

Adverse effects of diatomaceous earth on immature duration, mortality, adult deformity and salivary gland morphometrics in *Musca domestica* L.

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Abstract

Larvicidal bioassays against the third-instar larvae of the housefly *Musca domestica* were used to determine an LC₅₀ of 0.6636 mg diatomaceous earth (DE) 100 mL⁻¹ larval food medium. The lethal concentration thus estimated was then used to assess the effects of the DE on immature durations and mortalities, along with adult deformities of the houseflies from parental through F₂ generations under laboratory conditions. Results show that the DE product not only lingered larval and pupal durations significantly (P<0.001), but it also killed significantly greater number of larvae and pupae (P<0.001) and induced significant adult deformities (P<0.001) in three successive generations. Moreover, the immature growth inhibitions following DE treatments were highly significant compared to the controls (P<0.001). All these DE-induced alterations could be interpreted in terms of the drastic changes in the morphometrics of the larval salivary glands of the insects. The present findings therefore have practical implications in relevance to the control strategies of this vector species of public health and veterinary importance.

Key words: Diatomaceous earth (DE), *Musca domestica*, immature durations, mortality, adult deformity, salivary glands, vector control strategy.

Introduction

The common houseflies *Musca domestica* L. (Diptera: Muscidae) are known as one of the most serious pests at human and animal dwellings worldwide (Mündi, 1994), which can spread a deadly pathogenic bacterium (Sasaki et al., 2000) as well as transmit antibiotic-resistant bacteria (Macovei and Zurek, 2006). Adult houseflies are mechanical vectors of numerous dreadful diseases including typhoid fever, cholera, amoebic dysentery, diarrhoea, salmonellosis, anthrax and helminthic infections in man (Brazil et al., 2007; Fasanella et al., 2010; Scott et al., 2014), and they have been shown to be disease pathogen transmitters via their vomit, faeces and contaminated external body parts (Pest Control Solutions, 2012). Conventional chemical and synthetic insecticides have been used extensively for many years for controlling this commensal vector species (Cao et al., 2006; Malik et al., 2007). But the indiscriminate and unregulated uses of

such insecticides have adverse effects like development of insect resistance and residual effects on humans, animals and the environment (Acevedo et al., 2009; Pezzi et al., 2011). These problems, coupled with acute neurotoxicity to man and his domesticated animals, have stimulated the search for alternative mechanical control agents like sticky cards and traps (Hogsette et al., 1993; Kaufman et al., 2005), integrated approach (Axtell, 1970; Rutz et al., 2001) and diatomaceous earth DE (Weinzierl and Jones, 2000; eHow contributor, 2011; Rahman, 2012), that have been shown to be valuable for controlling houseflies. This approach could lead to gradually decreased uses of chemical and synthetic insecticides against this vector species of public health and veterinary importance.

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 (Korunic, 1998). 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 (Quarles, 1992; Fields, 2000). The insecticidal activity of DE, thus, results from their abrasiveness or absorptive characteristics or both. It damages the insects' water barrier by scratching or cutting the cuticle, absorbs fats, ultimately disrupting the cuticle's waterproof nature; finally dehydration usually causes the insects' death (Weinzierl and Jones, 2000). The fine powder of DE absorbs lipids from the waxy outer layer of insects' exoskeletons, causing them to dehydrate. The 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 that means it must be uncalcinated, and have a mean particle size below about 12 µm (Capinera, 2008).

The effects of DE on a wide range of stored product beetles including *Tribolium castaneum* (Arnaud et al., 2005; Hossain et al., 2010; Kabir et al., 2011; Reza et al., 2012), *T. confusum* (Mewis and Ulrichs, 2001; Athanassiou et al., 2004), different species of *Sitophilus* (Fields and Korunic, 2000; Mewis and Ulrichs, 2001; Stathers et al., 2004; Ulrichs et al., 2006; Islam et al., 2010; Kabir et al., 2011), *Callosobruchus maculatus*

(Stathers *et al.*, 2004; Islam *et al.*, 2010), *Rhyzopertha dominica* (Kavallieratos *et al.*, 2007; Kostyukovsky *et al.*, 2010), *Plodia interpunctella* (Mewis and Ulrichs, 2001), cockroaches and silver fishes (Faulde *et al.*, 2006), and also against flour moth *Ephestia kuehniella* (Athanassiou, 2006) have been reported by previous workers. Moreover, DE was reported to work against slugs (Fields *et al.*, 2002) and bedbug, house dust mite, ant and flea infestations (Faulde *et al.*, 2006).

Apart from the aforesaid arthropod and molluscan pests, DE was used as feed additives that provide control of internal parasites and fly larvae including house fly, stable fly and blow fly in animal manure (Weinzierl and Jones, 2000). Recently, Rahman (2012) and Islam and Rahman (2016) have reported on the negative impacts of DE on various reproductive traits in the house fly *Musca domestica*. Since environmental and human health problems associated with the use of synthetic pesticides have prompted the demand for non-polluting, biologically specific insecticides, the present study was aimed to examine the actions of DE against such an important vector species as *M. domestica* to evaluate DE's larvicidal impact, coupled with DE-induced changes in the larval and pupal durations, immature mortalities, adult deformities, and salivary gland morphometrics in the test insects.

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 (Morgan *et al.*, 1981; Islam and Aktar, 2013). 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. 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 larval and pupal durations and mortalities, and deformities in the emerging adults. 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% SiO₂, 3% Al₂O₃, 1% Fe₂O₃ and 1% Na₂O. 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.

Larvicidal bioassay and estimation of LC₅₀

The larvicidal bioassay with the above-mentioned DE concentrations was conducted as follows. After going through an initially pilot experiment in which five doses namely 0.0mg, 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 0.0mg, 0.2mg, 0.4mg, 0.6mg and 0.8mg for the larvicidal bioassay against the third-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 the released larvae was assessed and the corresponding LC₅₀ value was estimated as per standard procedures (Finney, 1978; WHO, 2005), with some minor modifications as required.

Immature durations, mortalities and adult deformities

The LC₅₀ of DE as determined above (*i.e.*, 0.6636mg DE 100mL⁻¹ larval food) was used to assess the cumulative larval and pupal durations (h) and mortalities (%), and deformities (%), if any, in the emerging adults of *M. domestica* from the parental to F₂ generations. The immature growth inhibition values (h) were calculated by deducting DE-treated values from the corresponding control values in all three successive generations. These experiments were replicated five times.

Salivary gland morphometrics

In this final experiment, the larval food media were treated again with the LC₅₀ of DE. Freshly eclosed and mated females were allowed to lay their eggs on 9-cm diameter Petri dishes in the culture cages. Control Petri dishes with untreated food were also maintained for comparison. In the end, salivary glands from the mature third-instar larvae were separated out using a binocular dissecting microscope at 40× magnification, and morphometric data

on the gland lengths (mm) and widths (mm) were recorded for 20 larvae from each of the control and DE-treated lines.

Statistical analyses

For preliminary processing of the raw data, means and standard deviations (mean \pm SD) were calculated for the control and DE-treated groups. DE toxicity in terms of the LC_{50} value and the resulting slope of regression line were calculated by probit analysis (Finney, 1978) using a software called *GWBASIC*. One-way analysis of variance (ANOVA) was used for the data on immature durations and growth inhibition, immature mortalities, adult deformities and salivary gland morphometrics, where the levels of significance were set at $P < 0.05$, and where applicable, the means were separated using Fisher's least significant difference (LSD) tests (Steel and Torrie, 1984). All statistical analyses were performed using SPSS version 16.0 for Windows.

Results

Estimation of LC_{50}

An LC_{50} of 0.6636mg DE $100mL^{-1}$ larval food was estimated against the third-instar larvae of *M. domestica* (Table 1). Accordingly, the log dose-probit mortality response was plotted (Fig. 1), in which the regression equation $Y = 2.2690 + 3.3594X$ showed a highly significant coefficient of correlation ($r = 0.8068$). Using this LC_{50} value, the effects of DE on immature durations, mortalities and adult deformities of the experimental insects were assessed from parental through F_2 generations under laboratory conditions.

Table 1: Estimation of LC_{50} from DE-treated third-instar larvae of *M. domestica*

Dos used	Log doses	Larv used	Larv kille	% Kille	% Correc	Empli probit	Exp probit	Work probit	Weigl probit	Fina probit
0.2	0.301	126	15	11.90	5	3.36	2.78	4.09	9.58	2.81
0.4	0.602	121	20	16.53	10	3.72	4.08	3.75	53.12	4.08
0.6	0.778	102	38	37.25	32	4.53	4.84	4.55	63.95	4.82
0.8	0.903	99	79	79.80	78	5.77	5.38	5.71	60.98	5.34

¹mg. $100mL^{-1}$ larval food; $LC_{50} = 0.6636$; Log $LC_{50} = 0.8213$; Regression equation= $Y = 2.2690 + 3.3594X$, correlation, $r = 0.8068$; lower and upper limits at 95% confidence were 0.4629 and 0.9487; $\chi^2 = 33.86$ at 2 df ($P < 0.001$).

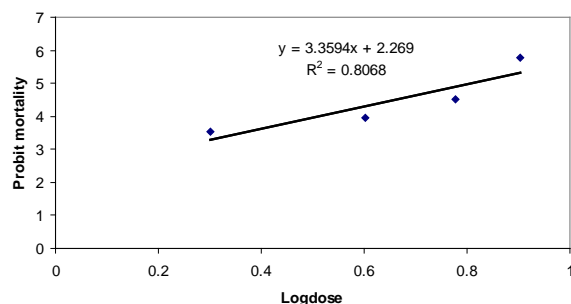


Fig 1: Regression line showing the dose-mortality response of DE against the 3rd-instar larvae of *M. domestica*

Immature durations, mortalities and adult deformities

Compared to the control lines, DE-treated lines showed increasingly higher larval and pupal durations, coupled with greater numbers of immature deaths (Fig. 2). The immature durations were lengthened from about 182h, 182h and 221h in the control lines to approximately 252h, 288h and 328h in the parental, F_1 and F_2 generations, respectively. The results indicated that DE was capable of inducing a highly significant immature growth inhibition of about 68h, 106h and 107h, respectively in the three successive generations (Table 2).

Moreover, in comparison with the immature mortalities of 0.15, 0.26 and 0.28 in the untreated control lines, the parental, F_1 and F_2 generations had significantly increased immature mortalities of 0.54, 0.52 and 0.55, respectively (Table 2). Adult deformities, on the other hand, showed an increasing trend of 0.02, 0.03 and 0.03, respectively in the three successive generations; although no adult deformity was recorded in the control lines (Table 2). DE-treated adult deformities ranged from deformed head, wings and legs (Fig. 3b), small size (Fig. 3c), much reduced wings and abdomen (Fig. 3d) to enlarged head and eyes (Fig. 3e) an overall reduction in all body parts (Fig. 3f). All these results clearly demonstrate the adverse effects of DE on such vital life-history traits as immature durations and mortalities, and adult deformities in *M. domestica* under study.

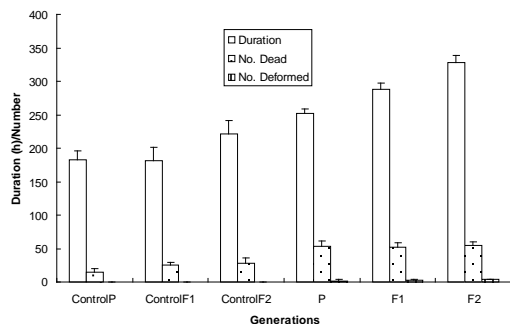


Fig 2: Immature durations and deaths, and number of deformed adults in the control and DE-treated *M. domestica* during parental through F₂ generations (values are mean \pm SD for five replicates)

Table 2: DE-induced changes in immature growth inhibition and mortality, and adult deformity in *M. domestica*

Generations	Growth Inhibition (h)	Immature Mortality (%)	Adult Deformity (%)
P			
Control	0.0 \pm 0.0 ^a	0.15 \pm 0.05 ^a	0.0 \pm 0.0 ^a
DE-treated ¹	68.2 \pm 16.9 ^b	0.54 \pm 0.08 ^b	0.02 \pm 0.01 ^b
F ₁			
Control	0.0 \pm 0.0 ^a	0.26 \pm 0.03 ^c	0.0 \pm 0.0 ^a
DE-treated ¹	106.2 \pm 18.0 ^c	0.52 \pm 0.07 ^b	0.03 \pm 0.08 ^c
F ₂			
Control	0.0 \pm 0.0 ^a	0.28 \pm 0.08 ^c	0.0 \pm 0.0 ^a
DE-treated ¹	106.8 \pm 12.6 ^c	0.55 \pm 0.05 ^b	0.03 \pm 0.01 ^c
F _{5,24} -values	80.44***	38.37***	17.96***

Values are mean \pm SD for five replicates; P, F₁ and F₂ refer to parental, F₁ and F₂ generations, respectively; ¹0.6636 mg of DE 100mL⁻¹ larval food medium; mean \pm SD values with dissimilar superscript letters differ significantly by LSD tests (P<0.05); ***= P<0.001.

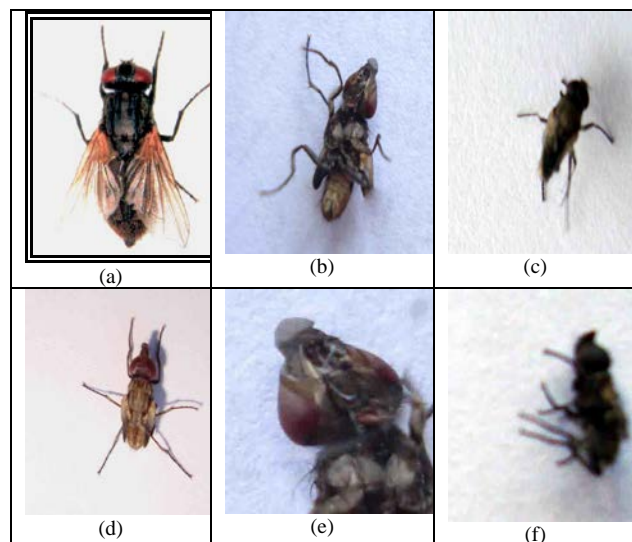


Fig 3: Normal adult in the control line (a) and variously deformed adults in the DE-treated lines (b-f) of *M. domestica*

Salivary gland morphometrics

Salivary glands of the untreated and DE-treated larvae are shown in Fig. 4. The lengths and widths of the salivary glands in the third-instar larvae of the experimental flies revealed that DE treatments increased the gland lengths appreciably (F= 17.66; P<0.001), although changes in the gland widths were statistically insignificant (Table 3). The results suggest that the larvicidal effect, as well as other adverse effects of the DE product on immature durations, growth inhibition, immature mortalities and adult deformities might be interpreted in terms of the drastic changes in the larval salivary glands of the experimental insects.

Table 3: Salivary gland morphometrics in the control and DE-treated third-instar larvae of *M. domestica*

Third-instar larvae	Length (mm)	Width (mm)
Control (n= 20)	265.7 \pm 40.6	42.0 \pm 8.6
DE-treated (n= 20)	322.5 \pm 44.9	45.4 \pm 4.9
F _{1,38} -values	17.66***	2.37ns

***= P<0.001; ns= not significant

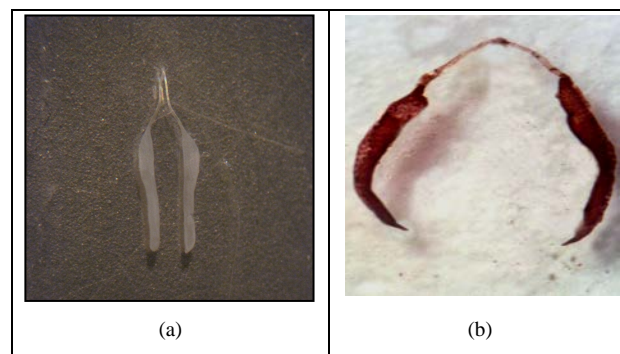


Fig 4: Salivary glands in the control (a) and DE-treated (b) third-instar larvae of *M. domestica*

Discussion

In the present study a thorough investigation has been made 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 such vital reproductive features of the houseflies as immature durations and mortality, and adult deformity, which ensures inhibition of the population build-up of the insects.

Previous researchers reported that overall reproduction, survivorship and physiology of a number of different pest species are inversely affected by DE treatments. Thus, DE at doses from 8-32 mg/g food at 24-, 48-, 72-, 96- and 120h exposure periods was found repellent against *T. castaneum* (Hossain *et al.*, 2010; Reza *et al.*, 2012), and population build ups in *T. castaneum* and *S. oryzae* were checked by DE treatments (Kabir *et al.*, 2011). The estimated LD₅₀ values of DE were found to vary from 2.60 ppm to 42.73 ppm against *C. maculatus* and *S. oryzae* (Islam *et al.*, 2010). DE can also be used successfully for the control of infestations with American and German cockroaches *Periplaneta americana* and *Blattella germanica* as well as silverfish *Lepisma saccharina* (Faulde *et al.*, 2006). Moreover, findings on the efficacy and persistence of DE against four common tropical storage pests *Prostephanus truncatus*, *S. zeamais*, *C. maculatus* and *Acanthoscelides obtectus* revealed increased parental mortality and reduced F₁ progeny emergence (Stathers *et al.*, 2004), which nicely corroborate to the present results.

DE was found lethal to adult mealworms *Tenebrio molitor* and *T. confusum*, but their larvae were unaffected; it was lethal to the first-instar larvae of *P. interpunctella*, but not lethal to older larval stages (Korunic, 1998; Fields and Korunic, 2000). Contact with DE caused adult *S. granarius*, *T. molitor* and *T. confusum* to lose weight and reduce their water contents (Rigaux *et al.*, 2001). However, two week-old larvae of *T. confusum* were more sensitive to DE than *P. interpunctella* at the same age (Mewis and Ulrichs, 2001), and the efficacy of DE against the adults of *T. castaneum* and *S. granarius* was found satisfactory (Cook, 2003). These findings are in well agreement with the present results.

Three commercially available DE products were reported to give significant protection against *S. oryzae*, *T. castaneum* and *R. dominica* in which significant adult mortalities were noted at 500, 1000 and 1500 ppm dose levels. ((Kavallieratos *et al.*, 2007; Kostyukovsky *et al.*, 2010). These findings are in good agreement with those reported here for houseflies in terms of larval and pupal durations and mortalities following DE treatments from parental to F₂ generations.

Reports on the effects of DE on dipteran insects are relatively scarce⁴ (Weinzierl and Jones, 2000). Circumstantial evidence, however, indicate that various body characteristics of insects including houseflies are negatively affected by DE (Garrett, 2008; eHow contributor, 2011; Griep, 2012; Safe Solutions Inc., 2012). Recently, Rahman (2012) has reported DE-induced changes in reproductive parameters in *M. domestica*, and

Islam and Rahman (2016) have demonstrated that egg-laying, egg-to-adult developmental period, adult emergence and female ratio of houseflies are significantly deteriorated by DE treatments in the larval food. The present results lend support to these findings. But no satisfactory explanation could be offered right now on DE-induced changes in the salivary glands morphometrics of the experimental flies reported here for the first time. It could be conjectured that the inert dust might have compelled certain physiological alterations, which might have triggered the observed changes in salivary gland measurements. Unfortunately, no literature is available to compare the data. Nevertheless, the present results are encouraging due to the fact that immature durations and mortalities, adult appearance and survival of the experimental insects were profoundly affected by the DE product. Further experiments with DE treatments in the household dumps and farm premises are designed and solicited for execution in the near future.

Conclusion

The present findings clearly demonstrate that DE at LC₅₀ of 0.6636 mg.100mL⁻¹ larval food could be used as an efficient larvicide against *M. domestica*. Owing to its low mammalian toxicity, DE dust could be utilized to reduce immature as well as adult survival of the houseflies under domestic and field conditions. It could be spread through the areas where houseflies are frequent, for example, abandoned food, manure and other places where they lay eggs and larvae grow up. The present results suggest that DE could be used for housefly larvicide, repellent and/or contact insecticide. It would decrease hatching, kill the larvae and/or reduce the number of the emerging adults, thus inhibiting population build-up of this commensal vector species of public health and veterinary importance.

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