Natural Resources Institute

Kent, United Kingdom

Project

Small-Scale Farmer Utilization of Diatomaceous Earths During Storage

Report

POTENTIAL INSECTICIDE EFFICIENCY OF AFRICA'S DIATOMACEOUS EARTHS

Report prepared by:

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Report

Potential insecticide efficiency of Africa's diatomaceous earths

Objective

Using the rapid assessment method (Korunic Z. 1997: Rapid assessment of the insecticidal value of diatomaceous earths without conducting bioassays. J. Stored Prod. Res. Vol. 33, No. 3, pp. 219-229, 1997) to determine the potential insecticide efficiency of five diatomaceous earth (DE) samples sent via Natural Resources Institute, UK, on January 28, 2003. DE samples have been collected from Tanzania, Zimbabwe and South Africa for use in a research project "Small-scale farmer utilization of diatomaceous earth during storage" funded by the United Kingdom Department for International Development Crop Post Harvest Programme.

Executive summary

The rapid assessment method was developed as a preliminary screen of raw DE materials of a high purity in order to select DE with promising insecticide efficiency and to eliminate DE with low efficiency or without any at all. The results of testing may serve for **the rough prediction** of the insecticide efficiency of DE samples without conducting bioassays. Then bioassays are performed only with DE samples with promising, a good insecticidal efficiency. Bioassay testing is still needed because the rapid assessment method cannot give an accurate answer what DE is the best among selected ones.

According to the outlined criteria for the rapid assessment method and obtained results, among the 5 DE samples, there are 2 groups of DE; a) a group with very good efficiency, similar or equal to Protect-It®; b) group with low or no efficiency against rice weevil (RW) and red flour beetle (RFB).

a) The first group with very good efficiency, similar to standard DE formulation
   - Sample B

b) The second group with very low efficiency against test insects
   - Samples A, C, D and E

According to the results of the rapid assessment of the insecticidal values of 5 DE samples, only one sample from the first group have to be subjected to bioassays to give us an accurate answer what concentrations need to be used for the successful control of test insects. However, because of very low purity of samples A, C and D and missing data for silicon dioxide content (chemical composition of DE) and particle size distribution, we decided to conduct some preliminary bioassays with all 5 samples.

Rapid assessment method

DE samples received from Natural Resources Institute, UK, on January 28, 2003, and their quantities and moisture contents are listed in Table 1.

Table 1. DE formulations received from Natural Resources Institute, UK

DE formulation	Grams received and moisture (m.c.)
A	1490 – raw material with 14% m.c.
B	440 – milled sample with 6% m.c.
C	460 – raw material with 5% m.c.
D	1685 – raw material with 6% m.c.
E	1545 – milled sample with 1% m.c.
DE used as standards	
Protect-It®	Commercial sample with 4% m.c.

Samples A and D contain a high percentage of foreign materials (different oxides, bentonite, kaolin, etc.). Also, sample C contains a significant amount of foreign materials, too. It is a great probability that these samples were collected from the surface. We believe that the purity of samples is increased significantly at deeper layers. According to our experience, the impurity may have a significant impact on the results of the rapid assessment method.

Results

The method is described in details in the publication of Korunic (1997) and was fully applied in performing the testing of five DE samples and 1 additional DE used as standard. Therefore, in this report we present the results and comments only.

Loose and tapped density

Table 2. DE loose and tapped density – comparative values only

DE	Average density (g/L)	
	loose	tapped
A	416	572
B	153	220
C	250	308
D	833	833
E	323	466
Protect-It®	210	265

<u>Groups according to tapped density</u>

Group 1. <300 g/L
- Sample B; Protect-It®

Group 2. >300 g/L
- Samples A; C; D; E.

pH value

Table 3. DE pH values

Formulation	pH value
Dest. water	6.02
A	8.91
B	6.34
C	8.36
D	9.71
E	10.67*
Protect-It®	6.80

* sample E may contains a significant amount of Ca oxide and a lower percentage of SiO_2

<u>Groups according to pH values</u>

Group 1. pH <9
- Samples A, B, C and Protect-It®

Group 2. pH >9

- Samples D and E.

Test weight (bulk density)

Table 4. Test weight (bulk density) measurement

DE Sample	Average kg/hL ± std. dev.*	Difference (kg/hl)
Untreated	80.55 ± 0.09 a	-
A	78.08 ± 0.06 e	2.47
B	76.97 ± 0.09 g	3.69
C	78.89 ± 0.09 c	1.63
D	78.49 ± 0.1 d	1.97
E	79.44 ± 0.08 b	1.11
Protect-It®	77.06 ± 0.1 g	3.49

*ANOVA (Tukey). P = 0.05. Means followed by the same letter are not significantly different

Groups according to test weight reduction

Group 1. > 2.5 kg

- Samples B and Protect-It®

Group 2. <2.5 kg

- Samples A, C, D and E

Adherence to wheat kernels

Table 5. Adherence of DE to wheat kernels - 1000 ppm - 0.1g/100 grams

DE	Free dust; grams at the bottom	Adherence (%)
A	0.0272	72.8
B	0.02	82.0
C	0.02	78.0
D	0.0435	56.5
E	0.041	59.0
Protect-It®	0.0175	82.5

Groups according to the percentage of adherence to kernels

Group 1. >80%

- Sample B and Protect-It®

Group 2. <80%

- Samples A, C, D and E

The simple criterion for the rapid assessment of the insecticidal potential of DE

The most efficient DEs have the greatest influence on bulk density (at 50 ppm the reduction of weight mass above 2.5 kg/hl), the lowest tapped density (300 g or less in one liter volume), the highest adherence to the wheat kernels (80% or higher) and pH value below 9.

The data for SiO_2 content, particle size distribution and the shape of diatoms are missing. They could assist in making a rough prediction of the insecticidal value of DE as well. In order to make a rapid and rough prediction of the potential insecticidal value of DE (good activity against insects, and not promising DE with lower or very low activity against insects), the criteria outlined in Table 6 were used.

Table 6. The prediction of the potential insecticidal activity based on the results of rapid assessment procedures

DE	Wheat bulk density 50 ppm >2.5 kg + <2.5 kg -	Tapped density grams in liter <300g/L + >300 g/L -	Adherence to wheat kernels (%) >80 + <80 -	pH <9 + >9 -	Prediction of the efficacy
A	**-**	**-**	**-**	**+**	Not good
B	**+**	**+**	**+**	**+**	Very promising
C	**-**	**-** (+)	-	**+**	Not good
D	**-**	**-**	**-**	-	Not good
E	**-**	**-**	**-**	-	Not good
Protect-It®	**+**	**+**	**+**	**+**	Standard; good

Comments

Due to the criteria in Table 6, DE formulations with the highest efficacy against RW and RFB have all four properties marked with a plus (+), and as activity against insects is decreased, DE has more properties marked with a minus (-). The most important properties are grain bulk density reduction, DE tapped density and adherence of DE particles to wheat kernels. If these DE properties are designated +, then the prediction is that this DE will have a good activity against insects; with two + marks, for the mentioned properties and + marks for pH value, this DE is still promising with medium to low activity against insects. But, if two of three, or all three important properties are marked with a minus (-), then the prediction is that this DE, at the acceptable concentration, is not effective enough to control insects.

According to the results in Table 6, there are two groups of DE:

a) group with a good efficiency, similar to standard DE
b) group with low or no efficiency against rice weevil (RW) and red flour beetle (RFB).

a) The first group with a good efficiency, similar to standard DE formulation

- Sample B

b) The second group with very low or no efficiency against test insects

- Samples A, C, D and E.

The rough prediction of the LC_{50} (lethal concentration) insecticidal value (IE) of DE

Rice weevil

The prediction of the insecticidal value (IE) of DE- LC_{50} (lethal concentration) against rice weevil (RW) at 25^0 C on Hard Red Spring wheat with 14% moisture content after exposure period of 5 days, according to the IE values is:

IE = **up to 20,** then the prediction for LC_{50} is **up to 400 ppm**.
DE belongs to the group with very good activity against the RW. It has a good chance to be accepted by the grain and milling industry.

IE = from **21 to 40**, then the prediction for LC_{50} is about **400 ppm to 700 ppm**.
DE belongs to a group with medium or lower activity. The chances of being accepted by the grain and milling industry are lower.

IE = from **41 to 60**, then prediction for LC_{50} is about **700 ppm to 1000 ppm**.
DE has some activity against insects. There is a little chance of the product being accepted by the grain and milling industries.

IE = >**60**, then prediction for LC_{50} is **above 1000 ppm**.
Activity against insects is very low. It is not suitable to be used as an insecticide.

Red flour beetle

The prediction of the insecticidal value of DE- LC_{50} (lethal concentration) against red flour beetle (RFB) at 25^0 C on Hard Red Spring wheat with 14% moisture content after exposure period of 14 days according to the IE values is:

IE = up to **40**, then the prediction for LC_{50} is up to **700 ppm.**
DE has a good activity against RFB. It has a chance of being accepted by the grain and milling industry.

IE = from **41 to 60**, then the prediction for LC_{50} is from about **700 ppm to 2500 ppm**.
DE activity is medium or low. There is little chance of it being accepted by the grain and milling industries.

IE = >**60**, then the prediction for LC_{50} is **above 2500 ppm**.

There is no chance the DE will be used as insecticide.

Table 7. The rough prediction of the LC_{50} insecticidal value of DE

Formulation	Prediction for RW; LC_{50} after 5 days		Prediction for RFB; LC_{50} after 14 days	
	IE value*	ppm	IE value	ppm
A	163.6	>1000	53.8	700 - 2500
B	- 8.13	<400	2	<700
C	47.9	700-1000	46.2	700 -2500
D	114.1	>1000	128.1	>2500
E	101.4	>1000	206.7	>2500
Protect-It®	0.7	<400	7.5	<700

*IE means insecticidal efficiency. DE with lower IE values are often more effective than DE with higher IE values.

Comments

According to the results for the prediction of the LC_{50} insecticidal value of DE (Table 7), among 5 samples, there are 3 groups of DE:

a) group with a good efficiency, similar to standard DE or even better
b) group of DE with medium to low efficiency
c) group with low or no efficiency against RW and RFB.

a) The first group with a good activity against **RW**
- Sample B

b) The second group with low efficacy against **RW**
- Sample C

c) The third group with very low efficiency against **RW**
- Samples A, D and E

a) The first group with a good activity against **RFB**
- Sample B

b) The second group with low efficacy to **RFB**
- Samples A and C

c) The third group with very low efficiency against **RFB**
- Samples D and E

Rapid assessments conclusions

The results of the rapid assessment indicated clearly that the sample B contains diatoms with very high potential efficiency against insects. Its potential efficiency is comparable with the efficiency of enhanced DE formulation Protect-It®. Also, the results of rapid assessment indicated that a sample C may be a little bit more effective than samples A, D and E. According to the results shown in Tables 6 and 7, the sample B has to be subjected to bioassays to give us an accurate answer about the concentrations that cause 50, 90 and 100% mortality of test insects under different conditions. However, because of significant foreign materials present in A and D samples and in some extend in C sample, the rapid assessment method cannot give the real prediction of the potential insecticide values of diatoms themselves. The method has been developed as a preliminary screen of raw DE materials with a high purity. Therefore, because of the one-time introductory testing of these samples, low purity of some samples, data for silicon dioxide content (chemical composition of DE) and particle size distribution, we decided to carry out a simple, preliminary bioassays with all 5 samples and Protect-It® as a standard.

Bioassay

Brief description of protocol for preliminary bioassays

Unsexed adults old 7 – 21 days of *Sitophilus oryzae* (L.), the rice weevil (RW), *Tribolium castaneum* (Herbst), the red flour beetle (RFB) and *Rhyzopertha dominica* (F.), the lesser grain borer bred in laboratory cultures at 30 ± 1 ^{0}C, 70 ± 3% r.h., were used in bioassays.

Uninfested, clean Hard Red Spring wheat, at 13.2 % m.c., was mixed with DE using concentrations 0 and 1000 ppm . After adding a weighed quantity of dust to preconditioned 0.5 kg of grain in each jar, the jars were closed tightly and thoroughly shaken for one minute. Untreated grain served as control (0 ppm). After mixing, each quantity of grain treated with a single concentration of DE was divided into five replications with 100 grams of grain. Test insects (50 adults of mixed sex, old 1 to 3 weeks were introduced into replications (jars), with each species being tested separately. The replications were held in a growth chamber at 30 ± 1^{0}C and 70 ± 5% r.h. After the exposure period of three and six days, the content of each jar was sifted using a 2 mm opening sieve to separate insects from the grain. The numbers of alive and dead insects were counted and a statistical analysis, ONE Way ANOVA, was carried out.

The results of bioassay 1 – efficacy of 5 DE samples and standard against test insects

Table 8. The efficiency of different DE formulations against rice weevil (RW) and red flour beetle (RFB) on wheat after exposure of 3 days. DE formulations were applied at **1000 ppm.**

Treatment	Mean mortality (%) ± std. dev. after 3 days	
	RW	**RFB**
Untreated	Unexpected, very high mortality of RW in untreated replications (32.0 ± 12.0). The test is not valid.	0.0 ± 0.0 a
A		0.0 ± 0.0 a
B		**61.5 ± 11.0 b**
C		2.7 ± 2.3 a
D		0.0 ± 0.0 a
E		2.6 ± 2.3 a
Protect-It®		**76.5 ± 12.7 b**

ANOVA (Tukey). P = 0.050. Means in the column followed by the same letters are not significantly different.

Table 9. The efficiency of different DE formulations against rice weevil (RW) and red flour beetle (RFB) on wheat after exposure of 6 days. DE formulations were applied at **1000 ppm.**

Treatment	Mean mortality (%) ± std. dev. after 6 days	
	RW	**RFB**
Untreated	Unexpected, very high mortality of RW in untreated replications (32.0 ± 12.0). The test is not valid.	0.0 ± 0.0 a
A		17.5 ± 21.6 ab
B		**100.0 ± 0.0 c**
C		28.5 ± 6.5 b
D		4.0 ± 4.0 ab
E		6.6 ± 8.3 ab
Protect-It®		**100.0 ± 0.0 c**

ANOVA (Tukey). P = 0.050. Means in the column followed by the same letters are not significantly different.

The results of bioassay 2 – efficacy of different fractions (particle size) of sample B

The diatoms median particle size from about 2 to 30 microns is cited in references as a very important DE property affecting insecticide activity. We found out that some DE formulations with smaller particles gave a significantly higher efficacy against the same species of insects than larger particles (e.g. marine DE “Celite 209”). However, different fractions of some other formulations gave the same results (no significant differences) (e.g. marine DE “Macedonia). In order to find out if the efficiency of DE samples B and C are affected by particle size distribution, we conducted a preliminary testing with different fractions of samples B and C:

- **fraction 1** containing particles from 0 to 150 microns
- **fraction 2** containing particles from 45 to 150 microns

- **fraction 3** containing particles smaller than 45 microns.

In order to compare the efficiency of sample C against test insects, in a test conducted with sample C we included DE formulation that is used in the production of Protect-It® (DE P.-It). Test insects were rice weevil and lesser grain borer. At the time of conducting of the test, we didn't have enough adults of red flour beetle.

The results are presented in Table 10 to 13.

Table 10. The efficiency of different fractions of DE **B** against rice weevil and red flour beetle after exposure of 1 day on wheat treated with 1000 ppm

Treatment	Mean mortality (%) ± std. dev. after 1 day	
	RW	RFB
Untreated	0.0 ± 0.0 a	0.0 ± 0.0 a
Fraction 1	11.2 ± 2.8 a	69.7 ± 0.0 b
Fraction 2	8.3 ± 2.2 a	57.4 ± 6.4 b
Fraction 3	8.9 ± 8.6 a	74.0 ± 18.5 b

ANOVA (Tukey). P = 0.050. Means in the column followed by the same letters are not significantly different.

Table 11. The efficiency of different fractions of DE **B** against rice weevil and red flour beetle after exposure of 3 days on wheat treated with 1000 ppm

Treatment	Mean mortality (%) ± std. dev. after 3 days	
	RW	RFB
Untreated	0.0 ± 0.0 a	0.0 ± 0.0 a
Fraction 1	100.0 ± 0.0 b	98.7 ± 0.3 b
Fraction 2	100.0 ± 0.0 b	100.0 ± 0.0 b
Fraction 3	100.0 ± 0.0 b	100.0 ± 0.0 b

ANOVA (Tukey). P = 0.050. Means in the column followed by the same letters are not significantly different.

Table 12. The efficiency of different fractions of DE **C** against rice weevil and red flour beetle after exposure of 1 day on wheat treated with 1000 ppm

Treatment	Mean mortality (%) ± std. dev. after 1 days	
	RW	LGB
Untreated	0.0 ± 0.0 a	0.0 ± 0.0 a
DE P.-It	**22.0 ± 5.9 b**	**91.2 ± 5.2 c**
Fraction 1	0.7 ± 1.6 a	2.4 ± 3.5 a
Fraction 2	0.0 ± 0.0 a	1.6 ± 2.1 a
Fraction 3	5.0 ± 3.6 a	9.5 ± 4.8 b

ANOVA (Tukey). P = 0.050. Means in the column followed by the same letters are not significantly different.

Table 13. The efficiency of different fractions of DE **C** against rice weevil and red flour beetle after exposure of 3 days on wheat treated with 1000 ppm

Treatment	Mean mortality (%) ± std. dev. after 3 days	
	RW	**LGB**
Untreated	4.0 ± 2.8 a	3.2 ± 3.3 a
DE P.-It	**100.0 ± 0.0 c**	**100.0 ± 0.0 c**
Fraction 1	52.6 ± 9.3 b	31.0 ± 7.9 b
Fraction 2	39.2 ± 12.8 b	27.1 ± 3.9 b
Fraction 3	41.8 ± 2.4 b	35.3 ± 11.7 b

ANOVA (Tukey). P = 0.050. Means in the column followed by the same letters are not significantly different.

Conclusions

The results of bioassay 1 for RFB are in a good agreement with the results of rapid assessment method. Based on the results of both assessment methods (rapid assessment and bioassay), it is obvious that DE sample B posses an excellent insecticide performance comparable with the efficiency of standard DE formulation that belong to the group of the best DE currently used as insecticides in the world. Samples A, C, D and E belong into a group of DE with very low efficiency against insects.

The first results of testing different particle size of samples B and C do not indicate that their particle size distribution is correlated with the efficiency against rice weevil, red flour beetle and lesser grain borer (sample C). This indicates a great probability that efficiency of DE sample C can't be increased by using formulation with a smaller particle size.

Because of the often inconsistency in the results obtained by performing several bioassays with different DE samples or with numerous DE samples taken from the same source, the results of our bioassays need to be confirmed. It would be useful to have new A, C and D samples collected from deeper layers with a high purity and again to perform a rapid assessment method and bioassays.