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Screening of Promising Maize Varieties Against Maize Weevil (*Sitophilus zeamais* Motschulky) Under Storage Condition

Sarjit Pandey[1,a,*], Dharma Raj Katuwal[1,b], Rashmi Aryal[2,c]

[1]*Institute of Agriculture and Animal Science, Tribhuvan University, Kathmandu, Nepal*
[2]*University of Bologna, Bologna, Italy*
[*]*Corresponding author*

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ABSTRACT

The maize weevil (*Sitophilus zeamais* Motschulsky) causes significant quantitative and qualitative losses during storage. To identify resistant varieties of maize against this pest, an experiment was conducted in a Completely Randomized Design (CRD) with 11 varieties in free and no-choice conditions. The study measured weight loss, mean bored grain, debris, and weevil numbers at 30, 60, and 90 days. The findings showed that BG13Y-POP, Manakamana-7, and RML-19/RML-6 were the most resistant varieties, with weight loss percentages of 1.99%, 1.47%, and 1.74%, respectively, and final weevil numbers of 104, 72, and 73. Ganesh-2 and ZM-401 were the most susceptible varieties, with weight losses of 7.34% and 6.05%, respectively. The maximum debris weight was found in RML-761/RL-105 (1.98 g), while the minimum was found in Manakamana-7 (0.26 g). The highest number of bored grains was observed in Ganesh-2 (81), while the lowest number was observed in Rampur-4 (51). Similarly, ZM-401 (158) and Ganesh-2 (165) exhibited the highest weevil population, while the lowest count was found in Rampur-4 (72). Overall, using resistant varieties, such as Manakamana-7, BG13Y-POP, and RML-19/RML-6, can be an effective approach for reducing post-harvest losses from weevil infestation.

[a] *pandeysarjit@gmail.com* *https://orcid.org/0009-0002-4938-1097*
[b] *asbinkatwal21@gmail.com* *https://orcid.org/0000-0002-6925-5728*
[c] *rashmiaryal77@gmail.com* *https://orcid.org/0009-0009-9121-7531*

Introduction

Maize (*Zea mays* L.) is an important cereal crop and ranks as the third most important globally, with an annual production of 1,210 million tons cultivated on 205.8 million hectares, achieving a productivity rate of 5.8 tons per hectare (FAOSTAT, 2021). In Nepal, maize holds the position of the second most important cereal crop, with an annual production of 2.99 million tons grown on 979 thousand hectares, and a productivity rate of 3.06 tons per hectare (MOALD, 2020/21). It contributes 6.83% to the Agricultural Gross Domestic Product (AGDP) in Nepal, making it the second-highest cereal crop after rice (MOALD, 2020/21). Maize cultivation is widespread across Nepal, with the crop being particularly successful in the mid-hills and Terai regions. Nonetheless, maize production is primarily concentrated in hilly areas, and the size of farms tends to be smaller compared to those in the Terai region (Gairhe et al., 2021).

Post-harvest loss due to insect infestation is a major challenge facing maize production worldwide, with losses ranging from 1-5% in developed nations to 20-50% in developing nations (Nukenine, 2010). The maize weevil (*Sitophilus zeamais*), Angoumois grain moth (*Sitotroga cerealella*), and larger grain borer (*Prostephanus truncatus*) are the most prevalent insect species that attack stored maize grains, and they have a rapid rate of reproduction that can lead to significant damage within a single season (Ojo et.al., 2016). Despite improvements in production, this persistent problem continues to impact maize farmers, especially in developing countries.

The maize weevil (*Sitophilus zeamais:* Coleoptera: Curculionidae) is one of the most harmful pests of grains, cereals, and other stored items. It can cause significant qualitative and quantitative damage to untreated stored maize, resulting in grain weight loss ranging from 20% to 90% (Muzemu et al., 2013). Poor storage practices in Nepal are the primary reason for maize seed deterioration, leading to a 10% to 20% quantitative loss during storage (Bhandari et al., 2015). The use of resistant cultivars may be the most effective pest management strategy to mitigate such losses, especially in existing integrated pest management programs (Keba and Sori, 2013).

Maize weevil damages stored maize, making it unsuitable for human consumption and the market, thus reducing pest damage is crucial for grain preservation (Bergvinson and Garcia-Lara, 2004). However, the majority of farmers in Nepal grow hybrid varieties, which are more susceptible to pest infestation and post-harvest loss. Although insecticides have been used to control the maize weevil, their overuse has led to insecticide-resistant populations (Ribeiro et al., 2003). Moreover, botanicals used for biological control have been shown to degrade grain quality. Therefore, there is a pressing need to identify resistant maize varieties that can limit weevil damage. This is crucial for maintaining grain preservation and attaining food security and food safety (Khakata et al., 2018). Therefore, it is essential to select resistant varieties as a long-lasting solution to prevent weevil infestation, taking into account the dual goals of food security and food safety. The use of resistant varieties, in conjunction with other control techniques, could establish an integrated pest management program that is safe, effective, and environmentally friendly.

Materials and methods

Location of the Experiment

The experiment was conducted at the Institute of Agriculture and Animal Science (IAAS), Lamjung Campus, Lamjung, Nepal, during August- November, 2018. The site is situated at an elevation of 610m, with 28.13° N latitude and 84.42° E longitude.

Experimental Design

For this experiment, the treatments were organized using a Completely Randomized Design (CRD) with three replications for each maize variety. The maize varieties used in the research were collected from National Maize Research Program (NMRP) Rampur, Chitwan, as well as from local farmers in Sundarbazar, Lamjung.

Weevil Culture

The starting culture of *S. zeamais* used in the experiment was obtained from the stock at Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur. The weevils were reared on a susceptible maize variety at the entomology lab of IAAS in Lamjung. To obtain a fresh weevil population for the experiment, 500 g of infestation-free maize grains were placed in a plastic jar, and 500 live weevils were added for incubation. The jar was securely covered with muslin cloth during incubation

Experimental Setup

All the maize samples were sun-dried to make them free from insects. The grain moisture content (GMC) of the oven-dried maize samples was determined using a WILE - Moisture Meter and adjusted to 14% moisture for all varieties. The experiment was carried out in both free-choice and no-choice tests under laboratory conditions, at a temperature range of 20-25°C and relative humidity (RH) of 75±5%.

Free Choice Test

The experiment involved testing eleven different maize varieties, with 50 g of grain samples used for each variety. The experiment was arranged in a Completely Randomized Design using polythene bottles with a diameter of 5cm and a height of 7cm, with three replications. Four circular holes were made at the bottom of the bottles on all four sides, and no lids were used to allow the weevils to freely enter the bottle. The bottles were arranged in a circular manner inside a wider circular container with a diameter of 60cm and a height of 20cm. Then, 800 F2-progeny of *S. zeamais* (irrespective of sexes) aged 20 days were released in the center of the container. The wide container was covered with black muslin.

Table 1. Treatment details

S N	Treatment	Varieties
1.	T1	ZM-401
2.	T2	RML-761/RL-105
3.	T3	BG13Y-POP
4.	T4	RML-19/RML-6
5.	T5	DEUTI
6.	T6	RAMPUR COMPOSITE
7.	T7	RAMPUR-4
8.	T8	TLBR-7
9.	T9	MANAKAMANA7
10.	T10	GANESH-2
11.	T11	ARUN-4

No Choice Test

In this experiment, 50 g of maize samples were placed into polythene bottles with a diameter of 5cm and a height of 7cm. Then, 5 pairs of F1-progeny of *S. zeamais* (male and female) aged 20 days were introduced into each bottle as an inoculum. Similarly, 5 pairs of F2-progeny of *S. zeamais* (male and female) aged 20 days were introduced into each bottle as an inoculum. The mouth of the bottles was perforated with a black muslin cloth to ensure free air circulation. The experiment was arranged in a Completely Randomized Design with three replications.

Data Collection

For data collection, 50g of maize sample was used and data were collected at 30-day intervals for three months to determine the total number of damaged grains, weight loss percentage, grain debris, weevil attraction, and weevil emergence. The count and weight method of damaged and undamaged grain as adopted by Gewinner et al. (1996) was used to determine all parameters using a weighing balance. Weight loss is an essential parameter for determining resistance in maize grains as it indicates economic loss for the farmer (Dari et al., 2010; Derera et al., 2014).

Grain weight loss % was determined by using mathematical formula.

$$\text{Weight loss } \% = \frac{(\text{Wu} \times \text{Nd}) - (\text{Wd} \times \text{Nu})}{\text{Wu} \times (\text{Nd} + \text{Nu})} \times 100$$

Where, Wu=Weight of Undamaged grain
Nd=Number of damaged grains
Wd= Weight of damaged grain
Nu=Number of undamaged grains

Statistical Analysis

The data input and tabulation were carried out using Microsoft Excel, while R package was used for statistical analysis. Analysis of variance (ANOVA) was performed at a 0.05% level of significance.

Results

Effect of Maize Varieties on Weight Loss Percentage by S. zeamais

The weight loss percentage was significantly different (P<0.05) among the tested varieties during 30, 60, and 90 days after observations in free-choice conditions (Table 2). In 30 days after treatment, the maximum percent loss was recorded in Ganesh-2 (1.88%) whereas the lowest percent loss was recorded in TLBR-7(0.23%), RML-761/RL-105(0.23%), Rampur composite (0.22%). Similarly, the maximum percent loss in 60 days of observation was recorded in ZM-401(2.72%) followed by Ganesh-2, RML-761/RL-105, and the lowest percent loss in TLBR-7, Rampur composite, and Rampur-4 respectively. However, in 90 days of observation, the grain damage percent was recorded highest in Ganesh-2 (7.34%) followed by ZM-401(6.05%), RML-761/RL-105 (4.91%), Deuti (4.79%) and least loss were recorded in Manakamana-7(1.47%), BG13Y-POP (1.90%), RML-1/RML-6 (1.74%) respectively.

Under no choice condition, a significant difference was observed at 5% level among varieties for weight loss (Table 2). At 30 days, the highest weight loss was seen on Ganesh-2 which showed susceptibility. Low weight loss was seen on RML-761/RL-105, Rampur composite, TLBR-7, while Rampur 4, ZM-401, RML-19/RML-6, Manakamana-7 was statistically par with Rampur composite. At 60 days, the highest weight loss was seen on Ganesh-2 while ZM-401 and RML-761/RL-105 were statistically at par with Ganesh-2. At 90 days, the highest weight loss was seen on ZM-401 and RML-761/RL-105 were statistically par with ZM-401. Manakamana-7, Rampur composite, Rampur-4, and TLBR-7 was less susceptible as they had low weight loss at 90 days.

Table 2. Effect of maize varieties on weight loss % under free choice and no choice by *S. zeamais* in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Mean weight loss (%)					
		Free choice			No choice		
		30 days	60 days	90 days	30 days	60 days	90 days
1.	ZM-401	0.51bcd	2.72a	6.05ab	0.51bcd	2.55ab	6.64a
2.	RML-761/RL-105	0.23d	1.80abc	4.92abc	0.23d	2.45ab	6.03ab
3.	BG13Y-POP	0.82b	1.07cd	1.99d	0.82b	0.49c	1.93cd
4.	RML-19/RML-6	0.46bcd	0.41d	1.74d	0.46bcd	0.76bc	2.17bcd
5.	DEUTI	0.48bcd	1.07cd	4.79abc	0.49bcd	0.83bc	1.30d
6.	RAMPUR COMPOSITE	0.22d	0.69d	2.74cd	0.23d	0.59c	1.21d
7.	RAMPUR-4	0.32cd	0.60d	2.5cd	0.32cd	0.48c	1.08d
8.	TLBR-7	0.23d	0.27d	2.87cd	0.23d	0.55c	1.18d
9.	MANAKAMANA 7	0.25cd	0.77cd	1.48d	0.25cd	1.01bc	1.48d
10.	GANESH-2	1.89a	2.12ab	7.35a	1.89a	3.07a	5.40abc
11.	ARUN-4	0.67bc	1.27bcd	3.89bcd	0.69bc	0.97bc	1.75cd
F-TEST		***	*	**	*	*	*
LSD		0.44	1.14	2.53	0.045	1.63	2.36
CV (%)		47.31	41	40.63793	47.3	27	51.21

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, *= Significant at 5% level of significance, ** = significant at 1% level of significance, ***=significant at 0.1% level of significance.

Effect of Maize Varieties on Grain Damage by S. zeamais

The mean grain damage was significantly different (P<0.01) among the tested varieties during 30, 60, and 90 days after observations in free-choice conditions (Table 3). In 30 days after treatment, maximum grain damage was recorded in Ganesh-2(15) and RML 761/RL-105 (15) varieties whereas lowest percent loss was recorded in Manakamana-7(7), Rampur-4(7) and RML 19/RML-6(8). Similarly, maximum mean grain damage in 60 days of observation was recorded in Ganesh-2(38) followed by ZM-401(34), RML-761/RL-105(38), and the lowest percent loss in RML-19/RML-6(18), Rampur-4 (19), Arun-4(20) respectively. However, in 90 days of observation, maximum mean grain damage was recorded in Ganesh-2(80) followed by ZM-401(74), RML-761/RL-105(73) BG13Y-POP (72) respectively and least grain damage was RML-19/RML-6(44) followed by Rampur-4 (51) respectively.

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Table 3. Effect of maize varieties on number of grain damage on free choice and no choice by *S. zeamais* in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Mean number of grain damage					
		Free choice			No choice		
		30 days	60 days	90 days	30 days	60 days	90 days
1.	ZM-401	11abc	34ab	74ab	14a	40abc	79 ab
2.	RML-761/RL-105	15a	33abc	73ab	15a	43ab	67 bc
3.	BG13Y-POP	12ab	27bcd	72ab	6b	17d	51 d
4.	RML-19/RML-6	8c	18e	44d	2b	17d	44 d
5.	DEUTI	11abc	32abc	73ab	6b	22d	53 d
6.	RAMPUR COMPOSITE	9bc	23cde	57bcd	3b	23d	48 d
7.	RAMPUR-4	7c	19de	51cd	2b	25cd	53 d
8.	TLBR-7	9bc	25bcde	59bcd	3b	23d	69 ab
9.	MANAKAMANA 7	7c	20de	56bcd	4b	26cd	56 cd
10.	GANESH-2	15a	38a	80a	17a	47a	81 a
11.	ARUN-4	9bc	20de	65abc	6b	30bcd	55 cd
F-TEST		*	***	**	***	***	***
Grand mean		10.57	26.51	64.48	7.57	28.75	59.97
LSD		4.5	8.95	17.29	6.59	13.74	12.20
CV (%)		25.82	19.82	15.742	51.1	28	11.95

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, *= Significant at 5% level of significance, ** = significant at 1% level of significance, ***=significant at 0.1% level of significance.

Table 4. Effect of maize varieties on number of weevil emergence in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Final weevil number (free choice)	Final weevil number (no choice)
1.	ZM-401	158a	105 a
2.	RML-761/RL-105	135b	99a
3.	BG13Y-POP	104c	75 bc
4.	RML-19/RML-6	72f	68 bc
5.	DEUTI	87de	104 a
6.	RAMPUR COMPOSITE	79def	60 c
7.	RAMPUR-4	72f	72 bc
8.	TLBR-7	91cd	68 bc
9.	MANAKAMANA 7	73ef	60 c
10.	GANESH-2	165a	110 a
11.	ARUN-4	89cd	88 ab
F-TEST		***	***
Grand mean		102	83
LSD		13.99	23.32
CV (%)		8.011	16.43

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, ***=significant at 0.1% level of significance.

Effect of Varieties on Number of S. zeamais Progeny Emergence

In no-choice test, there were variations, and significant differences were observed at <0.01% level among the 11 varieties for weevil progeny emergence (Table 4). It ranged from 60 to 110 mean adult emergences, which was low in Rampur composite followed by Manakamana-7 indicating their tolerance to *S. zeamais*. Similarly, the mean number of weevils was high in Ganesh- 2 followed by ZM-401 and Deuti showing their susceptibility to *S. zeamais*. The remaining tested varieties were intermediate types. In free-choice test also, significant differences were observed at <0.01% level among the tested varieties (Table 4). It ranged from 72 to 165 mean weevil emergence. The mean number of progeny emergence was low in Rampur-4 and RML-19/RML-6 followed by Manakamana-7 indicating their tolerance to *S. zeamais*. Similarly, the mean number of progeny emergence was high in Ganesh -2 and ZM-401 followed by RML-761/RL-105 and BG13Y-POP by showing their susceptibility to the *S. zeamais*. The rest of the genotypes were intermediate types.

Effect of Maize Varieties on Grain Debris Release by S. zeamais

In no-choice test, the maize varieties were statistically significant at 1% level for grain debris release (Table 5). It ranged from 0.095g to 1.73g mean grain debris, which was low in BG13Y-POP in followed by Manakamana-7, Arun-4, and RML-19/RML-6 indicating their tolerance to *S. zeamais*. Similarly, the mean amount of grain debris release was high in RML-761/RL-105 followed by ZM-401, and Ganesh-2 showing their susceptibility to *S. zeamais*. The remaining tested varieties were intermediate types.

Under free-choice test, the maize genotypes were statistically significant at 1% level for grain debris release which ranged from 0.21g to 1.24g (Table 5). The amount of grain debris release was low in Arun-4 followed by RML-19/RML-6 showing their tolerance to *S. zeamais*. Similarly, the amount of grain debris release was high in Ganesh-2 followed by Deuti and ZM-401 indicating their susceptibility to *S. zeamais*. The remaining genotypes were intermediate types.

Table 5. Effect of maize varieties on grain debris released in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Final grain debris (free choice)	Final grain debris (no choice)
1..	ZM-401	0.87ab	1.74 a
2	RML-761/RL-105	0.54bc	1.90 a
3.	BG13Y-POP	0.28bc	0.10 c
4.	RML-19/RML-6	0.24c	0.33 c
5.	DEUTI	0.80ab	0.45 bc
6.	RAMPUR COMPOSITE	0.36bc	0.62 bc
7.	RAMPUR-4	0.15c	0.54 bc
8.	TLBR-7	0.28bc	0.43 bc
9.	MANAKAMANA 7	0.44bc	0.26 c
10.	GANESH-2	1.24a	1.47 ab
11.	ARUN-4	0.22c	0.41 c
F-TEST		**	**
Grand mean		0.49	0.75
LSD		0.54	0.89
CV%		65.39	23

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, ** = significant at 1% level of significance.

Table 6. Preference of *S. zeamais* at 30 days on selected maize genotypes in storage, IAAS, Lamjung, 2018/19

SN	Treatment	Preference
1	ZM-401	45.67a
2	RML-761/RL-105	43.67a
3	BG13Y-POP	20.33b
4	RML-19/RML-6	21.00b
5	DEUTI	23.00b
6	RAMPUR COMPOSITE	14.67b
7	RAMPUR-4	16.33b
8	TLBR-7	15.00b
9	MANAKAMANA 7	16.00b
10	GANESH-2	40.67a
11	ARUN-4	16.33b
F-TEST		***
Grand mean		24.79
LSD		15.48
CV (%)		36.67

Mean separation in columns followed by the same letters are not significantly different at P=0.05 Note: LSD= Least Significant difference, CV= Coefficient of Variation, ***=significant at 0.1% level of significance.

Effect of Maize Varieties on S. zeamais Preference

In free-choice test, there was a statistically significant difference at 1% level for the mean number of weevils attracted on tested genotypes at 30 days (Table 6). The mean number of weevils attracted to the different varieties ranged 14.66 to 45.67. The preference was high in ZM-401, RML-761/RML-105, Ganesh-2. Similarly, the preference was low in Rampur composite, Arun-4, TLBR-7. The remaining tested genotypes were intermediate types.

Discussion

The number of damaged grains, grain debris, weight loss, and weevil emergence were all significantly different between maize varieties, and weevil attraction is a crucial signal for determining a variety's vulnerability. According to Abebe *et al.* (2009), the susceptibility index is positively correlated with the number of F1-progeny, percentage of damaged grains, and grain weight loss. These variations in the susceptibility of the maize types reveal a variety's innate capacity to defend itself against *S. zeamais* attack. Physical factors like grain hardness, pericarp surface texture, and nutritional factors like amylose, lipid, and protein content or non-nutritional factors, particularly phenolic compounds, may all contribute to this resistance (Garca-Lara et al., 2004).

Two biochemical substances that take the form of phenolic amides, may operate as antibiosis agents against *S. zeamais* (Muzemu et al., 2013). According to Arnason *et al.* (2004), this phenolic promotes resistance through both structural and antibiosis effects. According to Garcia-Lara *et al.* (2004), resistant maize cultivars have robust pericarps with high concentrations of hydroxycinnamic acids. Additionally, it has been claimed that the effects of antibiotics made insects more agitated, which decreased eating and may have contributed to the low levels of grain damage and weight loss among resistant cultivars (Muzemu et al., 2013). Grain hardiness has been identified as the primary resistance factor for weevils (Bamaiyi et al., 2007). Tryptophan and lysine, two components of proteins that are resistant to the maize weevil, may have a negative impact on feeding behavior, host preference, or growth and development (Arnason et al., 2004). According to Arnason *et al.* (2004), protein content was inversely linked with a maize variety's susceptibility to *S. zeamais*.

The significant difference in the number of weevils that emerged among the varieties may be caused by antibiosis effects, such as the lack of essential nutrients and an unbalanced proportion of nutrients, which cause weevil larvae to die and, in some cases, weevil adults to die before laying eggs (Derera et al., 2001).

Antixenosis mechanisms, such as a smooth pericarp, which could discourage weevils from oviposition and feeding and also hinders mandibles from grabbing maize kernels, may be responsible for the reduced discharge of grain debris. It is also well known that seeds contain attractants. The isolation of many volatiles by including hexanoic acid, that are attractants to maize weevils may account for the variations in weevil attraction between types (Keba and Soli,2013). The flint-like quality of some kinds of grain may also contribute to resistance. Flint maize is resistant to weevil because it is hard, thick with vitreous endosperm, starch collected in the periphery, and poor moisture absorption (Suleiman et al., 2014).

Conclusion

This study aimed to identify resistant varieties of maize against the *S. zeamais* infestation. The results showed that BG13Y-POP, Manakamana-7, and RML-19/RML-6 were the most resistant varieties, while Ganesh-2 and ZM-401 were the most susceptible. The use of resistant varieties such as Manakamana-7, BG13Y-POP, and RML-19/RML-6 could significantly reduce post-harvest losses from weevil infestation. Farmers and other stakeholders can use this information to choose the most suitable maize varieties for storage and reduce losses caused by *S. zeamais*. Further research could explore the genetic and biochemical mechanisms underlying the resistance of these varieties to weevil infestation.

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