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SHORT-TERM, MID-TERM AND LONG-TERM EFFECTIVENESS OF SilicoSec® AGAINST COWPEA WEEVIL

SUMMARY

The insecticidal efficacy of SilicoSec®; a formulation of diatomaceous earth (DE); was evaluated against cowpea weevil (*Callosobruchus maculatus*) in cowpea seed at three storage periods: 0, 3 and 6 months. The DE formulation was applied at three dose rates: 0.1, 0.5 and 1 g/kg. The experiments carried out at 25 °C and 55% RH and continuous darkness. The mortality of *C. maculatus* adults was recorded 72 h after exposure to DE-treated seeds. After mortality count, all insects (dead and alive) were removed from vials and the vials were kept under same condition for a further 35 days to assess progeny production. For adult's mortality, significant difference was noted between dose rates and mortality increased with increase in dose rate. In contrast, no significant difference was observed between the storage periods and efficacy of DE formulation was not affected by increasing the storage period. At the rate of 1 g/kg, 100% mortality was recorded after 0 to 6 months storage. For progeny production, significant differences were recorded for dose rate, storage period and associated interaction. In all storage periods, highest number of progeny was recorded at 0.1 g/kg and increasing in dose rate significantly decreased the progeny production. The results obtained in this research recommend that SilicoSec® at the rate of 1 g/kg could be used to control cowpea weevil at 6 months storage period.

Keywords: SilicoSec®, Cowpea weevil, Storage period

INTRODUCTION

Callosobruchus maculatus (F.) (Bruchidae: Coleoptera), commonly known as the cowpea weevil is the major insect pest of wild range of stored legume seeds, especially of the cowpea *Vigna unguiculata* (L.) (Mbaiguinam et al., 2006). The insect lays its eggs on the seeds of cowpea, which hatch and produced larvae that bore into the seed cotyledons on which they feed. Infestation may reach 100% within 3–5 months of storage (Khalequzzaman and Rumu, 2010). Control of *C. maculatus* relies heavily on the use of synthetic insecticides and fumigants. Some of the known side effects of using chemical insecticides include increased costs, handling hazards, residue problems and development of tolerance by treated insects. These insecticides are also polluting and potentially dangerous to users. Therefore, the use of fumigants or residual insecticides to

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control storage insect pests should be discouraged. Such concerns have led to the development of non-chemical methods as alternatives for the control of storage pests that infest food commodities (Adebiyi and Tedela, 2012; Shazali et al., 2004).

One of the most promising alternatives is the use of diatomaceous earth (DE). Diatomaceous earths consist of the fossils of phytoplankton (diatoms), which are mainly composed of amorphous hydrated silicates. When insects come into contact with the DE particles, the waxy fat and lipids are adsorbed from their cuticles resulting in water loss, dehydration and death. DEs have extremely low toxicity to mammals and are commonly used in cattle, poultry, and dog feeds to combat internal parasites (Stathers et al., 2004; Athanassiou et al., 2005b).

Since DEs are classified in the category of inert dusts it is postulated that DE remains unaffected and that its insecticidal activity persists for a long period. If this is true, then DE can be used for the long-term protection of stored-products, which is one of the key elements of a stored product integrated pest management (IPM)-based strategy. Thus, although persistence is a "red flag" for conventional insecticides, it is a desirable characteristic for nontoxic compounds, such as DE (Athanassiou et al., 2005b).The aim of present study was to assess the efficacy and persistency of a formulation of DE (SilicoSec®) against cowpea weevil in cowpea seed at different storage periods.

MATERIAL AND METHODS

DE formulation. The DE formulation used was SilicoSec®. SilicoSec® (Biofa GmbH, Münsingen, Germany) is a freshwater formulation of DE. It is composed of 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O. The median particle size is 8-12 μm (Athanassiou et al., 2005a)

Insect cultures. Insects were obtained from laboratory cultures. The cultures had been maintained at the Laboratory of Entomology, Department of Plant Protection, Islamic Azad University, Qaemshahr Branch, for near two years, with no history of exposure to insecticides. *C. maculatus* was reared on cowpea seeds at 27-30°C and 60±5% relative humidity (RH) in incubator and continuous darkness. Cowpea grains were disinfested by storing at -22 °C for 48 h. All adults used in the experiments were 0–24 h old and of mixed sex. For getting 0–24 h old adults, seeds with pupa window were separated and after one day, adults that emerged were collected with a hand-made aspirator.

Bioassays. DE formulation was applied at four dose rates: 0 (control), 0.1, 0.5 and 1 g/kg of cowpea. Assessment of this formulation was carried out after 3 time of storage: 0 month (1 day) as short-term, 3 months as mid-term and 6 months as long-term. For each dose rate-storage period (12 combination in total), one kg of cleaned and disinfested cowpea was weighted and placed in separate cylindrical glass jars (3-L capacity with screwed lids). Appropriate quantity of SilicoSec® was added to each jar. Subsequently, the jars were shaken manually for approximately 5 min to obtain an equal distribution of DE on the entire cowpea seed mass. Immediately, four samples of 50g were taken from each

combination lot as replication. These samples placed in separate glass vials (8 cm height and 5 cm diameter). Then 25 adults were introduced into each glass vial that was covered with muslin cloth to provide sufficient aeration. The vials were then placed in incubators (In-VitroCell ES NU-5841, USA), set at 25 °C and 55% RH, in darkness. The number of dead adults was counted after 72 h exposure to treated seed.

After the mortality count, all adults (dead and alive) were removed from the vials and the vials were left in the incubators at the same conditions for an additional period based on the life cycle of cowpea weevil (28-35 days). The number of emerged adults was then counted in controls and treated vials, and they were introduced to the below formula for the calculation of percentage of progeny reduction (*P.R %*) (Aldryhim 1990):

$$\left[P.R\% = \frac{\text{No. progeny in control - No. progeny in treatment}}{\text{No. progeny in control}} \times 100 \right]$$

Data analysis. Mortality counts were corrected by using Schneider-Orelli's formula (Püntener 1981). To equalize variances, mortality percentage of adults and percentage of reduction in progeny production were transformed using the square root of the arcsine. The morality data were analyzed by the General Linear Model (GLM) of the Statistical Analysis System (SAS Institute 2000), with insect mortality as the response variable and dose rate and storage period as main effects. The same procedure was also followed for progeny production counts. The means were separated by Duncan's multiple range test P=0.05 (Frey, 2010).

RESULTS AND DISCUSSION

In all experiments, the adult's mortality within control group was very low and did not exceed 2%. For adult's mortality, only dose rate was significant (P<0.0001) but storage period and associated interaction (dose rate × exposure time) were not significant. The details are given in Table 1. The corrected mortality percentage of cowpea weevil's adult at the dose rate of 0.1 g/kg was very low (less than 10 %) at all storage period. The mortality rate increased with increase of dose rate and complete mortality was achieved at the highest dose rate at all storage periods (Figure 1).

Table 1- ANOVA parameters for adult mortality of cowpea weevil exposed to SilicoSec®

Source	**df**	**F value**	**P**
Dose rate	2	289.13	<0.0001
Storage period	2	0.20	0.816
Dose rate× Storage period	4	0.83	0.516
Error	27		
Corrected total	35		

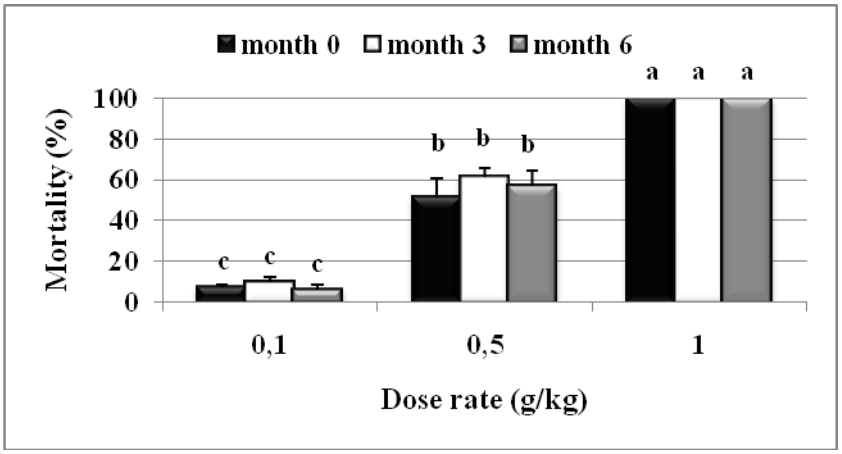

Figure 1 - Mean mortality (±SE) of *C. maculatus* adults exposed to cowpea treated with different dose rate of SilicoSec®.
Means followed by the same letter are not significantly different (Separated by Duncan's multiple range test at $P = 0.05$)

For progeny reduction, all main effects and associated interaction were significant (Table 2). Mean number of adult offspring produced (± SE) per vial in the control group was 178.06 ± 22.21. In all storage periods, the highest number of progeny production was recorded at 0.1 g/kg and increase in dose rate significantly decreased the progeny production. The highest rate of progeny reduction was recorded at 1 g/kg and after 3 months of storage that was 97.13 ± 1.02 % (Figure 2).

The results obtained in the present study demonstrated that SilicoSec® could be effective against cowpea weevil at the rate of 1 g/kg. Also, it could use as a persistent grain protectant because adults mortality was stable during 6 months of storage. Long-term protection of stored products is probably the most important issue to consider when an insect management strategy is planned (Vayias et al., 2006). Also, suppression of the subsequent generations is one of the basic characteristics of a successful grain protectant and considered as equal as parental mortality or even more important (Arthur, 1996; Sakenin Chelav et al., 2013). Our experiments indicated that SilicoSec® significantly reduced the number of emerged adults in F_1 generation in treated cowpea.

Table 2- ANOVA parameters for progeny production of cowpea weevil exposed to SilicoSec®

Source	**df**	**F value**	**P**
Dose rate	2	1230.83	<0.0001
Storage period	2	4.18	0.0261
Dose rate× Storage period	4	5.53	0.0022
Error	27		
Corrected total	35		

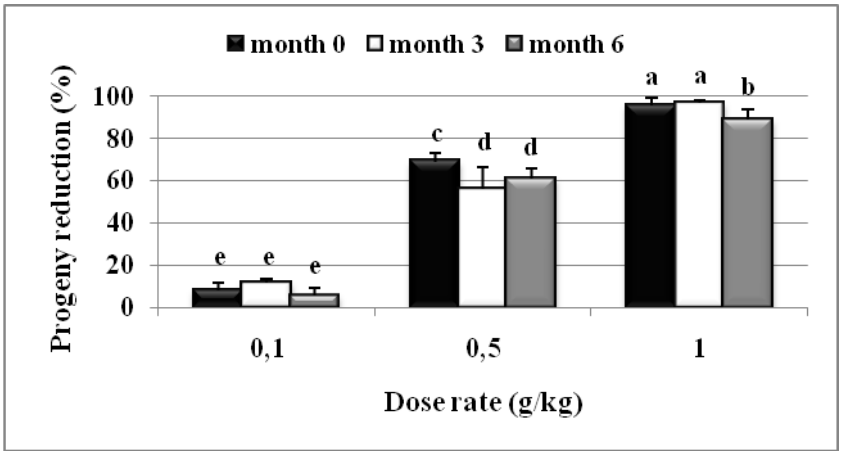

Figure 2 - Mean percentage (±SE) of reduction in progeny production in cowpea weevil exposed to cowpea treated with different dose rate of SilicoSec®. Means followed by the same letter are not significantly different (Separated by Duncan's multiple range test at $P = 0.05$)

Many researchers worked with DEs and according to conditions of experiments (temperature and relative humidity), the population of insect, proficiency of formulation (the medium particle size and percentage of SiO_2 in formulation) and types and varieties of commodities, the obtained results were different (Sakenin Chelav et al., 2013). Efficiency of different formulations of DE against cowpea weevil were reported by many researchers (Rohitha Presentha et al., 2002a,b; Stathers et al., 2004; Islam et al., 2010; Wakil et al., 2010; Shams et al., 2011; Parsaeyan et al., 2012; Tofel et al., 2012; Sakenin Chelav et al., 2013). Wakil et al (2010) indicated that DiaFil 610 (a new formulation of DE) at 30°C and 50% RH and dose rate of 0.8 g/kg caused 100% mortality in adults of *C. maculatus* after 5 days exposure. They recorded minimal progeny production at mentioned condition. Shams et al. (2011) indicated that SilicoSec® in dose rate of 0.5 g/kg caused 95% mortality in the adults of *C. maculatus* 2 days after exposure. In contrast, Tofel et al. (2012) in their investigations demonstrated that mortality of *C. maculatus* adults exposed to SilicoSec® was different in three varieties of cowpea and recorded 100% mortality at the dose rate of 2 g/kg 4 days after exposure. With the same formulation, we recorded complete mortality at the dose rate of 1 g/kg 3 days after exposure. We assume that the difference observed between the results of these two series of tests and our work may be attributed to differences in condition of experiment, commodity cultivars and insect population. The efficacy and persistence of Dryacide® and Protect-It® (two commercially available and enhanced formulations of DE) against cowpea weevil were studied by Stathers et al. (2004). They demonstrated that these two formulations at the recommended label rate (0.3 and 1 g/kg for Protect-It® and Dryacide®, respectively) could protect the cowpea treated seed for a storage period of 6 months. These results confirm our results and suggest that DEs can be used for long-term preservation of stored cowpea.

CONCLUSION

According to the results obtained in this experiment, SilicoSec® can be recommended for control of *C. maculatus* in cowpea at the dose rate of 1 g/kg.

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KRATKOROČNA, SREDNJOROČNA I DUGOROČNA EFIKASNOST SILICOSEC ® PROTIV LUCERKINOG ŽIŽKA

SAŽETAK

Procijenjena je efikasnost insekticida SilicoSec® u obliku diatomejske zemlje (DE), protiv lucerkinog žižka (*Callosobruchus maculatus*), kod sjemena lucerke tokom tri perioda skladištenja: 0, 3 i 6 mjeseci. Formulacija DE je primjenjena u tri doze: 0.1, 0.5 i 1 g/kg. Eksperiment je sproveden na 25°C i 55% RH u konstantnom mraku. Smrtnost odraslih *C. maculatus* zabilježen je 72 sata nakon izlaganja DE-tretiranom sjemenu. Nakon izračunavanja mortaliteta, svi insekti (mrtvi i živi) su uklonjeni iz fiole i fiole su držane pod istim uslovima za još 35 dana radi procjene proizvodnje potomstva. Kod smrtnosti odraslih, značajna razlika zabilježena je između doze i stope mortaliteta koja se povećava sa povećanjem doze. Nasuprot tome, nije uočena značajna razlika između perioda skladištenja, a povećanje perioda skladištenja nije uticalo na efikasnost DE formulacije. Kod doze 1 g/kg, zabilježen je mortalitet od 100% nakon 0 do 6 meseci čuvanja. Za proizvodnju potomstva, značajne razlike su zabilježene kada je riječ o različitim dozama, periodima skladištenja i povezanim interakcijama. U svim periodima skladištenja, najveći broj potomstva zabilježen je na 0,1 g/kg i povećanje doze značajno smanjuje proizvodnju potomstva. Rezultati dobijeni u ovom istraživanju preporučuju da se SilicoSec ® po stopi od 1 g/kg koristiti za kontrolu lucerkinog žižka za period skladištenja od 6 mjeseci.

Ključne riječi: SilicoSec ®, lucerkin žižak, period skladištenja