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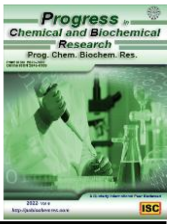

Original Article

Residual Toxicity of *Piper Nigrum* L. Powder Against the Susceptibility of Stored Maize Cultivars to Infestation by *Sitophilus Zeamais* Motschulsky (Coleoptera: Curculionidae)

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Highlights:

► The present work throw light on current levels of susceptibility prevailing in maize cultivars to maize weevil, can be tackled by intelligent selection of the cultivar of maize to be stored as well as improving conditions by using protection for maize grains stored.

► *P. nigrum* powder has revealed insecticidal as well as propitious protective effect on maize against weevils invasion for up to six months' storage.

ABSTRACT

Maize (*Zea mays* L.) cultivars grown in Egypt are not only numerous, but also show a wide diversity of exposure to stored grain pests causing damages that may threaten food security. Thus, the aim of this study was to assess the level of susceptibility of maize cultivars to infection by *Sitophilus zeamais* (Motschulsky), a voracious insect pest of stored maize. Likewise, to evaluate the efficacy of black pepper, *Piper nigrum* L. powder as an insecticide against this pest to protect stored maize. The susceptibility index values showed that cultivars THW-310 and THY-352 were more susceptible to infection by *S. zeamais*, and the levels of some amino acids were higher than cultivars SHY-162 and SHW-10, which showed relatively low infection. In all maize cultivars, application of *P. nigrum* powder reduced the severity of *S. zeamais* infestation, significantly improved seedling growth, and % germination after six months of maize storage, compared to the untreated samples. The promising *P. nigrum* powder could be recommended for use as a part of an integrated pest management program in stored maize, particularly for a long storage periods.

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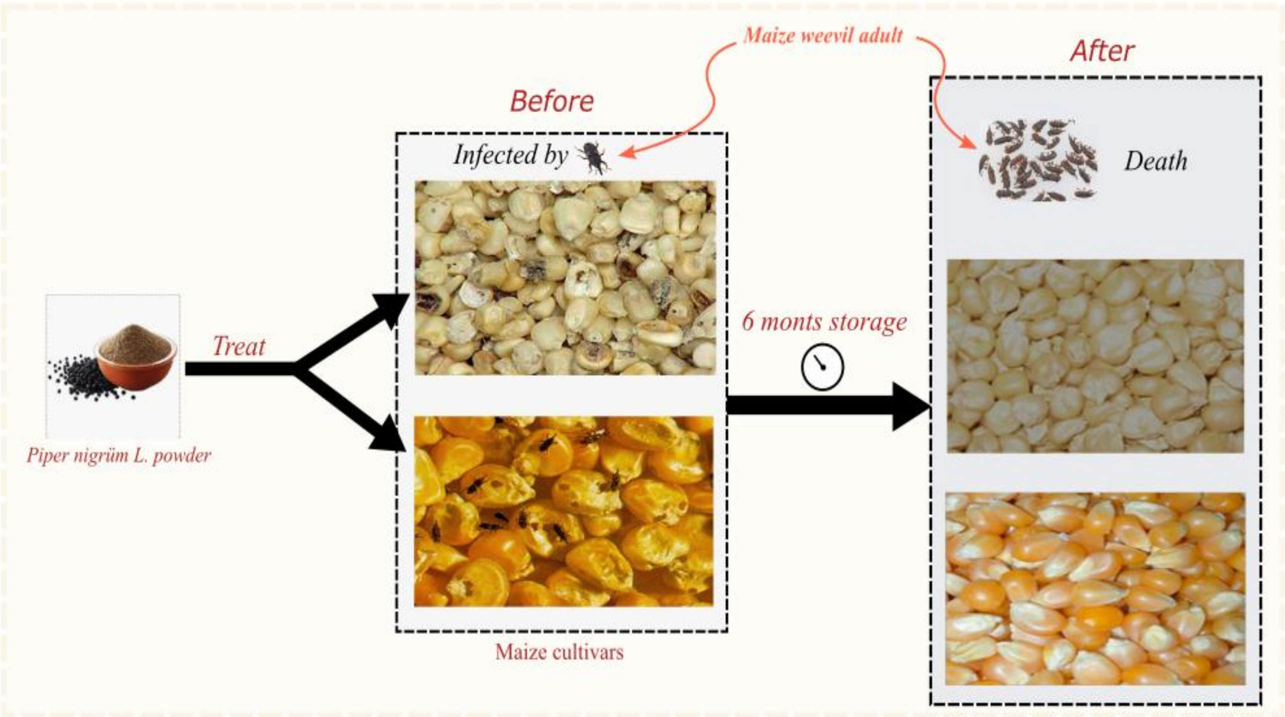
GRAPHICAL ABSTRACT



Schematic overview of the susceptibility of stored maize cultivars to maize weevil infestation and control by *Piper nigrum* L. powder as a bio-pesticide

1. Introduction

Maize is among the three most important cereal crops in the world, and is known as the queen of cereals. Maize provides between 50% and 70% of the dietary protein for humans, it is also one of the major crops used for feeding farm animals [1]. Several studies have shown that post-harvest insect pests are considered as the most important and cause heavy losses up to 20–30% in stored maize [2,3]. The seeds are basic inputs for the maize crops and their quality is important to obtain high productivity. It is essential that the seeds are not subjected to rapid spoilage during storage mainly by insect pests so as not to threaten food security [4]. Considerable success has been achieved by maize breeders in identifying and selecting maize hybrids which are tolerant or resistant to pre-harvest diseases and pests. With optimism that similar success can be achieved in post-harvest pests, where a large amount of maize is lost during storage [5]. The maize weevil, *S. zeamais* is one of the most destructive insect pests of stored food grains and causes huge losses in both quantity and quality [6].

In most systems, the protection of stored grain from pests depends mainly on the use of synthetic insecticides and fumigants. Although it is an effective control method, it has several problems such as residues and resistance, and this poses a great challenge [7]. To reduce the use of hazardous pesticides, development of effective and safe pest control alternatives to stored grains is required. One such alternative approach is the use of plant products and their by-products (botanical insecticides) which are receiving increasing attention in stored product pest management [8]. Botanical insecticides are used as powder, essential oil, solvent extract, repellent agents, and fumigants to control the stored insects [2-4]. *P. nigrum* has a large number of traditional and modern applications ranging from food additives to use as a potential insecticide against stored grain pests [9,10]. There are no studies on the relationship between the susceptibility of different maize cultivars to infection and their amino acid content. Clarification of this relationship is important to provide information to maize breeders that will enable them to combine high quality maize with a high degree of resistance. Hence, this study was undertaken to evaluate the variability in amino acids content of maize cultivars, and their correlations with susceptibility to *S. zeamais* infestation. *P. nigrum* powder has also been evaluated as a bio-pesticide to protect stored maize from infestation by weevils.

2. Materials and Methods

2.1. Maize weevil culture

The adults of maize weevil (*S. zeamais*) used in bioassays were obtained from infested maiz at the local vendor. In the laboratory, the infested maize were placed in glass jars (2 L capacity) and covered with muslin cloth. The maize were kept under controlled conditions (27 ±2 °C, 65 ±5% RH, and complete darkness) until the emergence of adults. Emerged adults (males and females) were transferred to glass jars (2 L capacity) containing sterilized clean maize grains. Seven-day-old adults were used in all bioassays.

2.2. Maize cultivars

Four maize cultivars were evaluated: Single Hybrid White 10 (SHW-10), Single Hybrid Yellow 162 (SHY-162), Triple Hybrid White 310 (THW-310), and Triple Hybrid Yellow 325 (THY-352) were selected as the most widely used cultivars by farmers. Maize cultivars used in these experiments were provided by the Ministry of Agriculture, Egypt.

2.3. Inherent susceptibility test

To evaluate susceptibility, sets of 20 *S. zeamais* adults (ten couples) were transferred introduced into glass jars (500 mL) containing 100 g of each cultivar, covered with a muslin cloth and kept for 7 days for conditioning and to achieve the stable environmental conditions. Insects that did not react to the touch of a fine-tip brush were considered dead replaced from a spare conditioning replicates this first set was meant to condition the insects to the maize cultivars. After 7 days, the first set of maize was discarded and all insects were transferred to second set into glass jars (500 mL) containing 100 g of each variety and covered with muslin cloth as for the first set. The second set lasted 7 days, during which the insects were allowed to lay eggs. Insects that did not react to the touch of a fine-tip brush during second set period were considered dead and not replaced, and then the adults were removed after 7 days. This set was left until the adults emerged first generation (F1). Adults were counted at frequent interval daily for the following 4 weeks after oviposition, and then removed. The total number of adults and the time from oviposition to the emergence of 50 percent was noted. All sets were under controlled conditions (27 ± 2 °C, 65 ±5% RH, and complete darkness). Three replicates for each maize cultivar contained 13% moisture. The susceptibility index of each cultivar was calculated using the below-mentioned formula [11]:

Susceptibility index:

$$SI = \text{Loge}\,(TN \times 100)/D \quad (1)$$

Where, TN is Loge (total number of F1 adults) and D is the development period.

2.4. Amino acids content of maize cultivars

Amino acid contents of maize seeds, one of the characteristics that could be responsible for the susceptibility or relative resistance of each cultivar to *S. zeamais* infestation, were investigated. Accurately weighing 1g of homogenous dry powder of each cultivar of maize seeds, and mixed with 5 mL of a 3.5% (w/v) sulfosalicylic acid. The sample was homogenized for 5 min and centrifuged at 3500 rpm for 30 min. The supernatant was analyzed for free amino acids by using the ninhydrin reagent method of Yemm and Cocking [12].

2.5. Preparation powder of Piper nigrum

Two hundred gram of dry seeds of black pepper *P. nigrum* (purchased from local market) was dried in an oven dryer overnight (12 h) and grinded to a fine powder using an electric grinder. Powder of seed *P. nigrum* was stored in airtight containers to evaluate its efficacy as a bio-pesticide.

2.5.1. Residual activity of Piper nigrum

To evaluate the efficacy of *P. nigrum* powder to protect maize seeds (13% moisture content) from infestation with maize weevil, 4 g of *P. nigrum* was mixed with 1 kg of each cultivar of maize seed in jars with uniform distribution of the powder. From the stock treated maize, weighted 100 g, and stored in sealed jars for varying periods up to 6 months after treatment at 27 °C. Then, each jar introduced with fifty adult maize weevils and allowed to feed for 72 h prior to mortality determination.

2.6. Germination tests

To carry out the germination test, one hundred randomly selected seeds from each cultivar of maize seed treated with *P. nigrum* after 6 months post treatment. Seeds were placed in Petri dishes containing moistened soft paper. The number of germinated seedlings from each Petri dish was counted and recorded after 7 days of planting. Percent germination was computed as follows:

– Germination:

$$G\ (\%) = \left(\frac{N}{T}\right) X\ 100 \qquad (2)$$

Where, N is the number of seeds germinated and T is the total number of seeds.

2.7. Statistical analysis

All experiments in the present investigation were based on triplicate and the data were expressed as a mean of replicates ± standard deviation (SD). The statistical differences between maize cultivars were evaluated using One-way analysis of variance (ANOVA) with Tukey's multiple range was performed. The correlation between susceptibility index and amino acid content in each maize cultivar was determined using the Pearson's correlation coefficient.

3. Results

3.1. Inherent susceptibility of the cultivars

Difference susceptibility to *S. zeamais* infestation was observed between four maize cultivars evaluated in this work (Table 1). Cultivar factor had a significant effect on susceptibility parameters such as number of emerged insects, median development time, and susceptibility index. The results indicate that cultivar THW-310 was the most susceptible, producing 5.8 times as many progeny of F1 in cultivar SHY-162 and it was the least susceptible. For the most susceptible cultivar, the mean developmental period of *S. zeamais* was 36 days, while it ranges 29-32 days in the less susceptible cultivars. The results indicate that there is a significant difference between four tested maize cultivars for *S. zeamais* infestation. SHW-10 and SHY-162 cultivars that had the lowest susceptibility index (moderately resistant cultivars) have recorded few insects emergence in F1. In contrast, THW-310 and THY-352 cultivars (susceptible cultivars) that had the susceptibility index two-fold higher, and recorded the highest insect's emergence in F1.

Table 1. Mean value of susceptibility index, developmental period, and total number of progen for four maize cultivars

Cultivar	Susceptibility index	Time for emergence of 50% F1 progeny	Total No. of F1 progeny
SHW-10	7.62 ± 2.64[a]	32.0 ± 1.39[b]	19.0 ± 2.83[b]
THW310	11.69 ± 1.86[c]	36.0 ± 1.20[d]	75.0 ± 3.29[d]
SHY-162	7.12 ± 2.88[a]	29.0 ± 1.67[a]	13.0 ± 2.25[a]
THY-352	9.94 ± 3.39[b]	34.0 ± 1.50[c]	36.0 ± 2.62[c]

Each value is a mean ± standard deviation (SD) of three replicates. Mean followed by the same letter in the column are not significantly different at P < 0.05 (Tukey's-test).

3.2. Amino acids content of maize cultivars

The amino acids content of maize cultivars and their correlation with susceptibility index is summarized in Table 2. Aspartic and proline were found to be the most abundant amino acids, while leucine was the least. Pearson's correlation coefficient showed different binary correlations between amino acids and susceptibility index of cultivars in the same Table. The increase of aspartic, threonine, serine, glutamic, proline, glycine, alanine, valine, ammonia, and arginine contents were positively correlated with cultivars susceptibility index, while cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine and lysine were negatively correlated with the susceptibility index for cultivars. Eight out of eighteen amino acids had negative significant correlated with susceptibility index although the correlation was weak with most of the amino acids. Susceptibility index was positively correlated to valine and isolencine contents and significantly correlated to growth and development of *S. zeamais* in THW-310 and THY-352 cultivars. Hence, the more susceptible cultivars might have higher levels of valine and isolencine compared to their less susceptible cultivars, SHW-10 and SHY-162.

3.3. Residual activity of *Piper nigrum*

The increase in mortality due to the bioassay of the four cultivars maize treated with *P. nigrum* powder against *S. zeamais* is presented in Table 3, for 6 month**s** from treatment. The results of the weevil mortality test showed that cultivar SHY-162 showed strong toxicity towards mortality with 100% mortality for 2 months after exposure, followed by cultivar SHW-10. Comparing the mortality percentages of *S. zeamais* after 5 and 6 months of exposure revealed that there was a slight increase of mortality percentages in all cultivars compared with after 1 and 2 months' post treatment. In sum, there was significant difference between the values for mortality (P< 0.05) of the treated cultivars.

Table 2. Amino acids content of maize cultivars and their correlation with susceptibility index

Amino acids (mg/g)	Cultivar				
	SHW-10	THW-310	SHY-162	THY-352	[a] Pearson's correlation coefficient
Aspartic	4.65	5.99	4.16	5.24	0.30
Threonine	2.75	0.11	2.87	3.46	0.66
Serine	2.32	0.23	2.43	3.10	0.75
Glutamic	3.36	2.62	2.27	4.98	0.46
Proline	3.35	3.68	6.79	4.67	0.32
Glysine	1.53	1.59	1.55	1.66	0.96
Alanine	3.63	2.68	2.76	3.28	0.49
Cystine	0.54	1.23	0.29	0.21	- 0.67
Valine	0.25	1.39	0.20	0.98	- 0.15
Methionine	0.27	0.54	0.31	0.35	- 0.21
Isoleucine	0.32	1.97	0.24	0.84	- 0.80
Leucine	0.20	0.29	0.12	0.12	- 0.30
Tyrosine	0.44	0.72	0.59	0.30	- 0.62
Phenylalanine	0.69	1.13	0.93	0.73	- 0.47
Histidine	1.38	0.94	1.14	0.88	- 0.41
Lysine	1.18	1.14	1.22	1.14	- 0.30
Ammonia	3.49	3.45	2.80	2.79	0.43
Arginine	1.44	1.87	2.16	1.57	0.36

[a] Pearson's correlation coefficient between free amino acids and susceptibility index

Table 3. Effect of *Piper nigrum* powder and cultivar on mean mortality (%) of *Sitophilus zeamais*

Cultivar	Months of storage					
	1	2	3	4	5	6
SHW-10	100.0 ± 0.00b	94.72 ± 4.18b	80.65 ± 3.66b	66.00 ± 5.08b	53.71 ± 5.52c	32.11 ± 3.56c
THW-310	90.00 ± 2.50a	61.02 ± 1.73a	53.20 ± 5.02a	42.26 ± 3.42a	28.30 ± 1.63a	12.13 ± 1.86a
SHY-162	100.0 ± 0.00b	100.0 ± 0.00c	87.45 ± 4.54b	74.23 ± 5.13c	60.23 ± 1.52d	45.85 ± 1.85d
THY-352	93.60 ± 1.56a	67.00 ± 3.56a	57.49 ± 3.34a	44.17 ± 6.47a	30.51 ± 3.20b	20.03 ± 2.09b

Each value is a mean ± standard deviation (SD) of three replicates. Mean followed by the same letter in the column are not significantly different at P < 0.05 (Tukey's-test)

3.4. Seed germination and seedling growth

Residues effect of *P. nigrum* powder and cultivar on seed germination and seedling growth was studied (Table 4). The highest germination percentages of 88.65% and 96.00% were recorded for the treated cultivars SHW-10 and SHY-162 samples, respectively, after 6 months of storage, while the lowest germination percentage was 45.72% and 48.67% after 6 storage months for the untreated cultivars THW-310 and THY-352 samples, respectively. The treated samples showed no visual abnormalities in the morphology of the plants and they appeared healthy for all cultivars after 6 storage months, in contrast to those of the untreated samples. Higher germination percentage recorded in the treated samples in all cultivars of maize after 6 months of storage could be due to the strong and positive correlation between the percentages of grain damage and germination capacity in which damaged grains are low in germination capacity.

Table 4. Effect of *Piper nigrum* powder and cultivar on seed germination and seedling growth of maize

Cultivar	Germination (%)	Radical (cm)	Plumule (cm)
Control	65.02 ± 1.12d	3.35 ± 0.15c	2.27 ± 0.20c
SHW-10	88.65 ± 1.06b	4.86 ± 0.14b	3.56 ± 0.87b
Control	45.72 ± 1.73e	2.15 ± 0.45d	1.07 ± 0.97d
THW-310	60.94 ± 1.65d	3.63 ± 0.22c	2.53 ± 0.31c
Control	77.25 ± 1.67c	4.79 ± 0.57b	3.75 ± 0.11b
SHY-162	96.00 ± 1.73a	6.88 ± 0.52a	5.73 ± 0.12a
Control	48.67 ± 1.87e	2.50 ± 0.27d	1.43 ± 0.26d
THY-352	65.85 ± 1.49d	3.98 ± 0.31c	2.84 ± 0.24c

Each value is a mean ± standard deviation (SD) of three replicates. Mean followed by the same letter in the column are not significantly different at P < 0.05 (Tukey's-test)

4. Discussion

Based on the susceptibility index values, the *S. zeamais* infestation was affected by maize cultivar. It was also observed that cultivars THW-310 and THY-352 were most preferred while cultivars SHY-162 and SHW-10 were the least preferred by *S. zeamais*. This may be due to the grain characteristics rendering the weevil more toward a particular maize cultivar. These findings are in agreement with Bhanderi *et al.* [13] screened 12 maize genotypes and reported that the susceptibility index varied from 9.00 to 15.33 in different genotypes. A similar study was conducted by Astuti *et al.* [14] reported that susceptibility index values varied from resistant to moderately resistant in six examined maize cultivars.

Differences in susceptibility levels between these cultivars might be explained by the differences in the amino acid content, but are unlikely to be the main explanation. Hence, the more susceptible cultivars THW-310 and THY-352 might have higher levels of valine and isolencine compared to their less susceptible cultivars SHW-10 and SHY-162. In an investigation by Hedin *et al.* [15], it was found that free amino acids were more important than all other factors evaluated in determining the resistance of maize cultivars to *Spodoptera frugiperda* larvae, while there is a positive correlation between susceptibility to insects and both protein content and total amino acids in different maize cultivars, as reported by Narang *et al.* [16]. Similar observations of a relationship between higher levels of amino acids in maize cultivars and significantly lower infestation with aphids was also reported by Faria *et al.* [17].

Another important aspect of this study is the evaluation of insecticidal property of *P. nigrum* powder to protect stored maize from infection by weevil. Results showed highly significant differences with *S. zeamais* mortality rate among the cultivars treated with *P. nigrum* powder for storage periods of up to 6 months. Similar observation on efficacy of *P. nigrum* powder on *S. zeamais* and *Sitophilus oryzae* was reported by Choden *et al.* [18], Ismail [19]. Damage to grains was minimal in the treated samples after 6 months of storage compared untreated samples in all cultivars. These significant variations in the cultivars germination show that the *P. nigrum* powder was significantly effective in protecting maize against weevil invasion for up to six months' storage. This was further confirmed by Santos *et al.* [20] reported that infection of maize seeds with *S. zeamais* decreased germination and quality of seeds. Similar trends were reported by Emeribe *et al.* [21] powder of *P. nigrum* at 0.4, 0.3 and 0.2 mL/g were significantly ($P \leq 0.05$) more effective in the control of *S. zeamais* in storage than 0.1 mL/g within 12-48 hours and 12-72 hours, respectively, with no damage to maize grain compared to the control group.

5. Conclusion

Maize susceptibility index values for the four tested cultivars, showed that two cultivars were more susceptible to *S. zeamais*, and that the levels of some amino acids were higher than the other two cultivars which showed a relatively low infestation. *P. nigrum* powder revealed good efficacy against *S. zeamais*, without negatively affecting germination percentage and seedling growth of maize. The comparison of results noted that the *P. nigrum* powder was significantly effective in protecting maize against weevil invasion for up to six months' storage. Therefore, it is recommended to use it to enhance the infection resistance and increase maize storage safety.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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