Efficacy assessment of diatomaceous earth against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on gram at different temperature and relative humidity regimes

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Abstract

The efficacy of diatomaceous earth against *Callosobruchus maculatus* (Coleoptera: Bruchidae) was evaluated on stored gram under laboratory conditions. The bioassay was conducted at 25 and 30°C in combination with 50 and 60% r.h. Diatomaceous earth (DE) formulation (Diafil 610), at the dose rates of 200, 400, 600 and 800 ppm was admixed with gram grains. Fifty unsexed adults of *C. maculatus* were released in each jar and treatments replicated thrice. Mortality data was recorded after 2, 3 and 5 days of exposure intervals and after every count the dead individuals were removed, and the commodity was maintained for an additional period of 25 d, in order to record the emergence of F1 adults. The results showed that all treatments were highly effective against the bruchids; however, the highest mortality (100%) was observed at 30°C and 50% relative humidity at 800 ppm of DE with minimal progeny production.

Keywords: Diatomaceous earth, *Callosobruchus maculatus*, Temperature, relative humidity, Gram

1. Introduction

The pulses are considered to be an important source for fulfilling the protein needs for low income groups of the population in many regions of Asia. *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a primary grain beetle (Cherry et al., 2007) which is widely distributed throughout the world and also causes considerable damage in stored gram. The losses in seed weight due to this beetle was estimated at 55-60% (Gujar and Yadav, 1978) and also 45.5-66.3% in protein content in India; similarly, 60% losses due to *C. maculatus* were reported in Nigeria during three months of cowpea storage (Caswell, 1981). Moreover, weight losses up to 30% occurred after six month of storage which made 70% of the grain unfit for human consumption (Singh and Jackai, 1985). Also, in Benin, 100% losses was inflicted due to *C. maculatus* and *Bruchidus atrolineatua* (F.) after few months of storage (Kossou et al., 2001). This situation makes necessary the application of control measures, in order to minimize the losses caused by this pest.

Admixture of diatomaceous earth (DE) formulations with dry grain is an excellent method of protecting stored products as they can be used as replacement to traditional chemicals. They have very low mammalian toxicity, are non reactive, leave no residues on grains (Cook and Armitage, 2000); control the insecticide resistant pests and long-lasting (Vayias et al., 2006). DEs absorb the wax layers of the insect's cuticle, causing desiccation and mortality due to water loss (Subramanyam and Roesli, 2000; Korunic and Fields, 2006). There are several commercially available DE formulations which have been successfully evaluated as stored grain protectants against a wide range of insect species (Korunic, 1998; Arthur, 2000; Fields and Korunic, 2000; Subramanyam and Roesli, 2000; Athanassiou et al., 2003; 2004; 2005; Stathers et al., 2008).

In Australia, Germany, USA, UK and other European countries DEs are used with success against different stored grain insect pests; however, in the Asian sub-continent DEs are not in use for stored-product protection. The purpose of this study is to evaluate the efficacy of DE at different concentrations, temperature and relative humidity levels against *C. maculatus*. The capacity for progeny production in the treated substrate was also recorded.

2. Materials and methods

2.1. Test insect

Callosobruchus maculatus was reared in the IPM laboratory in the Department of Agric. Entomology, University of Agriculture, Faisalabad (Pakistan) on the mung beans (*Vigna radiata* L.) in plastic jars, at 28 ± 2°C and 55-60% r.h. with photoperiod 14:10 L:D.

2.2. DE formulation

The DE used was DiaFil 610 (Celite Corporation, USA), which is a white fresh water DE containing 89% amorphous silicon dioxide, 4.0% Al_2O_3, 1.7% Fe_2O_3, 1.4% CaO, less than 1% of MgO and K_2O and 3% moisture. The median particle size is 10 microns, specific gravity is 2.2, surface area is 35.7 m2/g, pH is 8 and crystalline silica is >0.1%.

2.3. Bioassay

The study was conducted under two different temperatures (25 and 30°C) in combination with 50 and 60% r.h. The dose rates applied were 200, 400, 600 and 800 ppm. DE was mixed thoroughly for 2-3 minutes with the gram grains separately. Then, for each dose, the jars were kept undisturbed for 30 minutes so as to allow the dust to settle down. A lot of 150 grams of treated grains was divided into three parts of 50 g for each dose rate, so 15 cups (replicates) including untreated control were prepared for each set of temperature and relative humidity. Fifty unsexed adults of *C. maculatus* per treatment were placed into each jar and the opening of the jars was tightly closed with muslin cloth to avoid beetle escaping. The mortality data were recorded after three exposure intervals (2, 3 and 5 d) and after every count the dead adults were removed from the jars. After the last count the jars were kept for 25 d more in order to record progeny production of *C. maculatus*.

2.4. Statistical analysis

The mortality of *C. maculatus* was corrected by using Abbott's (1925) formula, and then the mortality data was subjected to the statistical analysis (MINITAB) and the means were separated by Tukey-Kramer test at $P = 0.05$. The control mortality was not included in the analysis as there was less than 3% mortality in the control jars. The same procedure was followed for the analysis of progeny data, but in this case control progeny was also included.

3. Results

3.1. Mortality of C. maculatus

The analysis of variance showed that most of the main effects were significant; however, their interactions were non significant (Table 1). The highest beetle mortality after 2 d of exposure (72.6%) was recorded where the highest dose rate of DE was applied at 30°C and 50% r.h. (Fig. 1). Similarly, after 3 d of exposure at the same conditions, the highest dose of DE gave 92.6% mortality (Fig. 2). The DE treated grains which were kept for 5 d exhibited 81.1% mortality at 25°C and 60% r.h. (Fig. 3) which was lower than the mortality at 25°C and 50% r.h. The highest mortality (100%) was recorded at 30°C and 50% r.h. and the lowest (91.7%) at 30°C and 60% of r.h.

Table 1 ANOVA for main effects and their associated interactions for mortality of *C. maculatus* after 2, 3 and 5 d of exposure of Diafil 610 (total df = 47; blank spaces in columns of *P* values are non-significant).

Parameters	**df**	**2 d**		**3 d**		**5 d**	
Temperature	**1**	**42.71**	**<0.001**	**18.28**	**<0.001**	**3.35**	**0.07**
Relative humidity	1	86.63	<0.001	13.82	0.001	3.90	0.05
Dose	3	137.59	<0.001	35.29	<0.001	6.12	0.002
Temperature x relative humidity	1	0.63	0.43	0.12	0.73	0.00	0.98
Temperature x dose	3	1.32	0.28	1.03	0.39	0.08	0.96
Relative humidity x dose	3	0.38	0.76	0.02	0.99	0.01	0.99
Temperature x relative humidity x dose	3	0.08	0.97	0.03	0.99	0.00	1.00
Error	32	-	-	-	-	-	-

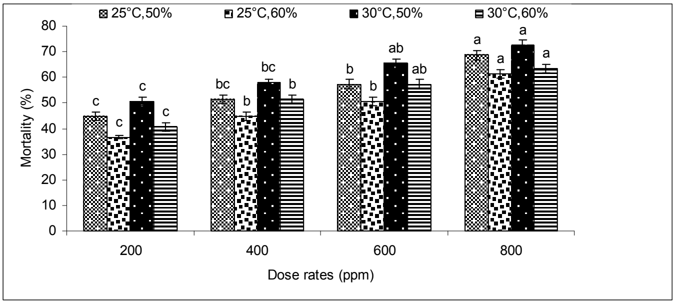

Figure 1 Means comparison of the data regarding the mortality (% ± SE) of *Callosobruchus maculatus* at different dose rates of diatomaceous earth in stored gram after 2 d of exposure (means followed by the same letters are not significantly different with each other in the temperature and relative humidity).

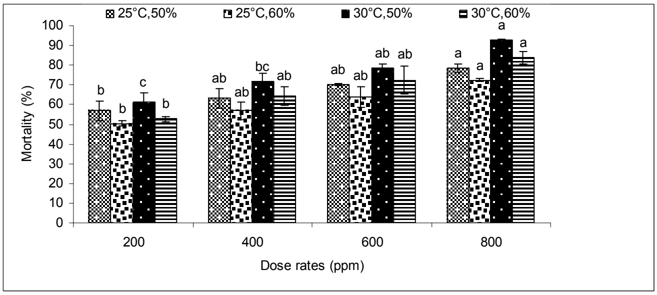

Figure 2 Means comparison of the data regarding the mortality (% ± SE) of *Callosobruchus maculatus* at different dose rates of diatomaceous earth in stored gram after 3 d of exposure (means followed by the same letters are not significantly different with each other in the temperature and relative humidity).

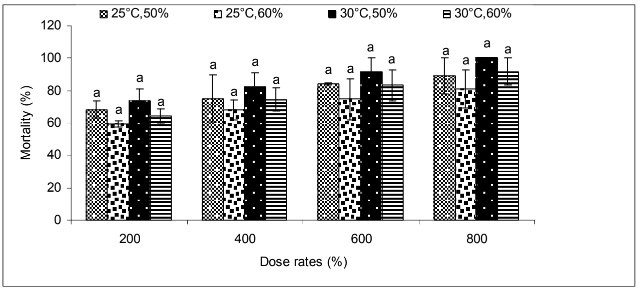

Figure 3 Means comparison of the data regarding the mortality (% ± SE) of *Callosobruchus maculatus* at different dose rates of diatomaceous earth in stored gram after 5 d of exposure (means followed by the same letters are not significantly different with each other in the temperature and relative humidity).

3.2. Production of F1

The analysis of variance showed that all the main effects were significant (temperature $F_{1,59}$ = 685.9, *P* <0.001; relative humidity $F_{1,59}$ = 182.4, *P* <0.001; dose rates $F_{4,59}$ = 1498.6, *P* <0.001) and their interactions were not significant. The maximum numbers of offspring were in the control jars (94.3 adults per jar) which were significantly different from all other treatments (Fig. 4). Maximum dose (800 ppm) of DE showed minimum development of the bruchids (5.3 adults per jar) as compared to minimum dose rate of DE.

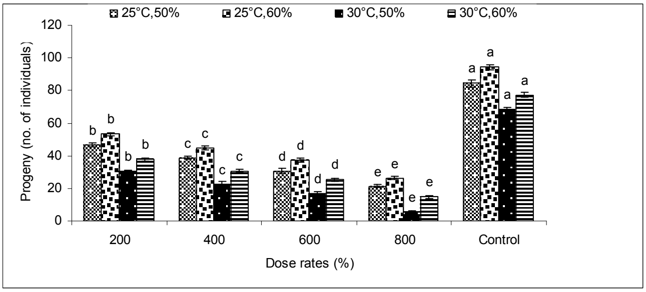

Figure 4 Means comparison of the data regarding the production of progeny (number of live adults ± SE per jar) of *Callosobruchus maculatus* at different dose rates of diatomaceous earth in stored gram (means followed by the same letters are not significantly different with each other in the temperature and relative humidity).

4. Discussion

The diatomaceous earths have proved successful for the management of different stored grain insect pests and can be replaced with the conventional insecticides (Islam et al., 2009) but their overall efficacy depends upon different factors, such as type and concentration of DE, grain moisture content, temperature, insect species, insect density and type of grain commodity (Korunic, 1997; Rigaux et al. 2001; Fields et al., 2003; Korunic and Fields, 2006). Among these factors the temperature and relative humidity plays an important role in determining the efficacy of DE against stored grain insect pests (White and Loschiavo, 1989). We have concluded in the present study that the DE used (Diafil 610) proved effective at high temperature and low relative humidity for the control of *C. maculatus*. These findings are in agreement with findings from previous studies (Arthur, 2000; Fields and Korunic, 2000; Mewis and Ulrichs, 2001; Vayias and Athanassiou, 2004; Vayias and Stephou, 2009). Generally, water loss in the insect's body may be increased when humidity or moisture is low (Fields and Korunic, 2000; Stathers et al., 2004). However, Akbar et al. (2004) reported that there was no significant effect of relative humidity on the efficacy of DE against the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae).

High temperature enhances the effectiveness of DE (Korunic, 1996; Mewis and Ulrichs, 2001) as mortality is increased with the increase of temperature (Arthur, 2000; Athanassiou et al. 2004). Our results for *C. maculatus* also demonstrated similar findings on the effect of temperature on DE efficacy. The possible reason of higher mortality at higher temperature would be due to the fact that at high temperature the mobility of the insect increases which provide more chance for the attachment of DE with the cuticle (Fields and Korunic, 2000). Also, there is enhanced metabolic activity which eventually increases the loss of water from the body of an insect (Ceruti et al. 2008). However, Athanassiou et al. (2007) showed that there was high mortality of the insect pest at 20°C in comparison with 30°C.

Longer exposure interval suppresses the progeny emergence in the treated substrate provided the dry conditions prevails (Athanassiou et al., 2003; 2005). Also, also the higher dose rate was negatively correlated with the production of F1 of *Sitophilus* spp. (Paula, 2001). The present trial undoubtedly supports the statements of other researchers as there was less progeny at high dose rates and longer exposure intervals.

In storage structures, DEs may prove good alternative to the traditional fumigants and synthetic insecticides, in an effort to provide residue-free commodities for the consumers. The results clearly indicated that the *C. maculatus* was controlled at higher temperature and lower relative humidity with less production of progeny at the higher dose rates of DE. The utmost effort should be focused on storing the grain commodities at higher temperature and lower r.h. combinations in order to benefit for the application of a DE-based strategy.

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18, 265-267.

Akbar, W., Lord, J.C., Nechols, J.R., Howard, R.W., 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97, 273-280.

Arthur, F.H., 2000. Impact of accumulated food on survival of *Tribolium castaneum* on concrete treated with cyfluthrin wettable powder. Journal of Stored Products Research 36, 15-23.

Athanassiou, C.G., Kavallieratos, N.G., Andris, N.S., 2004. Insecticidal effect of three diatomaceous earth formulations against adults of *Sitophilus oryzae* (Coleoptera: Curculinoidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae) on oat, rye and triticale. Journal of Economic Entomology 97, 2160-2167.

Athanassiou, C.G., Kavallieratos, N.G., Peteinatos, G.G., Petrou, S.E., Boukouvala, M.C., Tomanoviae, Z., 2007. Influence of temperature and humidity on insecticidal effect of three diatomaceous earth formulations against larger grain borer (Coleoptera: Bostrychidae). Journal of Economic Entomology 100, 599-603

Athanassiou, C.G., Kavallieratos, N.G., Tsaganou, F.C., Vayias, B.J., Dimizas, C.B., Buchelos, C.Th., 2003. Effect of grain type on the insecticidal efficacy of SilicoSec against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Crop Protection 22, 1141-1147.

Athanassiou, C.G., Vayias, B.J., Dimizas, C.B., Kavallieratos, N.G., Papagregoriou, A.S., Buchelos, C.Th., 2005. Insecticidal efficacy of diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. Journal of Stored Products Research 41, 47-55.

Caswell, G.H., 1981. Damage to stored cowpea in northern part of Nigeria. Samaru Journal of Agricultural Research 1, 11-19.

Ceruti, F.C., Lazzari, S.M.N., Lazzari, F.A., Pinto Junior, A.R., 2008. Efficacy of diatomaceous earth and temperature to control the maize weevil on stored maize. Scientia Agraria Curitiba 9, 73-78.

Cherry, A.J., Abalo, P., Hell, K., Korie, S., 2007. Farm-scale trials to compare the entomopathogenic fungus *Beauveria bassiana* with pirimiphos methyl + deltamethrin and essential oil of lemon grass for protection of stored cowpea against *Callosobruchus maculatus* (Coleoptera: Bruchidae). Annals of Applied Biology 151, 1-10.

Cook, A.D., Armitage, M.D., 2000. Efficacy of a diatomaceous earth agaisnt mite and insect population in small bins of wheat under conditions o flow temperature and high humidity. Pest Management Science 56, 591-596.

Fields, P., Allen, S.Z., McLaughlin, A., Stathers, T., 2003. Standardised testing for diatomaceous earth. In: Credland, P.F., Armitage, D.M., Bell, C.H., Cogan, P.M., Highley, E. (Eds). Advances in Stored Product Protection, Proceedings of Eight International Conference on Stored-Product Protection, 22-26 July 2002, York, UK. CAB International, Wallingford, UK, pp. 779-784.

Fields, P., Korunic, Z., 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. Journal of Stored Products Research 36, 1-13.

Gujar, G.T., Yadav, T.D., 1978. Feeding of *Callosobruchus maculatus* (Fab.) and *Callosobruchus chinensis* L. in green gram. Indian Journal of Entomology 40, 108-112.

Islam, Md. S., Hasan, Md. M., Lei, C., Mucha-Pelzer, T., Mewis, I., Ulrichs, C., 2009. Direct and admixture toxicity of diatomaceous earth and monoterpenoids against the storage pests *Callosobruchusmaculatus* (F.) and *Sitophilus oryzae* (L.). Journal of Pest Science. DOI: 10.1007/s10340-009-0276-7.

Korunic Z., Fields, P.G., Kovacs, M.I.P., Noll, J.S., Lukow, O.M., Demianyk, C.J., Shibley, K.J., 1996. The effect of diatomaceous earth on grain quality. Postharvest Biology and Technology 9, 373-387.

Korunic, Z., 1997. Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioasssays. Journal of Stored Products Research 33, 219-229.

Korunic, Z., Fields, P., 2006. Susceptibility of three species of *Sitophilus* to

diatomaceous earth.: In: Lorini, I., Bacaltchuk, B., Beckel, H., Deckers, D., Sundfeld, E., P., d.S.J., Biagi, J.D., Celaro, J.C., Faroni, L.R.D.A., Bortolini, L.d.O.F., , Sartori, M.R., Elias, M.C., Guedes, R.N.C., da Fonseca, R.G., Scussel, V.M. (Eds), Proceedings of the Ninth International Working Conference on Stored Product Protection, 15-18 October 2006, Campinas, Brazil, Brazilian Post-harvest Association, Campinas, Brazil,

Korunic, Z.,1998. Diatomaceous earths, a group of natural insecticides. Journal of Stored Products Research 34, 87-97.

Kossou, D.K., Gbèhounou, G., Ahanchédé, A., Ahohuendo, B., Bouraïma, Y., van Huis, A., 2001. Indigenous cowpea production and protection practices in Benin. Insect Science and its Application, 21: 123-132.

Mewis, I., Ulrichs, C., 2001. Treatment of rice with diatomaceous earth and effects on the mortality of red flour beetle *Tribolium castaneum* (Herbst). Journal of Pesticide Science 74, 13-16.

Paula, M.C.Z., 2001. Manutencão da qualidade do arroz armazenado: monitoramento e controle de insetos. Curitiba, 74 f. Tese (Doutorado em Entomologia) – Curso de Pós–Graduacão em Entomologia, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Rigaux, M., Haubruge, E., Fields, P.G., 2001. Mechanisms for tolerance to diatomaceous earth between strains of *Tribolium castaneum* (Coleoptera: Tenebrionidae). Entomologia Experimentalis et Applicata 101, 33-39.

Singh, S.R., Jackai, L.E.N., 1985. Insect pests of cowpea in Africa: their life cycle, economic importance and potential for control. In: Singh, S.R. and Rachie, K.O. (Eds). Cowpea Research, Production and Utilization., John Wiley and Sons, Chichester UK, pp. 217-231.

Stathers, T.E., Denniff, M., Golob, P., 2004, The efficacy and persistence of diatomaceous earths admixed with commodity against four tropical stored product beetles. Journal of Stored Products Research 40, 113-123.

Stathers, T.E., Riwa, W., Mvumi, B.M., Mosha, R., Kitandu, L., Mngrara, K., Kaoneka, B., Morris, M., 2008. Can diatomaceous earth have potential as grain protectants for small-holder farmers in sub-sharan Africa? The case of Tanzania. Crop Protection 27, 44-70.

Subramanyam, Bh., Roesli, R., 2000. Inert dusts. In: Subramanyam, Bh., Hagstrum, D.W. [Eds]. Alternatives to Pesticides in Stored-Product IPM, Kluwer Academic Publishers, Boston, USA, pp. 321-380.

Vayias, B.J., Athanassiou, C.G., 2004. Factors affecting efficacy of the diatomaceous earth formulations SilicoSec against adults and larvae of the confused beetle *Tribilium confusum* Du Val (Coleoptera: Tenebrionidae). Crop Protection 23, 565-573.

Vayias, B.J., Athanassiou, C.G., Kavallieratos, N.G., Tsesmeli, C.D., Buchelos, C.T., 2006. Persistence and efficacy of two diatomaceous earth formulations, and a mixture of diatomaceous earth with natural pyrethrum against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) on wheat and maize. Pest Management Science 62, 456-464.

Vayias, B.J., Stephou, V.K., 2009. Factors affecting the insecticidal efficacy of an enhanced diatomaceous earth formulation against three stored-product insect species. Journal of Stored Products Research 45, 226-231.

White, N.D.G., Loschiavo, S.R., 1989. Factors effecting survival of the merchant grain beetle (Coleoptera: Cucujidae) and the confused flour beetle (Coleoptera: Tenebrionidae) exposed to silica aerogel. Journal of Economic Entomology 82, 960-969.