

Research Article

Toxicity of diatomaceous earth on seed weevil, *Sitophilus oryzae* L. and its effect on agro-morphological characters of maize seeds

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Abstract

Sitophilus oryzae L. (Curculionidae; Coleoptera) is considered to be a serious internal feeder of stored cereals. The use of insecticides results in the development of resistance among the pests and residues in the produce. Diatomaceous Earth (DE) is from a natural source, environment-friendly, safe to humans and natural enemies. In addition, it is highly effective against a wide range of stored pest species and has no toxic residues on the treated seeds. The promising alternative to synthetic insecticides is the application of DE in storage pest management under physical control. With this background, the present study was aimed to find the efficacy of DE against rice weevil, *S. oryzae* L. and their effect on the agro-morphological characters of maize (*Zea mays* L.) seeds. Contact toxicity bioassays were carried out with different concentrations of DE against *S. oryzae*. The results of the bioassay studies revealed LD₅₀ at the concentration of 1.27 mg/100 gm of maize seeds. Further, 100 per cent mortality was achieved at the dose of 15 mg/100 gm of maize seeds within six days of exposure. The effect of DE on the germination provided a significant increase in germinability of maize seeds (LD₅₀= 94%, LD₉₅= 98% and control= 96%). DE at the concentration of LD₉₅ had a beneficial effect on the seedling parameters, especially germination% (98%) and seedling length (53.02 cm) of maize. The present study concluded that DE could be effectively utilised as an alternative management tool to chemical insecticides in the management of rice weevil under storage conditions.

Keywords: Agro-morphological characters, Diatomaceous earth, Maize, Seeds, *Sitophilus oryzae*, Toxicity

INTRODUCTION

Diatomaceous earth (DE) is inert dust with amorphous silica as a major component and is made up of fossilised diatoms. The dust colour varies from white to red. Another major component present in the dust is calcium in addition to minor elements like sodium, phosphorous, zinc etc. (Subramanyam and Roesli, 2000). It is an odourless, non-inflammable inert material with pH vary-

ing from slightly acidic to alkaline (4.4-9) (Korunic, 1997). The average size of DE particles ranges from 2.5 to 30 microns. It is said that DE possesses entomotoxic activity because of amorphous silica in uniform size (Korunic, 1997). The major advantage of the usage of inert dust for stored pest management is that they are safe for humans and natural enemies. Hence, DE can be used as a natural insecticide for the long-term protection of stored produces (Korunic *et al.*,

2017; Ziaee et al., 2019). The possible mechanism behind the insecticidal activity is that they cause abrasion of the integument, especially the waxy layer by strong adherence onto the body of the insect. Insect mortality is brought about by desiccation (Ebeling, 1971). The cuticular water loss rate (CWLR) was found to be higher in weevils treated with DE than those untreated weevils (Prasantha et al., 2015).

DE is used against various storage pests. Athanassiou et al. (2004) compared the effect of DE on the adults of *Sitophilus oryzae* and *Tribolium confusum* where adult mortality pattern remained same for the three different concentrations i.e., 0.75, 1, and 1.5 g of DE/kg of grain. Islam et al. (2010) investigated the effects of Protect-It® and SilicoSec® formulations of DE against *Callosobruchus maculatus* and *S. oryzae*. Protect-It® was more effective against *C. maculatus*, whereas SilicoSec® was more effective against *S. oryzae*. Badii et al. (2014) reported that efficacy of DE altered with a change in RH (Relative Humidity). It was found that DE was more effective against *C. maculatus* at 50 % RH than at 80 % RH. Modified formulations of DE were found to be more effective than original DE. Modified DE formulations like Al-DE and Ca-DE had a more toxic effect on the adults of *Sitophilus granarius* in wheat kernels (El-Aziz and El-Ghany, 2018).

Commercial formulations available in the market are Dryacide, Dicalite, Diacide, DiaFil, Insecolo, Insectigone, Insecto, Kenite, Melocide, Organic Plus, Perma-Guard, Protect-It, SilicoSec, Shellshock, etc. (Subramanyam and Roesli, 2000). The insect species that are more subtle to DE belongs to the genus *Cryptolestes* whereas the most tolerant insects belong to the genus *Prostephanus*. In addition, *Oryzaephilus* is said to be less sensitive, *Sitophilus* as less tolerant and *Tribolium* and *Rhyzopertha* as the most resistant ones to DE (Maceljski and Korunic, 1972; Desmarchelier and Dines 1987; Fields and Muir, 1996; Korunic et al., 1997; Korunic and Fields, 1998, 2006).

Cereals are grown to a larger extent to meet out the food demand of humans. Among them, maize (*Zea mays*) is considered to be a vital crop as it serves as human food and livestock feed. It is grown worldwide and plays a prominent role in the coarse grain trade. According to the Ministry of Agriculture & Farmers welfare, Government of India, 2018-19 maize production and productivity are 9.18 m ha, 27.23 MT and 2965 kg ha⁻¹, respectively. Maize plays a prominent role in coarse grain trade worldwide. The major constraint with maize cultivation is post-harvest loss which accounts for significant yield loss in terms of both production and productivity. Both biotic and abiotic factors bring about post-harvest losses. Storage insect pests play a major role in imposing damage to the maize seeds. More than 37 species of insects inflict damage in storage (Tadese,

1997). Among them, *S. oryzae* L. causes more economic damage both qualitatively and quantitatively. The use of chemicals to control the pest, led to harmful effects in the environment and resistance development in insects. In this case, DE can be considered as an alternative to chemical insecticides, mainly in the storage environment. If preservation of germination is considered a primary factor in storage, then DE can be chosen to enhance or preserve the germinability of the stored seeds (Omobowale and Akomolafe, 2021). DE can be a preferred seed protectant as a preventive measure rather than a curative one. The current study was aimed to study the toxic effect of diatomaceous earth against rice weevil, *S. oryzae* and its effect on agro-morphological characters of maize seeds.

MATERIALS AND METHODS

Rearing of *Sitophilus oryzae*

The weevils of *S. oryzae* were collected from the infested cereal produces from storage godowns at the Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore, for mass culturing. The weevils were reared on maize grains at room temperature of 27 ± 3°C. Subculturing was done at 15 to 20 days intervals to maintain a continuous supply of insects. Uniform aged adults of one week old were used for bioassay studies. The study material of diatomaceous earth was purchased from SGP industries, Bikaner, India. DE is mainly composed of amorphous silica, alumina and iron oxide.

Toxicity assessment of DE against *S. oryzae*

Bioassay was performed in a small plastic container and 100g of maize seeds were taken in each container. Maize seeds were treated with DE at different concentrations (0.25 mg, 0.5 mg, 0.75 mg, 1 mg, 1.5 mg, 2 mg, 3 mg, 4 mg, 5 mg, 7.5 mg, 10 mg, 12.5 mg and 15 mg) and a control (without any DE treatment) was maintained. Then the containers were shaken manually for approximately 1min to achieve equal distribution (Subramanyam and Roesli, 2000). Fifteen pairs of adults were introduced into each container. Four replications were maintained for each treatment in a completely randomized design. Experiments were carried out at 27 ± 3°C, 70% RH. Insect mortality (lack of locomotion and or response to repeated probing) was recorded at 24 h intervals for seven days. The corrected mortality was worked out by the formula given by Abbott. The observations on the percent mortality were subjected to probit analysis and LD₅₀ value was worked out.

$$\text{Corrected (\%) mortality} = (X - Y) / (100 - X) \times 100 \quad \text{.....Eq. 1}$$

X = Percentage mortality in DE treated treatments.

Y = Percentage mortality in the untreated check.

Seed germination assessment

Seed germination studies were carried out by following the methodology given by ISTA (International Seed Testing Association) and Govindaraju et al. (2020). Maize seeds were sterilised with 10% sodium hypochlorite solution. The seeds were washed and treated with two different concentrations of DE solutions (LC₅₀ and LC₉₅). A control was maintained by soaking the seeds in water. Twenty-five seeds from each treatment were taken and kept on the germination sheet in equidistant manner. Then, they were rolled and tightened using rubber band. Each treatment was replicated seven times. At the end of 7 days, the seedling parameters like shoot length (cm), root length (cm), dry matter production (g), germination percentage (%) and vigour index were recorded.

Germination percentage (%) = Number of strongly Germinated seeds / Total number of seeds x 100

Vigour index = Germination (%) x Seedling length
..... Eq. 2

Statistical analysis

The data were analyzed by completely randomized design (CRD) using SPSS statistical software. Probit regression analysis was carried out by Finney's method (1971). Analysis of variance (ANOVA) was done to determine whether significant difference exists between treatments.

RESULTS AND DISCUSSION

Toxicity assessment against *S. oryzae*

The results of the bioassay studies revealed the entomotoxic ability of diatomaceous earth against *S. oryzae*. Probit regression analysis displayed LD₅₀ as 1.27 mg/100 g and LD₉₅ as 86.11 mg/100 g (Table 1.). Calculated chi-square value was less than the tabular value which indicated that the data perfectly fit into the probit model. 1.27 mg of DE was able to kill 50% of the insect population in 100 g of seeds within a period of 7 days. These results are in accordance with the findings of Athanassiou et al. (2004, 2005). They have reported that DE at the dose of 1 and 1.5 g/kg resulted in higher mortality in the adults of *S. oryzae* and *T. confusum*. Fig.1. shows the light microscopic images of DE treated and untreated rice weevil. DE adheres onto the surface

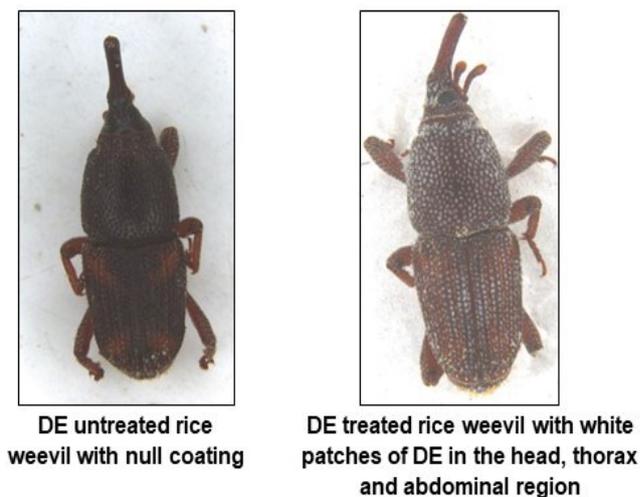


Fig. 1. Difference in DE treated and untreated insects

of rice weevil when it comes in contact with DE treated seeds. By adhering to the integument, DE gets coated on the whole body especially on the head, thorax, abdomen and its appendages. Similarly, the effect of commercial formulation of amorphous DE (Fossil Shield®) on confused flour beetle, granary weevil, mealworm and Indian meal moth was higher at the dose of 2 and 4 g/m² and caused more lethal effects to adults than late larval stages (2-3 weeks old). In general, DE on contact with insects' cuticle causes disruption and dehydration (Mewis and Ulrichs, 2000). Erturk et al. (2020) founded that wettable powder formulation of DE - Detech® was highly working against *S. oryzae* at the concentration of 2 g/m². The present results showed LD₅₀ value was ten times lower than the previous studies indicating that DE was more toxic at low dose i.e., high insecticidal efficacy.

Toxicity pattern of DE against *S. oryzae*

The cumulative pattern of mortality of DE at different concentrations against *S. oryzae* in maize seeds is shown in table 2. Among the various doses used, higher mortality of 80% was obtained at 7.5 mg/100 g on the 7th day of exposure. On 1st day after release (DAR), more insect death (6.67%) was seen at the concentration of 3 mg/100 g with the mortality of 20%, 40% and 60% in the subsequent days. At the highest dose of 15 mg/100 g, cent percent mortality was achieved within six days of exposure. However, higher mortality at lower dose of 0.25 mg/100 g was only 26.67%. The results revealed that insecticidal activity increased with con-

Table 1. Probit regression analysis of mortality data of DE against *S. oryzae*

S. No.	Particulars	LD ₅₀ mg/100 g of seeds (95 % fiducial limits)	LD ₉₅ mg/100 g of seeds (95 % fiducial limits)	Slope	χ ² *	Degrees of freedom
1.	DE	1.27 (0.74-2.16)	86.11 (10.84-683.96)	1.011	0.147	6

* - Calculated χ² value was less than the tabular value (p=0.05), indicating that the data fit the probit model

Table 2. Toxicity pattern of DE against *S. oryzae*

S. No.	Dose/ 100g	No. of insects used	% Mortality (Cumulative mortality)						
			1 day	2 days	3 days	4 days	5 days	6 days	7 days
1.	0.25 mg	30	0.00 ^c ±0.00	0.00 ^f ±0.00	3.33 ^g ±0.05	6.67 ^g ±0.05	13.33 ^h ±0.14	20.00 ^h ±0.14	26.67 ^k ±0.36
2.	0.5 mg	30	0.00 ^c ±0.00	3.33 ^g ±0.03	6.67 ^g ±0.01	13.33 ^f ±0.03	20.00 ^h ±0.25	26.67 ^h ±0.34	33.33 ^h ±0.12
3.	0.75 mg	30	0.00 ^c ±0.00	3.33 ^g ±0.03	6.67 ^g ±0.06	13.33 ^f ±0.07	20.00 ^h ±0.18	26.67 ^h ±0.41	40.00 ^h ±0.54
4.	1 mg	30	0.00 ^c ±0.00	3.33 ^g ±0.02	6.67 ^g ±0.08	13.33 ^f ±0.10	20.00 ^h ±0.04	26.67 ^h ±0.29	46.67 ^h ±0.67
5.	1.5 mg	30	3.33 ^b ±0.01	6.67 ^d ±0.09	13.33 ^d ±0.13	20.00 ^e ±0.31	26.67 ^h ±0.26	33.33 ^g ±0.50	53.33 ^g ±0.47
6.	2 mg	30	3.33 ^b ±0.01	6.67 ^d ±0.06	13.33 ^d ±0.12	20.00 ^e ±0.16	33.33 ^g ±0.08	40.00 ^f ±0.46	60.00 ^f ±0.93
7.	3 mg	30	6.67 ^a ±0.01	13.33 ^b ±0.18	20.00 ^b ±0.01	26.67 ^c ±0.15	40.00 ^e ±0.60	46.67 ^d ±0.24	66.67 ^e ±0.64
8.	4 mg	30	3.33 ^b ±0.03	10.00 ^c ±0.08	16.67 ^c ±0.27	23.33 ^d ±0.27	36.67 ^f ±0.50	43.33 ^e ±0.68	66.67 ^e ±0.27
9.	5 mg	30	6.67 ^a ±0.10	13.33 ^b ±0.12	20.00 ^b ±0.28	26.67 ^c ±0.26	40.00 ^e ±0.67	46.67 ^d ±0.68	73.33 ^d ±0.49
10.	7.5 mg	30	6.67 ^a ±0.07	13.33 ^b ±0.02	20.00 ^b ±0.22	26.67 ^c ±0.29	43.33 ^d ±0.41	50.00 ^c ±0.26	80.00 ^c ±1.24
11.	10 mg	30	6.67 ^a ±0.03	13.33 ^b ±0.13	20.00 ^b ±0.18	33.33 ^b ±0.00	46.67 ^c ±0.11	66.67 ^b ±0.09	86.67 ^b ±0.19
12.	12.5 mg	30	6.67 ^a ±0.07	20.00 ^a ±0.24	33.33 ^a ±0.05	46.67 ^a ±0.24	73.33 ^b ±0.67	100.00 ^a ±0.05	100.00 ^a ±0.41
13.	15 mg	30	6.67 ^a ±0.06	20.00 ^a ±0.14	33.33 ^a ±0.01	46.67 ^a ±0.49	80.00 ^a ±1.28	100.00 ^a ±0.23	100.00 ^a ±0.41
14.	Control	30	0.00 ^c ±0.00	0.00 ^f ±0.00	0.00 ^g ±0.00	0.00 ^h ±0.00	0.00 ^k ±0.00	0.00 ^l ±0.00	0.00 ^l ±0.00
		SEd	0.0723	0.1256	0.1544	0.2007	0.4762	0.6060	0.9301
		CD (0.05)	0.1459	0.2535	0.3117	0.4051	0.9612	1.2233	1.8775

Means ± SE within a column followed by the same letter is not significantly different from each other at 5% level of significance (LSD)



Fig. 2. Difference in germination of seeds in different treatments (DE-LD₅₀, DE-LD₉₅, Control)

centration and time of exposure of dusts with the insects. By enhancing the formulations of DE (i.e., DE + bitterbarkomycin, DE + Abamectin) higher toxicity can be achieved at very lower doses (≤ 100 ppm). At the same time, DE can be effectively included in an integrated storage pest management programme (Shah and Khan, 2014). DE can be effectually combined with insecticide Spinosad and *Trichoderma harzianum* to protect wheat grains from rice weevil (Gad et al., 2020). Diatomaceous earth has also been found to be compatible with the fungus *Metarhizium anisopliae* used against *S. oryzae* (Athanasios et al., 2008).

Effect of DE on agro-morphological characters of maize seeds

The germination studies of maize seeds by roll towel method confirmed the significant differences among the treatments used (LD₅₀, LD₉₅ and control). The observed seedling parameters such as germination%, root length, shoot length, seedling length, vigour index and dry matter production are listed in table 3. Germination per cent was higher (98%) in LD₉₅ concentration, whereas it was only 94% in LD₅₀ concentration. Thus,

% germination differed significantly @ 0.05 level of significance in the two different concentrations. On taking seedling length (Shoot length + Root length) into consideration, it was higher (53.02 cm) in LD₉₅ concentration when compared with control (46.99 cm) and LD₅₀ concentration (47.37 cm) (Fig.2.). Vigour index was maximum (5195.96) at LD₉₅ concentration whereas it was lower in untreated control (4511.04). Diatomaceous Earth is rich in minerals like silica, alumina and iron oxide and considered beneficial for plant growth that enhances seedling relative growth rate, vigour and seedling cotyledon growth (Sun et al., 2021). By taking this into consideration, the current results showed higher seedling growth rates in LD₉₅ concentration, indicating that amorphous silica has been absorbed by the maize seeds and thus actively involved in the germination process. Similarly, higher dry matter production was noticed in LD₉₅ treatment (2.6 g) than LD₅₀ concentration and (2.5 and 2.1 g, respectively).

Further, DE is mainly composed of amorphous silica and it can be used as a supplement to silica in the fertilization of crops for biotic and abiotic stresses. Pati et al. (2016) proved that the application of DE enhanced

Table 3. Effect of DE on germination and seedling parameters in maize (7th day)

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Vigor index	Initial fresh weight (g)	After dry weight (g)
Control	96 ^{ab} ±0.94	21.30 ^c ±0.06	25.69 ^b ±0.22	46.99 ^b ± 0.43	4511.04	14.2 ^c ±0.03	2.1 ^c ±0.01
DE LD ₅₀	94 ^b ±1.11	22.29 ^b ±0.24	25.08 ^c ±0.13	47.37 ^b ± 0.20	4547.52	16.2 ^b ±0.17	2.5 ^b ±0.02
DE LD ₉₅	98 ^a ±0.18	26.26 ^a ±0.21	26.75 ^a ±0.09	53.02 ^a ± 0.63	5195.96	18 ^a ±0.20	2.6 ^a ±0.03
SEd	1.8389	0.2650	0.2182	0.6444		0.2159	0.0294
CD (0.05)	4.0066	0.5775	0.4755	1.4041		0.4705	0.0640

Means ± SE within a column followed by the same letter is not significantly different from each other at 5% level of significance (LSD)

the development and uptake of nutrients in field crops. Similarly, Mills-Ibifofori *et al.* (2019). revealed that DE supplementation in ornamentals resulted in positive effects against stress tolerance in flower and leaf features development. The earlier findings are in accordance with the present results. Therefore, treating DE in storage acts as an effective protectant against storage pests and as a potential supplement to silicon fertilizer which may be considered as an added advantage.

Conclusion

Contact toxicity studies of DE on *S. oryzae* revealed LD₅₀ at the concentration of 1.27 mg/100 g and LD₉₅ at the concentration of 86.11 mg/100 g. Among the treatments, LD₉₅ was more effective in the case of both entomotoxic and germination studies. At LD₉₅ concentration, almost 95% of the weevils were killed, thus making it an effective dose. Maximum mortality could be obtained within 5 days of exposure and insect mortality increased with an increase in dosage and period of exposure. In addition to toxicity, DE positively affected the agro-morphological characters (shoot length, root length, germination percentage, dry matter production and seedling vigour) of maize seeds. Thus, DE can be effectively deployed in the field of storage as a potent seed protectant against major storage pests like *S. oryzae*.

Conflict of interest

The authors declare that they have no conflict of interest.

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