)	29.80 ± 0.83 c	67.00 ± 0.95 d	26.72 ± 0.54 d
Pahari (white)	27.00 ± 0.70 d	72.20 ± 1.29 c	33.18 ± 0.27 c
LSD Value	1.1050	2.4709	0.5680

Each value is a mean ± standard error of five replications. Means followed by the same letters along the column are not significantly different at (P>0.05).

Table 2. Mean (± SE) weight loss, adult longevity and adult sex ratio of maize weevil on six different maize varieties

Maize varieties	Weight loss	Adult longevity	Adult Sex ratio (M/50F)
Jalal (white)	23.77 ± 0.23 b	64.80 ± 0.83 b	44.86 ± 1.67 ^{NS}
Azam (white)	26.12 ± 0.23 a	68.40 ± 1.14 a	44.48 ± 1.78
Sadaf (yellow)	16.88 ± 0.22 e	53.40 ± 1.14 e	46.60 ± 1.87
Zard local (yellow)	20.00 ± 0.25 d	61.20 ± 1.30 d	43.83 ± 0.84
Kashmiri (yellow)	19.95 ± 0.34 d	62.40 ± 1.40 cd	44.19 ± 0.37
Pahari (white)	22.22 ± 0.39 c	62.80 ± 0.83 c	46.15 ± 0.73
LSD Value	0.3757	1.4099	3.0844

Each value is a mean ± standard error of five replicates. Means sharing common letter along the column are not significantly different at P= 0.05.

weevil distribution was also recorded after 48 and 72 hours of investigations. Among the tested varieties Azam (white), Jalal (white) and Pahari (white) were found most preferred whereas; Sadaf (yellow), Kashmiri (yellow) and Zard local were found as the least preferred varieties.

***Germinations of maize seed after exposure to maize weevil*:** Germination percentage of maize grains after 45 days exposure to maize weevil was found different. Mean germination percentage ranged from 57-90% with a mean of 73.25% (Fig. 2). High germination percentage was found in variety Sadaf (yellow) (90%) followed by Kashmiri (yellow) (83.5%) and Zard local (yellow) (75%), when data were recorded after 11 days of the start of germination trial. Low germination percentage was recorded in Azam (white) (57%), Jalal (white) (63%) and Pahari (white) (71%) of tested varieties.

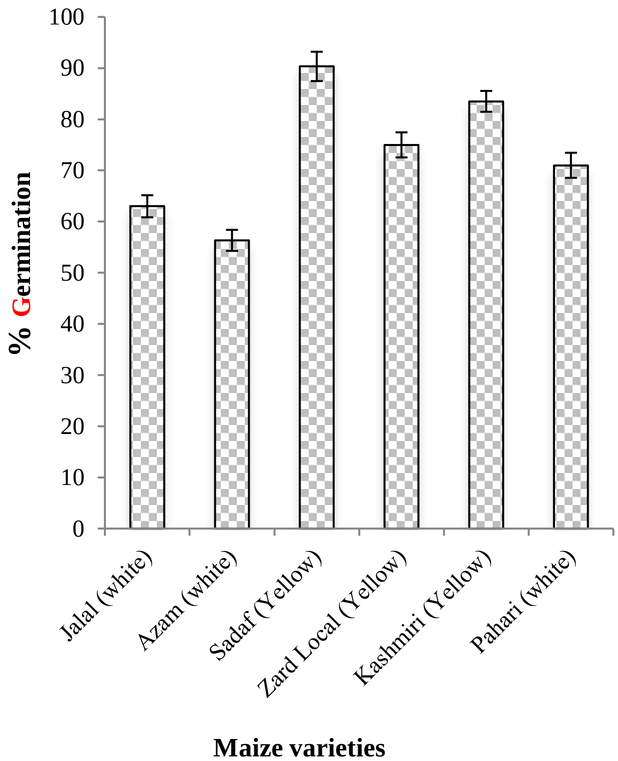

Figure 2. Percent germination of maize grains after exposure of 45 days to maize weevil

***Grain hardness (N) of different varieties of maize*:** The grains of tested varieties were placed under hardness tester machine to check the hardness which indicated that yellow varieties Sadaf, Zard local and Kashmiri had maximum hardness of 317, 302.33 and 296 N, whereas; white varieties *i.e.* Pahari, Jalal and Azam had minimum values of grain hardness *i.e.* 264.33, 251.66 and 238.33 Newton. It is evident that white varieties have thin pericarp while yellow varieties have maximum thickness of pericarp.

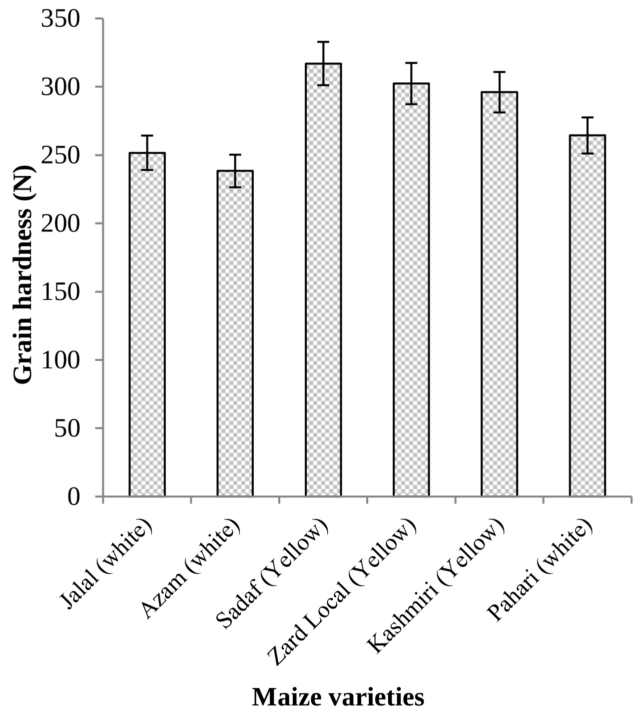

Figure 3. Grain hardness (N) of different varieties of maize crop using hardness tester machine

DISCUSSION

In our experiments we found substantial distinction among different maize varieties with respect to days to F_1 adult emergence, total number of F_1adults' emergence, percent infestation of maize grains, weight loss and adult longevity. Our results suggest that varieties with yellow color, having hard surface were found more tolerant to weevil's infestation than the white varieties. Among the tested varieties, the development of the test insect was more rapid on the variety Azam (white) and was found most susceptible showing minimum days to adult emergence, maximum number of F_1 progeny, maximum percent infestation and weight loss. These results are in conformity with the findings of Demissie *et al.,* 2014. They reported that resistant varieties result into longest developmental time, least adult emergence, minimum grain damage and grain weight loss.

Regarding total number of F_1 adult emergence all the tested maize varieties had significant effect on the progeny production of maize weevil. Maximum number of F_1 adult emergence was recorded in Azam (white) while minimum number of adults emerged in variety Sadaf (yellow). These results are confirmed by (Abebe *et al.*, 2009) they screened out different improved maize varieties for susceptibility to maize weevil. Resistant varieties showed low numbers of F_1 generation, low percentage of seed damage, seed weight loss and showed longer developmental time. Varieties having maximum F_1 generation showed short developmental time. Boost in F_1 generation linearly increased seed damage and weight loss showing an inverse relationship between susceptibility index, % seed damage and developmental time while number of F_1 generation, % seed damage and seed weight loss was positively related with susceptibility index. Keba *et al*., 2013 concluded that the variations in the number of weevil's emergence indicated differences in susceptibility to maize weevil attack among the tested varieties. The varieties having the maximum number of weevil's emergence demonstrated high susceptibility to maize weevil attack which is attributed to the lack of resistance mechanisms in or on the grains. Similarly, our results indicated that minimum weight loss was recorded in Sadaf (yellow) while maximum weight loss was witnessed in Azam (white). Similar findings were also reported by different scientists (Muzemu *et al.*, 2013; Nwosu, *et al.*, 2015). They evaluated five open pollinated varieties and one hybrid maize variety for tolerance/susceptibility against maize weevil. The resistant variety exhibited the properties of inhibition of maize weevil progeny, low weevil emergence, less weight loss and low grain damage and high germination percentage in contrast to vulnerable varieties which were contradictory in all these protocols. Usman, *et al.*, 2015 confirmed that grain hardness played a pivotal role in resistance to attack of maize weevil while, weak pericarp of kernel resulted in high infestation of target insects and reported huge amount of losses in mentioned contents. Akpodiete *et al*., 2015 found that the morphological characteristics of the different maize varieties could not be ignored in the presence of chemical contents such as phenolic and resistance against maize weevil.

Similarly, adult longevity was also affected when the test insects were reared on different varieties. Maximum longevity of maize weevil was recorded when it was reared on Azam (white) and Jallal (white) whereas; shortest longevity was recorded on Sadaf (yellow). According to (Babarinde *et al*., 2008, Bamaiyi *et al*., 2007) weak pericarp of the grain facilitates the insects in getting the protein and starch resultantly longevity of the insect is maximum. Therefore, the present findings confirmed that Azam (white) is the most susceptible variety having more number of adults emerged than the resistant one Sadaf (yellow). The tested varieties had no significant effect on the adult sex ratio of maize weevil. The mean sex ratio revealed that always lower number of males emerged as compared to female in all treatments. It was confirmed by (Derera, *et al*., 2010) they screened out the ovi-position period, number of weevil emergence and sex ratio on different maize genotypes and reported no significant difference between treatments regarding sex ratio (male/female). The germination test of tested varieties after 45 days exposure to maize weevil attack, showed that Sadaf (yellow), Kashmiri (yellow) and Zard local (yellow) varieties had high germination percentage which indicated their resistance against maize weevil attack which could not affect the embryo of the maize seed. Similarly, Azam (white), Jalal (white) and Pahari (white) varieties showed low germination percentage due to their susceptibility towards maize weevil attack. It was concluded that among the tested varieties two varieties showed tolerance & progeny suppression against maize weevil by high adult maize weevil mortality, less grain loss, low adult emergence and high germination percentage. Similarly, (Derera *et al*., 1999; Oyiga and Uguru, 2011) concluded that maize weevil deterrence from oviposition, feeding and hindrance of mandibles from gripping on smooth pericarp of maize kernel. Less damage to the smooth grain might be attributed to antixenosis mechanisms. The grain hardness test indicated that yellow varieties Sadaf, Zard local and Kashmiri had maximum hardness of grains whereas; white varieties *i.e.* Pahari, Jalal and Azam had minimum values of grain hardness. It is evident that white varieties have thin pericarp while yellow varieties have more thickness of pericarp. Similar results were documented by Zakka *et al*. (2012), they found variation in grain hardness of seventeen varieties. They concluded that hybrid variety MASYN-VAR-3F2 showed maximum grain harness of 275.12 N whereas; Akparike and Bende had minimum grain hardness of 116.62 N and 91.65 N.

Hence, it is suggested that the use of resistant genotypes against maize weevil like Sadaf (yellow) is a viable option to reduce the problem of infestation and damage caused by maize weevil during storage in developing countries including Pakistan.

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