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Diatomaceous earth-induced alterations in the reproductive attributes in the housefly *Musca domestica* L. (Diptera: Muscidae)

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ABSTRACT

Using concentrations of 0.0, 0.2, 0.4, 0.6 and 0.8mg diatomaceous earth (DE) 100 mL^{-1} larval food medium, a mean LC_{50} value of 0.6636mg was determined for the 3^{rd}-instar larvae of the housefly *Musca domestica*. Time-course mortalities of the larvae at the determined LC_{50} level were assessed at 24h, 48h and 72h post-treatments. Finally, DE-induced changes in such vital reproductive attributes as egg-laying, egg-hatch, larval duration, number of dead larvae, pupal duration, number of dead pupae, number of adults emerged and female ratios from parental through F_2 generations were recorded. Results indicated that DE could be used as an efficient larvicide against *M. domestica* and it was capable of inducing deleterious effects on all the reproductive parameters at the determined LC_{50}. These findings have potential implications because the present DE concentration might be utilized for the control of this important vector species under household as well as field conditions.

Introduction

Diatomaceous earth (DE) is a geological deposit consisting of the fossilized skeletons of numerous species of siliceous marine and fresh water unicellular organisms, particularly diatoms and other algae[1]. It is made up of almost pure amorphous silicon dioxide. It has been recognized as an effective mechanical insecticide due to its abrasive and physico-sorptive properties. It works mainly by absorbing the waxy cuticle of insects upon contact, causing death by desiccation[2-3].

The insecticidal activity of DE results from their abrasiveness or absorptive characteristics or both. It damages the insects' water barrier by scratching or cutting the cuticle, absorbing fats, disrupting the cuticle's waterproof nature and finally dehydration usually causes the insects' death[4]. The fine powder of DE absorbs lipids from the waxy outer layer of insects' exoskeletons, causing them to dehydrate. Arthropods die as a result of the water pressure deficiency. In order to be effective as an insecticide, DE must not be heat-treated prior to application, in other words, it must be uncalcinated, and have a mean particle size below about 12 µm[5]. However, DE is sometimes mixed with an attractant or other additives to increase its effectiveness[6].

The effects of DE on a wide range of stored product beetles including Rhyzopertha dominica[7-8], Tribolium castaneum[9-14], T. confusum[15], Sitophilus species[6-7, 13, 15-17], Callosobruchus maculatus[6,16],, Plodia interpunctella[15], and cockroaches and silver fishes[18] have been reported by previous workers.

Apart from the above coleopteran pests, DE was used as feed additives that provided control of internal parasites and fly larvae including house fly, stable fly and blow fly in animal manure[4]. Moreover, it has been reported to work against cockroaches, silverfish, bedbug, house dust mite, ant and flea[18], gastropods such as slugs[19] and also against flour moth infestations[20].

Among vertebrates, DE has been used to control internal parasites in ruminants[2], and DE with or without pesticides was used in integrated pest management (IPM) programmes because of their low toxicity to mammals[17]. It was found to be an anthelmintic agent in crossbred steer calves[21] and cattle[22], and it has potential to be an effective treatment to help control parasites and improve production of free-range commercial layer hens[23]. Since environmental and human health problems associated with the use of synthetic pesticides have prompted the demand for non-polluting, biologically specific insecticides, the current study tested the action of DE against such an important insect vector as the housefly, *Musca domestica* to evaluate DE's larvicidal efficacy as well as its changes in terms of some vital reproductive attributes of the test insect.

Materials and Methods

Collection and colonization of the test insects

The adult houseflies, *Musca domestica* L. (Diptera: Muscidae) were collected from Binodpur fish market near Rajshahi University (RU). Soon afterwards the flies were provided with milk soaked in sterilized cotton pads, transported to the Genetics Research Laboratory, Department of Zoology, RU, and cultured in 50cm × 30cm × 200cm cages made up of wood and nylon nets for colonization. To produce consistent quality houseflies for experiments, the methods devised by previous workers were followed with a slight modification[24-25]. In brief, the food medium was prepared by mixing 9g powdered milk, 5g baker's yeast and 100mL water. The adults were provided with 9cm-diameter Petri dishes containing cotton wools soaked in prepared food medium. The cotton wools were changed every 24 hour to prevent dehydration and unpleasant odour of the culture medium. The adult flies released in the cages were fed on the food medium, allowed to mate and lay eggs.

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The larvae hatched out in the Petri dishes, fed and kept growing until transformed into pupae. The pupae were then collected in Petri dishes and transferred to the adult rearing cages for eclosion. To eliminate spontaneous mutations, if any, the houseflies were reared for two successive generations. Then adult flies of approximately the same age were used as parents for estimating the reproductive attributes. All the experimental flies were reared in the laboratory at 25°-28°±2° C, 75-80% uncontrolled RH and 8:16 light: dark photo regime.

Diatomaceous earth (DE) and its treatment protocol

A commercial DE product, Silicosec®, was procured from Agrinova GmbH, Germany. It is a relatively new formulation of fresh water origin that contained approximately 92% SiO_2, 3% Al_2O_3, 1% Fe_2O_3 and 1% Na_2O. The average particle size was between 8 and 12 µm. Concentrations of 0.2mg, 0.4mg, 0.6mg and 0.8mg of DE 100 mL^{-1} of the larval food media were made by dissolving the DE product in distilled water. A control line was maintained for comparison.

Estimation of LC_{50} values for the 3rd instar larvae of *M. domestica*

The larvicidal bioassays with the above-mentioned DE treatments were conducted as follows. After going through an initially pilot experiment in which five doses namely 0.0, 0.5mg, 1.0mg, 1.5mg and 2.0mg of DE 100 mL^{-1} of the larval food media were applied, the final doses were selected as 0.0, 0.2mg, 0.4mg, 0.6mg and 0.8mg for the larvicidal bioassays against the 3rd-instar larvae of *M. domestica*. Larvae were released in 9-cm diameter Petri dishes provided with cotton pads soaked in the treated food media. For each bioassay, 72h post-treatment mortality of 100 larvae was assessed and the corresponding LC_{50} value was estimated as per standard procedures[26-27]. The experiment was replicated five times, from which the mean LC_{50} value was estimated.

Time-course mortalities of the 3rd instar larvae of *M. domestica* using LC_{50} of DE

The LC_{50} of DE as determined above (0.6636mg of DE 100mL^{-1} larval food media) was used to confirm and ensure that the dose acts as an effective larvicide for the test insects *M. domestica*. Thus the time-course larval mortalities were counted after 24h, 48h and 72h post-treatment. This experiment was replicated five times, where each replication consisted of 20 3rd-instar larvae of *M. domestica*.

Estimation of reproductive attributes in *M. domestica* from parental through F_2 generations following DE treatments

In this experiment, the larval food media were treated again with the LC_{50} of DE and freshly eclosed and mated females were allowed to lay their eggs on 9-cm diameter Petri dishes in the culture cages. Data were collected on such important reproductive attributes as fecundity (24h egg-laying), hatchability (% egg-hatch), larval duration (in h), number of dead larvae, pupal duration (in h), number of dead pupae, number of adults, female ratio (number of females ÷ total number of adults) and number of deformed adults. The attributes were recorded from parental through F_2 generations. A control line for each generation and five replications were maintained.

Statistical procedures

For preliminary processing of the raw data, means and standard deviations (mean ±SD) were calculated for each treatment group. LC_{50} toxicity values and the slope of regression lines for the DE were calculated by probit analysis[26] (Finney, 1978) using a software called *GWBASIC*. One-way analysis of variance (ANOVA) was used, where the levels of significance were set at P<0.05, and the means were separated using Fisher's least significant difference (LSD) tests[28]. All statistical analyses were performed using SPSS (version 16.0 for Windows).

Results

Estimation of LC_{50}

A mean LC_{50} of 0.6636mg of DE 100mL^{-1} larval food was calculated from five replicated trials against the 3rd-instar larvae of *M. domestica* (Table 1). The dose-mortality response showed a regression equation of Y= 2.2690 + 3.3594X, indicating a significant correlation (r= 0.8068) between the log dose and probit mortality (Fig. 1). This estimated LC_{50} of DE was further used for assessing the time-course mortalities of the housefly larvae and for evaluating some reproductive attributes of the adult flies under laboratory conditions.

Table 1. Estimated LC_{50} values following DE treatments on the 3rd-instar larvae of *M. domestica*.

Replications	LC_{50} values*	Regression equations	Confidence limits (Lower Upper)	Chi-squared (2 df)
1	0.6457	Y = 2.3383 + 3.9999X	0.3897 1.0698	64.3941
2	0.7211	Y = 2.5894 + 3.3770X	0.5644 0.9215	9.0108
3	0.6627	Y = 2.1756 + 4.1732X	0.4629 0.9487	33.8567
4	0.6478	Y = 2.7558 + 3.3653X	0.4351 0.9645	28.0970
5	0.6408	Y = 3.1000 + 2.8697X	0.7211 0.9112	16.2690
Mean	0.6636	Y = 2.2690 + 3.3594X	Correlation r =0.8068	-

*mg.100mL^{-1} larval food

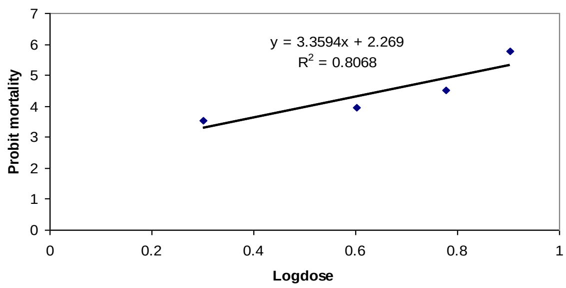

Fig 1. Regression line showing the dose-mortality response of DE against the 3rd-instar larvae of *M. domestica*.

Time-course mortalities of the 3rd instar larvae of *M. domestica*

The larvicidal effect of the LC_{50} of DE on the 3rd-instar of M. domestica revealed that DE ensured 4%, 23% and 27% larval mortalities respectively at 24h, 48h and 72h post-treatments, with an overall mortality of 54% larvae (Fig. 2). This suggested an efficient larvicidal property of the DE product under study.

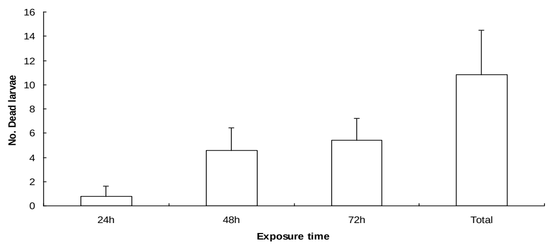

Fig 2. Time-course mortality of the 3rd-instar larvae of *M. domestica* following LC_{50} DE 72h post-treatment (mean ±SD values for five replicates).

Effects of DE on reproductive attributes of *M. domestica*

Results on the effects of the LC_{50} of DE on egg-laying, hatchability, immature durations and female ratio of the experimental insects are presented in Table 2. Compared to the control values, DE-treated parental, F_1 and F_2 generation females laid significantly reduced number of eggs ($F_{5,\ 24}$= 7.13; P<0.001), although the egg-hatch percentages were not affected. As expected, however, both larval and pupal durations were lengthened significantly ($F_{5,\ 24}$= 40.44 and 140.36; P<0.001 for both) following DE treatments in the parental through F_2 generations. In contrast, the female ratios were reduced significantly ($F_{5,\ 24}$= 5.36; P<0.01), indicating that DE had killed a greater number of females than males of the experimental insects.

Table 2. DE-induced changes in some reproductive attributes in *M. domestica*.

Generations	Fecundity[1]	Egg-hatch (%)	Larval duration (h)	Pupal duration (h)	Female ratios[2]
P					
Control	123.6±7.9^a	97.9±1.0^a	122.2±5.8^a	62.0±10.4^b	0.50±0.03^a
DE-treated[3]	108.6±4.8^b	96.9±1.0^a	170±5.8^d	82.4±4.1^c	0.40±0.03^c
F_1					
Control	126.2±6.3^a	97.8±0.6^a	122.0±10.2^a	59.6±10.6^b	0.50±0.02^a
DE-treated[3]	100.0±14.1^c	96.6±1.5^a	144.0±7.3^b	143.8±4.1^d	0.44±0.10^b
F_2					
Control	134.4±11.1^a	94.6±6.4^a	116.0±9.6^a	105.0±11.2^a	0.51±0.03^a
DE-treated[3]	111.0±15.8^b	97.9±0.7^a	159.6±7.3^c	168.2±6.6^e	0.40±0.04^c
F-values	7.13***	1.05ns	40.44***	140.36***	5.36**

P, F_1 and F_2 refer to parental, F_1 and F_2 generations, respectively; [1]24h egg-laying; [2]Number of females ÷ Total number of adults; [3]0.6636 mg of DE $100mL^{-1}$ larval food medium; mean± SD values with dissimilar superscript letters differ significantly by LSD tests (P<0.05); ns= not significant, **= *P*<0.01, ***= *P*<0.001; all F-values are at 5, 24 df.

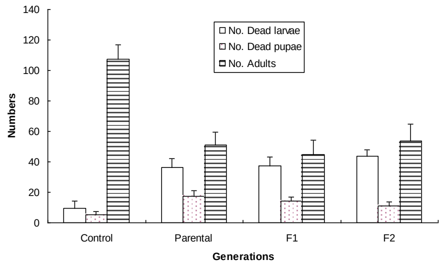

Fig 3. DE-induced immature mortalities and adult emergence in *M. domestica*

The efficacy of the DE used against the larvae, pupae and adults of *M. domestica* was quite evident from the results shown in Fig. 3. The number of dead larvae and pupae increased significantly from around 10 in the control to 36, 38 and 44 in the parental, F_1 and F_2 generations, respectively ($F_{5,\ 24}$= 30.43 and 17.24; P<0.001 for both). On the other hand, the number of emerging adults was reduced significantly ($F_{5,\ 24}$= 49.93; P<0.001) in all three generations.

Discussion

In the present study a thorough investigation has been carried out to assess the efficacy of DE against *M. domestica*, a cosmopolitan vector of many human diseases. Results clearly demonstrated that DE is not only an excellent larvicide, but it is also capable of inducing negative effects on some vital reproductive traits of the houseflies including egg-laying, adult emergence and female ratio.

Published reports show that activity of the commercial DE formulations affect growth and development of different species of the stored-product insects, and provide long-term protection to the stored grains. Thus, DE was found lethal to adult mealworms *Tenebrio molitor* and *Tribolium confusum*, but their larvae were unaffected; it was lethal to the 1st-instar larvae of *Plodia interpunctella,* but not lethal to older larval stages[1,7]. Contact with DE caused adult *Sitophilus granarius, T. molitor* and *T. confusum* to lose weight and reduced their water content[9]. Two week-old larvae of *T. confusum* were more sensitive to DE than *P. interpunctella* at the same age[15]. These findings are in good agreement with those reported here for houseflies in terms of larval, pupal and adult survival following DE treatments from parental to F_2 generations.

Two commercially available DE products were reported to give significant protection against *Rhyzopertha dominica* for periods of 40 weeks when admixed with farm stored maize, sorghum and cowpeas[8]. Moreover, the efficacy of DE against the adults of *Tribolium castaneum* and *Sitophilus granaries* was found satisfactory[10]. Findings on the efficacy and persistence of DE against four common tropical storage pests *Prostephanus truncatus*, *Sitophilus zeamais*, *Callosobruchus maculatus* and *Acanthoscelides obtectus* revealed increased parental mortality and reduced F_1 progeny emergence[16], which nicely corroborate to the present results.

Admixtures of DE and certain monoterpenoids increased the efficacy of the former where the estimated LD_{50} values varied from 2.60 ppm to 42.73 ppm against *Callosobruchus maculatus* and *Sitophilus oryzae*[6]. Population build ups in *T. castaneum* and *S. oryzae* were checked by DE treatments[13]. Recent reports indicated that DE can be used successfully for the control of infestations with American and German cockroaches *Periplaneta americana* and *Blattella germanica* as well as silverfish *Lepisma saccharina*[18]. Further, DE at doses from 8-32 mg/g food at 24-, 48-, 72-, 96- and 120h exposure periods was found repellent against *T. castaneum*[12,14]. Although reports on the effects of DE on dipteran insects are relatively scarce[4], however, the present results are encouraging due to the fact that fecundity, immature durations and mortalities, adult emergence and female ratios of the experimental insects were profoundly affected by the DE product. Further experiments in the household and farm premises are designed and solicited for execution in the near future.

Conclusion

DE is basically a lethal dust lined with microscopic razor sharp edges. Ingestion of this lethal powder by the insects causes them to dehydrate from the inside out, as well as shredding their inside, which happens quickly, usually within a few minutes. Since it is a mechanical insecticide, insects cannot develop an immunity or resistance to DE. Therefore, it can be used to control insects for a long time without the manifestation of insecticide resistance which is often reported for other insecticides. The main advantage of DE is its low mammalian toxicity. The present findings clearly demonstrate that DE at LC_{50} of 0.6636 $mg.100mL^{-1}$ larval food could be used as an efficient larvicide against *M. domestica*. In addition, it could also be utilized to reduce egg-laying, lengthen immature duration and inhibit population build-up of this important vector species.

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