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EVALUATION OF QUALITATIVE LOSSES OF MAIZE GENOTYPES TO *Sitophilus zeamais* (MOTSCH) (COLEOPTERA: CURCULIONIDAE) AND ITS RESPONSE TO PLANT EXTRACTS UNDER LABORATORY CONDITION

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The research work was carried out in the Grain Research, Training and Storage Management Cell (GRTSMC), Department of Entomology, University of Agriculture, Faisalabad to evaluate the qualitative losses caused by maize weevil (*Sitophilus zeamais*) on eight maize genotypes viz: NK-8441, MMRI-Yellow, 30K08, Agati-2002, Swl-2002, Ev-6098, Ev-1098 and Pak-Afghan and its response to plant extracts. The experiment was conducted in a walk-in environmental chamber maintained at 28, 32 and 35°C with 60-65% relative humidity. The experiment was performed using Completely Randomized Design with three replications for each treatment. Qualitative losses included percentage moisture content, crude protein, ash, fat and crude fiber. The data were recorded after 120 days. High moisture content was observed in MMRI-Yellow and lowest moisture content was observed in 30K08 while high percent amount of ash, fiber and fat was observed in MMRI-Yellow and low amount was observed in 30K08. At the end different concentrations of three plant extracts including *Terminalia chebula, Chicorium intybus, Glycyrrhiza glabra* were used to investigate the percent mortality of the tests insect. Percent mortality data was recorded after 24, 48 and 72 hours of treatment. Maximum mortality of 89.09% was observed by using of *Chicorium intybus* at 5% concentrations after an exposure period of 72 hours while minimum mortality of 25% was observed by using *Glycyrrhiza glabra* at 10% concentration after an exposure period of 24 hours.
Keywords: Qualitative losses, maize, genotypes, maize weevil, plant extract

INTRODUCTION

Cereals grains are rich source of protein and vitamins which contribute 1/4th part of the total energy obtained. Maize (*Zea mays* L.) is economically important crop of Pakistan. Based on area and production it is considered third important food crop after wheat and rice. It is used as a staple food in Pakistan. About 12-16 million tones of grains are lost due to improper storage including 4-5 per cent losses at market level while 7-10 percent losses are estimated at the farm level. These losses occur during improper storage and handling such as unsuitable harvesting methods and transportation. In maize 3-4 million tons of grains are lost per year (Basappa *et al.,* 2005). Among the stored grain insect pests of maize, the beetles are the most important. Maize weevil (*Sitophilus zeamais*) is very notorious pest of maize. In storage most of the losses occur due to this pest (Mugo *et al.,* 2012). According to (Abebe *et al.,* 2009) *S. zeamais*causes20-90% losses during storage in maize while the study of Osipitan and Lawal (2012) showed 80% infestation of stored maize due to *S. zeamais.*

S. zeamais is primary pest which feeds on sound grains by making irregular holes in grain kernals and reduced its nutritive value (Demissie *et al.,* 2003). *S. zeamais* also reduces the dry matter of the sound grains. The reductions of dry matter metabolize the grain kernel and increase the heat and carbon dioxide (Canepple *et al.,* 2003). Increased heat and moisture of grain produces hot spot areas which lead to fungal development (Hill, 1987). Single larva of *S. zeamais* consumes 5-7% part of a grain while adult can consume 30-50% (Umoetok, 2004).

In many countries use of conventional fumigation has stopped due to adverse effects of phosphine and methyl bromide on environment. Use of plant extracts or botanicals have advantage over the synthetic insecticides because plant extracts have novel mode of action, reduce cross resistance, easy to use and handle, no adverse effects on environment and easily available. These plant extracts also produce the fumigants effects. About 75 species of plants are reported to carry fumigant properties against a variety of insect pests (Pugazhvendan *et al.,* 2012). A resistance variety may be more useful when is used in combination with other control methods. The present studies were conducted to identify the response of different genotypes of maize to *S. zeamais* to assess resistance/ susceptibility and its response to different plant extract under laboratory condition.

MATERIALS AND METHODS

The present study was carried out in the Grain Research, Training and Storage Management Cell of the Department of Agri. Entomology, University of Agriculture, Faisalabad to evaluate the qualitative losses caused by maize weevil (*Sitophilus zeamais*) on eight genotypes of maize crop viz: NK-8441, MMRI-Yellow, 30K08, Agati-2002, SWL-2002, EV-6098, EV-1098 and Pak-Afghan. The samples of different maize genotypes/ varieties were taken from Maize and Millet Research Institute Yousafwala Sahiwal. The selected genotypes were treated with heat to eliminate any prior infestation before starting the experiment. Samples were washed with water and then dried in oven at high temperature to homogenize moisture. The experiment was performed using Completely Randomized Design having three replications for each treatment. Sample of each genotype weighing 50g were taken in glass jars. Adults of *S. zeamais* were obtained from the stalk cultures maintained at 27±1°C and 65%R.H and batches of 30 insects of each species were released separately in each jar. The experiment was conducted in a walk-in environmental chamber maintained at 28, 32 and 35°C with 60-65% RH.

***Chemical analysis*:** Moisture content was determined by drying 3g sample of maize flour in an air forced draft oven at a temperature of 105±5°C for 24 hrs till to constant weight as described by AOAC (1990). The moisture content was calculated according to the following formula:

$$\text{Moisture content (\%)} = (\text{WOS} - \text{WDS})/\text{WOS} \times 100$$

WOS = Weight of original sample; WDS = Weight of dried sample

Crude fat content were determined by using "Soxhlet Apparatus" along hexane as solvent (AOAC, 2006). The crude fat was determined according to the following formula:

$$\text{Crude fat (\%)} = (\text{WOS} - \text{WSE})/\text{WOS} \times 100$$

where WOS = Weight of original sample; WSE = Weight of sample after extraction

Crude protein was determined by Kjeldal method in AOAC (2006). The sample 3g was first digested with 25 ml concentrated sulphuric acid in the presence of digestion mixture for 5-6 hours or till light green or transparent colors. The sample was diluted to 250 ml with distilled water. The distillation was done by taking 10 ml of diluted sample and 10 ml of 40% NaOH solution in distillation apparatus. The ammonia thus liberated was collected in 2% boric acid solution containing methyl red as indicator. Finally, the sample containing ammonium burate was titrated against 0.1 N H_2SO_4 solutions till golden brown end point. The crude protein percentage was calculated by multiplying nitrogen with a factor 6.25 as given below.

$$\text{Crude protein} = \text{Nitrogen (\%)} \times 6.25$$

The crude fiber content were estimated by taking 3g fat free sample of each treatment and at first allow to digest in 1.25% H_2SO_4 for 30 minutes followed by washing and filtering the residue in distilled water and then start to digest in another solution containing 1.25% NaOH for 30 minutes followed by same washing and filtering treatment. Then received residue was allowed to dry and ignite in muffle furnace 3-5 hours at temperature of 550-650°C till grey or white ash as described in AOAC (2006). The percentage of crude fiber was calculated according to the following formula.

$$\text{Crude fiber (\%)} = \text{WLI/WS} \times 100$$

where WLI = Weight loss on ignition; WS = Weight of sample

The ash content is total inorganic matter and estimated by using oven dried 3 g sample of each treatment were charred on the ignite prior to place in muffle furnace at the temperature of 550-600°C for 5-6 hours or till to greyish or whitish ash formed. As given by AOAC (2006). The ash contents were calculated with the help of following formula.

$$\text{Ash content (\%)} = \text{WA/WF} \times 100$$

where WA = Weight of ash; WF = Weight of flour

***Management of insects by plant extracts*:** *Sitophilus zeamais* were collected from the old godowns and homogenous population was prepared in the Stored Grain Pest Management and Research Laboratory, University of Agriculture, Faisalabad. Culture was reared in sterilized flour and after 5 days the flour was sieved out and kept in the incubator until the emergence of F1 adults. The adults were allowed to mature for 3 weeks and then these adults were used for the experimental studies.

Fresh stem and leaves of *Terminalia chebula, Chicorium intybus, Glycyrrhiza glabra* were collected from botanical garden located in University of Agriculture, Faisalabad. Plant material was ground to get the homogenous powder and then 50g of powder was mixed with 100g of acetone and then rotary shaker was used to shake the mixture for 24 hours at least. After that the extract was filtered. The primary extract was sent to the rotary evaporator for the evaporation of excess acetone. The concentrate thus formed was served as the mother liquor. The experiment was carried in 80 mm Petri dishes and Whatmans filter paper was used in bioassay. Different concentrations of *Terminalia chebula, Chicorium intybus, Glycyrrhiza glabra* extracts were applied on the filter paper and then the filter paper was allowed to get dry. Twenty adults of test specimen were released in each petri dish and then the petri dish was covered with lid. Mortality of the adults was recorded three times after equal intervals of 24 hours. Extracts of both plants was used separately under controlled laboratory conditions.

RESULTS AND DISCUSSION

***Chemical analysis*:** The results (Table 1) show that there was a significant difference ($P<0.05$) for moisture content among the different maize varieties. The highest moisture content was observed in MMRI-Yellow (11.9%) which differed significantly from genotype 30K08 (11.52%)

Table 1. Comparison of interaction between treatment and temperature.

Treatments	Moisture Temperatures				Protein Temperatures				Fat Temperatures				Fiber Temperatures				Ash Temperatures			
	28°C	32°C	35°C	Mean	28°C	32°C	35°C	Mean	28°C	32°C	35°C	Mean	28°C	32°C	35°C	Mean	28°C	32°C	35°C	Mean
MMRI-Yellow	11.9 abc	12.03 ab	11.76 bc	11.90 b	6.31 a-d	6.12 b-e	5.90 e	6.11 d	0.52 b-f	0.31 fg	0.193 g	0.34 c	0.22 e-h	0.11 h	0.13 gh	0.15 e	0.31 a-f	0.11 f	0.11 f	0.18 d
Ev-6098	11.77 bc	11.81 abc	11.35 de	11.64 c	6.48 a	6.35 ab	5.98 de	6.27 cd	0.76 ab	0.46 d-g	0.330 fg	0.56 b	0.26 b-h	0.31 ab	0.23 e-h	0.27 cd	0.34 a-f	0.24 b-f	0.26 b-f	0.28 cd
Pak-Afghan	11.76 bc	11.81 abc	11.32 de	11.63 c	6.50 a	6.34 abc	5.94 e	6.26 cd	0.73 abc	0.39 efg	0.227 g	0.45 bc	0.26 b-h	0.27 b-h	0.26 b-h	0.26 cd	0.34 a-f	0.27 b-f	0.34 a-f	0.31 cd
Agati-2002	11.78 bc	11.78 bc	11.27 e	11.61 c	6.51 a	6.44 ab	6.12 b-e	6.36 bc	0.73 abc	0.37 efg	0.490 c-f	0.53 b	0.27 b-h	0.41 abc	0.26 c-h	0.31 bc	0.37 a-f	0.41 a-e	0.36 a-f	0.38 bc
Swl-2002	11.77 bc	11.74 bc	11.32 de	11.61 c	6.50 a	6.33 abc	5.95 e	6.26 cd	0.73 abc	0.44 efg	0.390 efg	0.52 b	0.28 b-h	0.37 a-e	0.25 c-h	0.30 c	0.34 a-f	0.25 b-f	0.25 b-f	0.28 cd
Nk-8441	11.8 bc	11.70 Bcd	11.32 de	11.60 c	6.51 a	6.33 abc	5.93 e	6.26 cd	0.72 a-d	0.35 fg	0.230 g	0.43 bc	0.24 d-h	0.16 fgh	0.21 e-h	0.20 de	0.35 a-f	0.17 def	0.16 ef	0.22 d
Ev-1098	11.76 bc	11.70 bcd	11.29 e	11.58 c	6.51 a	6.33 abc	6.00 cde	6.28 c	0.75 abc	0.44 efg	0.390 efg	0.53 b	0.26 b-h	0.23 ab	0.42 e-h	0.30 c	0.34 a-f	0.24 c-f	0.26 b-f	0.27 cd
30K08	11.6 cde	11.69 bcd	11.28 e	11.52 c	6.59 a	6.52 a	6.43 ab	6.51 ab	0.79 a	0.79 a	0.630 a-e	0.74 a	0.33 b-e	0.49 a	0.33 b-e	0.39 ab	0.46 a-d	0.48 abc	0.53 ab	0.49 ab
Control	12.2 a	12.2 a	12.20 a	12.20 a	6.60 a	6.60 a	6.60 a	6.60 a	0.80 a	0.80 a	0.800 a	0.75 a	0.4 a-d	0.40 a-d	0.40 a-d	0.40 a	0.60 a	0.60 a	0.6 a	0.6 a
TempMean	11.81 a	11.83 a	11.46 b		6.50 a	6.37 b	6.09 c		0.73 a	0.48 b	0.410 c		0.28 b	0.33 a	0.26 b		0.38 a	0.31 b	0.32 b	
TukeyhsdS.E	0.0339=0.0587=0.1017				0.0295=0.0511=0.0885				0.0227=0.0392=0.0680				0.0138=0.0240=0.0415				0.0245=0.0441=0.0763			

Means sharing similar letter in columns not significantly different by LSD Test.

Table 2. Comparison of means values of percentage mortality regarding effect of three different concentrations and three different times with extract of *Glycyrrhiza glabra, Chicorium intybus* and *Terminalia chebula* on adult of *Sitophilus zeamais* (Motsch).

Glycyrrhiza glabra			*Chicorium intybus*			*Terminalia chebula*		
Exposure Time (hours)	Conc. (%)	Mortality Mean ± S.E	Exposure Time(hours)	Conc. (%)	Mortality Mean±S.E	Exposure Time (hours)	Conc. (%)	Mortality Mean±S.E
24	10	25.00±2.88a	24	15%	38.33±7.26a	24	15%	46.66±1.66b
24	15	28.33±1.66a	24	10%	48.33±4.40ab	24	10%	48.33±1.66b
48	10	33.89±5.08a	24	5%	51.66±9.27ab	24	5%	51.66±4.40b
48	15	37.28±3.38a	48	15%	57.62±7.38abc	48	15%	69.49±2.93a
72	10	38.18±6.55a	72	15%	69.09±3.63abc	72	15%	74.54±1.81a
72	15	41.82±1.81a	48	10%	69.49±11.7abc	48	10%	74.57±2.93a
24	5	53.33±15.89a	72	10%	70.90±9.62abc	48	5%	76.27±3.38a
48	5	55.93±16.94a	48	5%	81.35±1.69bc	72	10%	78.18±5.45a
72	5	60.0±19.99a	72	5%	89.90±3.14c	72	5%	81.81±4.81a

Means sharing similar letter in columns not significantly different by LSD Test.

showing the lowest moisture content at 32°C. The genotype 30K08 did not differ significantly from all other genotypes. The results (Table 1) also showed that protein content varied from each other among the different varieties. The highest protein content was observed in 30K08 (6.51%) and the lowest was recorded in genotype MMRI-Yellow (6.11%). These both genotypes differ significantly from each other. The result (Table 1) also revealed that there were significant differences for the fat contents among the different maize varieties. The highest amount of fat contents were observed in genotype 30K08 (0.74%) and lowest for MMRI-Yellow (0.34%). These two genotypes differed significantly from each other. The genotype MMRI-Yellow was statistically at par with genotypes NK-8441 and Pak Afghan. The result (Table 1) also indicates that there were significant differences for the fiber contents among the different maize varieties. The highest amount of fiber contents were observed in genotype 30K08 (0.38%) and the lowest for MMRI-Yellow (0.15%). Significant differences for the ash contents among the different maize genotypes were also observed. The highest amount of ash contents was observed in genotype 30K08 (0.49%) while the lowest in genotype MMRI-Yellow (0.18%).

The results indicate highly significant changes in nutritional composition of maize grains of different maize genotypes. The results of present study concede with findings of Kapoor and Jood (1993). They examined that grain with higher moisture contents were favoured by insects. Modgill and Samules (2003) observed significant increase in crude fiber of infected grains. Khalil *et al.* (2010) reported that ash, fiber and protein contents significantly affected the infestation caused by *S. granaries*. The present results are not in conformity with Santiago and Mendoza (1983), who reported that there was no significant difference in protein content in susceptible and resistant maize varieties. Junior *et al.* (2000) observed high moisture content in low moisture maize grains

without insect and low protein content was observed in higher moisture maize grains infested with *S. zeamais.*

***Management of insects using plant extracts*:** The results (Table 2) indicate that interaction of concentration and time with extracts of *Glycyrrhiza glabra* did not differ significantly ($P<0.05$) from each other. Maximum mortality was observed (60.0 %) at 5% concentration at 72 hours of exposure time. The results (Table 2) also revealed that interaction of concentration and time with extract of *Chicorium intybus* significantly differ from each other higher mortality of S. *zeamais* was observed at 72 hours after application of plant extracts at 5% (89.9) concentration of plant extract of *Chicorium intybus*. The results (Table 2) also showed significant difference among the interaction of concentration and time. Maximum mortality was observed after 72 hours of application and 5% concentration (81.81) while the lowest mortality was recorded after 24 hours of application and 15% concentration (46.66).

The results (Table 3) represents the means values of three plant extract at different exposure time at different concentration on adults of *Sitophilus zeamais* (Motsch). It is evident from the results that maximum mortality was recorded (89.09%) at lowest concentration after 72 hours of exposure time in case of *Chicorium intybus* and followed by *Terminalia chebula* (81.81%) at 5% concentration and *Glycyrrhiza glabra* (55.93%) at same concentration.

The present study concede with work done in past by Asawalam (2006) who found plant extracts of *P. guineense*most effective against *S. zeamais* and high percentage mortality was obtained at low concentration and higher exposure time after 72 hours. The present finding can be compared with those of Kerdchoechuen*et al*. (2010) who reported 96% percentage mortality by *Osimum basilicum* after 72 hours of application of extract at 5 % concentrations. The present findings can, partially, be compared with those of Ogunsina *et al*. (2011) who achieved the control of *Sitophilus zeamais* by plant extract of *H. spicigera* after application of 48 hours at 5% concentration.Belmain *et al.* (2001) observed that plant extracts of *S. longipedunculata* was most effective against *Sitophilus zeamais*. In the present study three plant extracts were used at concentration of 5, 10 and 15% while the exposure period was 24, 48 and 72 hours. Maximum mean mortality was observed at lower concentration and higher exposure period. Maximum mean mortality was (56.42%) at 5% concentration and 72 hours exposure time in *Glycyrrhiza glabra* while in case of *Chicorium intybus* maximum mortality was observed (74.037%) at 5% concentration and 72 hours exposure period. In the case of *Terminalia chebula* maximum mortality was observed (69.91%) at 5% concentration and 72 hours exposure period. Comparative study of three extracts *Terminalia chebula*, *Chicorium intybus* and *Glycyrrhiza glabra* shows that maximum mortality was

Table 3. Comparison of means values of plant extract at different exposure time and different concentration on adult of *Sitophilus zeamais* (Motsch).

Plants	Exposure Time (hours)	Concentration (%)	Mean Mortality±S. E
Glycyrrhiza glabra	24	10	25.00±2.88 a
Glycyrrhiza glabra	24	15	28.33±1.66 ab
Glycyrrhiza glabra	48	10	33.89±5.08 abc
Glycyrrhiza glabra	48	15	37.28±3.38 a-d
Glycyrrhiza glabra	72	10	38.18±6.55 a-d
Chicorium intybus	24	15	38.33±7.26 a-d
Glycyrrhiza glabra	72	15	41.82±1.81 a-e
Terminalia chebula	24	15	46.66±1.66 a-f
Terminalia chebula	24	10	48.33±1.66 a-f
Chicorium intybus	24	10	48.33±44.0 a-f
Terminalia chebula	24	5	51.66±4.40 a-f
Chicorium intybus	24	5	51.66±9.27 a-f
Glycyrrhiza glabra	24	5	53.33±15.9 a-f
Glycyrrhiza glabra	48	5	55.93±16.9 a-f
Chicorium intybus	48	15	57.62±7.38 a-f
Glycyrrhiza glabra	72	5	60.00±20.0 a-f
Chicorium intybus	72	15	69.09±3.63 b-f
Chicorium intybus	48	10	69.49±11.7 b-f
Terminalia chebula	48	15	69.49±2.93 b-f
Chicorium intybus	72	10	70.90±9.62 b-f
Terminalia chebula	72	15	74.54±1.81c-f
Terminalia chebula	48	10	74.57±2.93 c-f
Terminalia chebula	48	5	76.27±3.38 c-f
Terminalia chebula	72	10	78.18±5.45 def
Chicorium intybus	48	5	81.35±1.69 ef
Terminalia chebula	72	5	81.81±4.81 ef
Chicorium intybus	72	5	89.09±3.14 f

obtained by *Terminalia chebula* (66.83%) at 5% concentration after 72 hours exposure period followed by *Chicorium intybus* (63.98%) and *Glycyrrhiza glabra* (41.53%).

***Conclusion*:** It is obvious from the present studies that high moisture content, percent amount of ash, fiber and fat was observed in MMRI-Yellow and maximum mortality of maize weevil was observed by using aqueous extract of *Chicorium intybus*. Hence, it is concluded that MMRI-Yellow is resistance genotype against maize weevil and *Chicorium intybus* can effectively be used for the management of maize weevil.

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