

The efficacy of diatomaceous earth in mixed formulation with other dusts and an insecticide against the pulse beetles, *Callosobruchus chinensis* L. and *Callosobruchus maculatus* (F.)

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Abstract: Effectiveness of diatomaceous earth (DE) and other inert dusts (kaolin powder, paddy husk ash, coal ash, alluvial soil, china clay) and a dust formulation insecticide carbaryl have been tested on the pulse beetles *Callosobruchus chinensis* L. and *C. maculatus* (F.). The bioassay of the dusts was done on adult beetles by mixing them with normal food (lentil and black gram seeds). The LD₅₀ of the combined doses of mixtures for *C. chinensis* have been calculated as 12703.57 and 859.36 ppm for DE+ kaolin powder; 2432.78 and 274.00 ppm for DE+ paddy husk ash; 3430.036 and 426.16 ppm for DE+ coal ash; 12563.47 and 652.29 ppm for DE+ alluvial soil; 2242.81 and 325.76 ppm for DE+ china clay; and 21.33 and 14.45 ppm for DE+ carbaryl after 24 and 48 h after treatment respectively. The LD₅₀ of combined doses of different mixtures for *C. maculatus* have been calculated as 3640.65 and 503.74 ppm for DE+ kaolin powder; 54946.68 and 987.2394 ppm for DE + paddy husk ash; 61029.04 and 3229.436 ppm for DE+ coal ash; 61029 and 4265.599 ppm for DE+ alluvial soil; 4648.786 and 642.278 ppm for DE+ china clay; and 24.12017 and 15.47023 ppm for DE+ carbaryl after 24 and 48 h after treatment respectively. The co-toxicity coefficient has been calculated and all ratios showed synergistic action. The highest co-toxicity coefficient was recorded as 88885.15 and 92107.22 in DE+ carbaryl at 24 and 48 h after treatment for *C. chinensis* and 78615.55 and 86004.88 in DE+ carbaryl at 24 and 48 h after treatment for *C. maculatus*.

Key words: Diatomaceous earth, *Callosobruchus chinensis*, *C. maculatus*, inert dust, carbaryl.

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Introduction

Inert dusts, especially DE dusts and silica gels, are suitable for disinfesting empty storage facilities and for grain treatment (Aldryhim, 1990). Their use is more appealing in view of the widespread development of resistance in stored-product insects to conventional pesticides. Fields (2000) studied that the minimum effective rate of dust needed for suppression of progeny production of bruchid beetles 50% less in mixed form with other dusts than that needed for complete mortality used alone.

Admixture of dust formulations of insecticides with grain is a widely used method of protection against stored product pests and has particular advantages when used to treat small batches for local storage (LaHue, 1978). In some cases insecticides are supplied by manufacturers as dust concentrates for dilution with locally available mineral bases; in other cases formulation is carried out in the user country using local mineral carriers and imported technical grade insecticide. The addition of suitable stabilizing agents is usually necessary to prevent rapid decomposition of the insecticide on the mineral surface, and a small proportion of amorphous silica is sometimes added to dust concentrates as an anti-caking agent.

The integration of non-chemical control methods

can mitigate problems related to residues in food and pest resistance by overuse of these products (Beckel *et al.*, 2004). The manipulation of grain temperature and the use of inert dusts such as diatomaceous earth are examples of promising non-chemical methods for the integrated management of insect pests of stored products (Flinn, 1998; Flinn & Hagstrum, 2002; da Conceição *et al.*, 2012). Diatomaceous earth is derived from sediment diatomaceous alga shell, and when in contact with the insects causes the removal of the wax layer of the cuticle, causing its death by desiccation (Korunic, 1998).

Diatomaceous earth mixed with grain via dusting or spraying, controls most of the pests effectively. This product works on larvae and adult insects, clinging to their bodies as they move on the surface or within the treated grain mass (Alves *et al.*, 2006). Moreover, it presents some advantages such as low toxicity to mammals and environment; it does not leave harmful residues in the treated product; it is effective against insect species resistant to insecticides, and it is persistent and stable at high and low temperatures (Collins, 2006).

However, studies are needed on the toxicity of diatomaceous earth in combination for populations of insect pests of stored products with

different standard of susceptibility to the insecticides currently in use. These studies are important because populations of the same species with different genotypes may show different responses to the same treatment (McKenzie, 1996; Li *et al.*, 2007).

Inert dusts, especially DE dusts and silica gels are suitable for disinfecting empty storage facilities and for grain treatment. Their use is more appealing in view of the widespread development of resistance in stored-product insects to conventional pesticides. On grain, different treatment techniques (treating partial layers) should be explored. Locally available different inert dusts are easily available and initiatives should be given to develop insecticides with these materials. Keeping these in mind the present study was undertaken to find out the toxicity of DE and some other inert dusts on the adult pulse beetles, *C. chinensis* and *C. maculatus*.

Materials and Methods

Test Insects

The pulse beetles *C. chinensis* and *C. maculatus* were collected from Shaheb Bazar, Rajshahi. Mass cultures were maintained in earthen pots and sub-cultures in glass jars (500 ml) or beakers (500 ml) with the food medium in the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. All the equipments were kept in an oven for sterilization, about six hours at 60°C. Lentil (*Lens culinaris* Medic.) and black gram (*Vigna mungo* (L.)) seeds were used as food medium for *C. chinensis* and *C. maculatus* respectively through the experiment. A large number of beetles were thus reared for continuous supply of the newly emerged adults.

Diatomaceous earth and others dusts

SilicoSecs was obtained from Agrinova GmbH (Germany). SilicoSec is a relatively new DE formulation of freshwater origin, and contains approx. 92% SiO₂, 3% Al₂O₃, 1% Fe₂O₃, and 1% Na₂O (McLaughlin, 1994). A dry lump of kaolin was purchased from the market. The lumps were crushed in boiling distilled water and then homogenized. The preparation was left to cool at 29°C. It was then filtered through a 53 µm mesh sieve, a piece of 45 µm fine steel gauze and a piece of cotton fabric (25 µm of fine-knit). The resulting suspension, called "kaolin milk", was left undisturbed for 3 days and protected from dust

with a fine steel gauze. The particulates were recovered by draining of the water and they were placed in the sun to dry. The dried material was crushed in a porcelain mortar and the powder was sifted (53 µm mesh) and stored in a container away from moisture.

Paddy husk and coal ashes were collected from rice mills and brickfields respectively. The ashes were sieved with a fine net (mesh 600) and placed in an incubator for an hour at 60°C to dry up excessive moisture. Alluvial soil was collected from the riverbed of the Padma, Rajshahi. The china clay was procured from the local market. Both soil and clay were powdered in a mortar and pestle and finally meshed and dried. For comparison the insecticidal activity of the inert dusts, a commercial insecticide dust formulation "Sevin 85 SP" of Bayer CropScience was used.

Experiments

For combined treatment DE was mixed in different ratios with kaolin powder, paddy hush, coal ash, alluvial soil, china clay and carbaryl. The ratios were 1:1, 1:5, 1:10, 1:20, 1:50 and 1:100 for kaolin powder, ashes and clays and for carbaryl it was 1:1, 1:2, 1:5 and 1:10. In all cases the unit of DE was 50 ppm excepting for carbaryl which was 5 ppm. The mixed formulations were used for the bioassay on the newly emerged adults of *C. chinensis* and *C. maculatus* with respective food and in each case a control batch was maintained on untreated food. The mortality of the beetles was recorded 24 and 48 h after treatment.

Data analysis

The mortality percentage was corrected using Abbott's formula (Abbott, 1925). Probit analysis was done according to Finney (1947) and Busvine (1971) using a software developed in the Department of Agricultural and Environmental Science, University of Newcastle upon Tyne, UK. Co-toxicity coefficients were calculated following Sun and Johnson (1960) as:

$$\text{Co-toxicity coefficient} = \frac{\text{LD}_{50} \text{ of the toxicant alone}}{\text{LD}_{50} \text{ of the toxicant in the mixture}} \times 100$$

Results and Discussion

The results of LD₅₀, 95% confidence limits, regression equations (Y) and χ^2 of diatomaceous earth in mass ratio mixtures with tested dusts on *C. chinensis* and *C. maculatus* are presented in Table 1.

The LD₅₀ and the co-toxicity coefficient for DE

and dusts has been separated as ratios and are presented in Table 2. The result shows that the LD₅₀ values of diatomaceous earth in mixture were decreased in different ratios as the different dusts were mixed. The synergism was in the order of DE: carbaryl> DE: china clay> DE: paddy hush ash> DE: coal ash> DE: alluvial soil> DE:

kaolin powder at 24 h and DE: carbaryl> DE: paddy hush ash> DE: china clay> DE: coal ash> DE: alluvial soil> DE: kaolin powder at 48 h for *C. chinensis* and the synergism was in the order of DE: carbaryl> DE: kaolin powder> DE: china clay> DE: paddy hush ash> DE: coal ash> DE: alluvial soil at 24 and 48 h for *C. maculatus*.

Table 1. LD₅₀, 95% confidence limits and regression equations of diatomaceous earth in mass ratio mixtures with tested dusts on adult pulse beetles.

Pulse beetles	Diatomaceous earth: Dust	Combined LD50 (ppm)	95% confidence limits		Regression equation	χ^2 (4 df)
			Lower (ppm)	Upper (ppm)		
<i>C. chinensis</i>	24 hours					
	Kaolin powder	12703.57	2380.47	67793.47	Y= 1.960363+ 0.7406656 X	0.3381
	Paddy hush ash	2432.78	1556.73	3801.82	Y= 0.1815667+ 1.423003 X	5.9025
	Coal ash	3430.03	1486.49	7914.71	Y= 1.966581+ 0.8580376 X	0.3197
	Alluvial soil	12563.47	911.16	173229.7	Y= 3.228549+ 0.4321551 X	0.0747
	China clay	2242.81	1218.96	4126.62	Y= 1.664071+ 0.9955641 X	0.4389
	Carbaryl	21.33	17.70	25.710	Y= 0.1733399+ 3.631431 X	2.4141
	48 hours					
	Kaolin powder	859.36	449.24	1643.86	Y= 2.835927+ 0.7375404 X	1.1209
	Paddy hush ash	274.00	151.06	496.99	Y= 2.507756 + 1.022351 X	3.0830
	Coal ash	426.16	253.33	716.87	Y= 2.298666 + 1.02729 X	1.7954
	Alluvial soil	652.29	296.75	1433.82	Y= 3.301301+ 0.6035645 X	0.5115
	China clay	325.76	208.27	509.54	Y= 1.698464 + 1.313831 X	0.8225
	Carbaryl	14.451	11.95	17.47	Y= 0.5489369 +3.837429 X	4.1731
<i>C. maculatus</i>	24 hours					
	Kaolin powder	3643.651	1299.44	10216.85	Y= 2.480977+0.7072854 X	1.2359
	Paddy hush ash	54946.68	2051.36	1471774	Y= 2.156692+0.5998614 X	0.2658
	Coal ash	61029.04	686.56	5424904	Y= 3.077287+0.4017759 X	0.4222
	Alluvial soil	61029.04	686.56	5424904	Y= 3.077287+0.4017759 X	0.0422
	China clay	4648.78	2040.53	10590.94	Y= 1.317287+1.004192 X	0.1762
	Carbaryl	24.12	19.95	29.14	Y= 0.0678453 +3.567871 X	2.4422
	48 hours					
	Kaolin powder	503.74	301.46	841.763	Y= 2.31615+0.9932041 X	1.2084
	Paddy hush ash	987.23	694.52	1403.312	Y= 0.5533071 + 1.484992 X	5.3390
	Coal ash	3229.43	686.94	15182.06	Y= 3.439218+0.444778 X	0.2714
	Alluvial soil	4265.59	978.81	18589.1	Y= 3.09993+0.5234382 X	0.4148
	China clay	642.27	410.31	1005.385	Y= 1.840817+1.125176 X	1.6445
	Carbaryl	15.47	12.85	18.614	Y= 0.4231234+3.847742 X	4.6092

Table 2. Co-toxicity coefficient of mixtures of Diatomaceous earth in mass ratio mixtures with tested dusts on adult *C. chinensis* and *C. maculatus*

Species	DE : Dust	Combined LD50 (ppm)	LD50 of DE in mixture (ppm)	Co-toxicity coefficient
<i>C. chinensis</i>	24 hours			
	Kaolin Powder	12703.57	396.986	149.117
	Paddy Hush Ash	2432.782	76.024	778.668
	Coal Ash	3430.036	107.188	552.277
	Alluvial Soil	12563.47	392.608	150.780
	China Clay	2242.814	70.087	844.628
	Carbaryl	21.33705	0.666	88885.150
	48 hours			
	Kaolin Powder	859.3629	26.855	1546.838
	Paddy Hush Ash	274.0051	8.562	4851.721
	Coal Ash	426.1612	13.317	3119.348
	Alluvial Soil	652.2975	20.384	2037.890
	China Clay	325.7676	10.180	4080.585
	Carbaryl	14.45133	0.451	92107.228
<i>C. maculatus</i>	24 hours			
	Kaolin Powder	3643.651	113.864	519.896
	Paddy Hush Ash	54946.68	1717.083	34.475
	Coal Ash	61029.04	1907.157	31.039
	Alluvial Soil	61029.04	1907.157	31.039
	China Clay	4648.786	145.274	407.488
	Carbaryl	24.12017	0.753	78615.551
	48 hours			
	Kaolin Powder	503.7491	15.742	2638.823
	Paddy Hush Ash	987.2394	30.851	1346.483
	Coal Ash	3229.436	100.919	411.620
	Alluvial Soil	4265.599	133.299	311.632
	China Clay	642.278	20.071	2069.670
	Carbaryl	15.47023	0.483	86004.886

In the present investigation the possibility of using DE as an extender or carrier for some other inert dusts or insecticidal dust formulations used to protect stored grain from insect attack. Formulations of this type would have the advantages of reducing levels of toxic residues and of having greater activity against insect populations with low levels of resistance to the chemical component of the formulation. Le Patourel and Singh (1984) showed that formulations of permethrin, cypermethrin and deltamethrin on absorptive silica had the additional advantage of a greater than additive action between the insecticidal components when tested against *T. castaneum* in grain of low moisture content.

The trials in the study indicate that SilicoSec can be used successfully as a protectant against adults of the bruchid beetles *C. chinensis* and *C. maculatus*. Regardless of the synergism of other factors, the effect of DE is dose-dependent (Fields & Korunic, 2000; Athanassiou *et al.*, 2003; Stathers *et al.*, 2004; Mahdi & Khalequzzaman, 2006), as in the case of residual insecticides used as grain protectants. However, the dose rate is more important in the case of inert dusts, given that the presence of dust in grain highly affects the physical properties of grain (Korunic *et al.*, 1996). In addition, dust formulations that are effective at high application rates are usually not acceptable, for health and environmental reasons (Subramanyam & Roesli, 2000). Nevertheless, higher application rates are recommended in cases of increased humidity, or as a surface treatment in bulked grain (Nickson *et al.*, 1994; Bridgeman, 2000; Subramanyam & Roesli, 2000; Cook & Armitage, 2002).

In the present study, the rates of 1600 ppm produced high mortality levels, though 100% was not achieved in most of the cases examined. However, given that pulse beetles can survive at application rates that are effective against other stored-grain beetle species (Aldryhim, 1990; Arthur, 2000a, b; Fields & Korunic, 2000), higher dose rates or longer exposure intervals are needed to achieve 100% mortality for adults of this species. The efficacy of SilicoSec is determined by the type of the product the dust is applied to. Athanassiou *et al.* (2003), using SilicoSec in dose-response tests against *S. oryzae* adults in peeled rice, paddy rice, barley and maize, found that mortality notably varied in different types of grain.

The efficacy of diatomaceous earth on the mortality of insect pests of stored products is usually affected by several factors among which stands out the temperature (Chanbang *et al.*, 2007). Generally, the increase in temperature favors the increase in the effectiveness of this product by stimulating the movement of insects within the grain mass, providing an increased contact of them, with the diatomaceous earth (Chanbang *et al.*, 2007; Vayias *et al.*, 2009). In addition, the insects have higher respiration rates at higher temperatures (Cotton, 1932), and consequently the greater water loss via spiracles promoting desiccation (Zachariassen, 1991). However, it was shown in some studies that the insect mortality can vary between species (Arthur, 2000a; Vayias & Athanassiou, 2004; Athanassiou *et al.*, 2005; Vayias *et al.*, 2009).

Increased exposure time is highly important for DE efficacy, since surviving individuals may disperse from the treated substrate and colonize untreated parts of the product mass (Subramanyam & Roesli, 2000). This fact must be seriously taken into account in cases of partially treated grain masses with DE, such as the surface treatment in grain bulks, when DE in the surface is used alone, as a barrier to infestation (Korunic & Mackay, 2000).

Conclusion

Diatomaceous earth is a potential alternative to be used in the development of strategies for management of resistance in insect pests of stored products, since a uniform response was observed among populations of *C. chinensis* and *C. maculatus*. In the present study, the rates of 1600 ppm produced high mortality levels, though 100% was not achieved in most of the cases examined. However, given that pulse beetles can survive at application rates that are effective against other stored-grain beetle species. Other dusts and clays used were inactive to *C. chinensis* and *C. maculatus* but in combination with DE they also provide some sort of synergistic effects.

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