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Efficacy of neem extract against the blowfly and housefly

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Abstract The blowfly, *Chrysomya megacephala* (Fabricius), and housefly, *Musca domestica* Linnaeus, are ubiquitous insects that have the potential to spread a variety of pathogens to humans and livestock. Pest management techniques for populations of these flies are needed. Currently, bioinsecticides, particularly those derived from plant origin, have been increasingly evaluated in controlling populations of medically important insects. In this study, an attempt was made to evaluate the efficacy and biological activity of a commercially available neem extract, containing 0.24% azadirachtin A, against *C. megacephala* and *M. domesitca*. Laboratory bioassays were performed using the feeding method of mixing neem solutions with fresh beef, once or multiple times, as food for rearing third instar. The laboratory tests showed that neem products significantly reduced larval and pupal survival, adult emergence, pupal weight, adult wing length, and fecundity on the subsequent generation, in a dose-dependent manner in both species. Efficacy was observed in the first generation and could extend to the second generation. Despite these reductions, reduction in total adult longevity was not evident for larvae fed once on neem solutions with fresh beef, and slightly earlier (≈1 week) mortality was observed in both species when they were fed as larvae on multiple doses. Adverse effects of this neem-based product toward *M. domestica* were slightly greater than those in *C. megacephala*. These data reinforced the efficacy of neem extract in reduced adult emergence and anti-fecundity in the subsequent generation. However, neem extract induced only low to moderate larval and pupal mortalities.

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Introduction

The blowfly, *Chrysomya megacephala* (Fabricius), and housefly, *Musca domestica* Linnaeus, are medically important insects worldwide. Their adults are not only pestiferous insects in the human environment (Zumpt 1965; Greenberg 1971), but also mechanical carriers and/or reservoirs of several pathogens, i.e., bacteria, viruses, protozoan cysts, and helminth eggs, which can cause disease in humans (Greenberg 1971; Monzon et al. 1991; Fotedar et al. 1992; Sulaiman et al. 2000; Maldonado and Centeno 2003; Sukontason et al. 2007). It has been recorded that the emergence of rhino-conjunctivitis in humans is specifically due to sensitization to the adult housefly (Focke et al. 2003). The larvae of these flies can also be myiasis-producing agents in humans and animals, thus leading to economic loss, particularly in agronomic livestock (Zumpt 1965; Zhu and Lin 1999; Bhatt and Jayakrishnan 2000; Kumarasinghe et al. 2000; Jiang 2002; Sehgal et al. 2002). Regarding this, fly-borne and fly-caused diseases lead to human health concerns and economic impact, particularly in countries with tropical and subtropical climates, in which

these fly species prevail. In Thailand, these two fly species account for ≈90% of the domestic flies collected (Sucharit et al. 1976; Tumrasvin et al. 1978; Sucharit and Tumrasvin 1981), and myiasis cases caused by *C. megacephala* or *M. domestica* have been reported (Sukontason et al. 2005).

Although chemical insecticides can effectively reduce fly populations, some serious side effects from these chemicals can result from residuals found in food, the environment, and non-target organisms. Long persistence of insecticides in the environment could create an accumulation of chemicals, which are magnified in food chains or food webs to eventually affect humans and animals. Long-term utilization can also cause insecticide resistance in target pest populations. These problems highlight the need for alternative control measures for the long-term management of pest populations, without harming the environment. Therefore, much effort has been focused on bioinsecticides, in particular those derived from plant origin, as a potential source of insect control agents. Of these, extract from the neem tree, *Azadirachta indica* (A. Juss) (Meliaceae), has been extensively documented as a promising agent with various insecticidal effects against many groups of insect. Example of this include: reduction of fecundity and post-embryonic development in the melonfly, *Bactocera cucurbitae* (Coq.), oriental fruit fly, *Bactocera dorsalis* (Hendel) (Singh 2003), and root weevil, *Diaprepes abbreviatus* (L.) (Weathersbee III and Tang 2002); fecundity and longevity of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Steffens and Schmutterer 1982; Di Ilio et al. 1999) and the bug, *Clavigralla scutellaris* Westwood (Mitchell et al. 2004); delay of growth development in the moth, *Helicoverpa armigera* (Hübner) (Ma et al. 2000); inhibition of feeding in the mosquitoes, *Culex tarsalis* Coquillett and *Culex quinquefasciatus* Say (Su and Mulla 1998a); ovicidal activity in the mosquitoes, *C. tarsalis* and *C. quinquefasciatus* (Su and Mulla 1998b); inhibition of oviposition in the mosquitoes, *Anopheles stephensi* Liston and *Anopheles culicifacies* Giles (Dhar et al. 1996); larvicidal activity in the horn fly, *Haematobia irritans* (L.), stable fly, *Stomoxys calcitrans* (L.), and housefly (Miller and Chamberlain 1989); and repellency effect in the sand flies, *Phlebotomus papatasi* (Scopoli) (Dhiman and Sharma 1994) and *Phlebotomus argentipes* Annandale and Brunetti (Sharma and Dhiman 1993), and mosquito, *A. culicifacies* (Sharma et al. 1993), which was also reviewed by Mulla and Su (1999). Despite the results just listed, probability of insect to product effect from *A. indica* is low (Vollinger and Schmutterer 2002). By taking the results of these works into account, the aim of this study was to assess the efficacy of the neem product from Thailand against *C. megacephala* and *M. domestica* in the laboratory, thereby providing the feasibility of this natural product for suppressing populations of these fly species in Thailand and elsewhere.

Materials and methods

Fly source and rearing

The adult *C. megacephala* and *M. domestica* used in this study were obtained from a laboratory colony reared in the insectarium at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai (at 17–21° N, 98–99° E), Thailand. Laboratory colonies were maintained at an ambient temperature of 24–28°C with a light/dark photoperiod of ≈12:12 h. Larvae were fed a fresh pork liver diet. Adults were reared on two kinds of food: (1) a mixture of 10% (*w*/*v*) sugar and multivitamin syrup solution and (2) fresh pork liver (used as both a food source and oviposition site; Sukontason et al. 2004).

Biopesticide and plant source

A formulated neem extract (Sadao Thai 111) was obtained from Thai Neem Products Co., Ltd (Thailand). This product was derived from the neem seed, *Azadirachta indica* var. *siamensis*, extracted with ethanol. The product was screened for levels of azadirachtin using high pressure liquid chromatography at the Department of Agriculture, Ministry of Agriculture and Cooperatives, Thailand. In this study, the product contained 0.24% azadirachtin A.

Laboratory bioassays

Experiment 1: feeding bioassay with a single dose of neem extract for C. megacephala and M. domestica

One hundred and fifty newly hatched larvae (the first instar), obtained from the same batch of eggs, were divided into five groups of 30 larvae each. The larvae in each group were introduced into separate transparent plastic boxes using a new camel-haired brush (No. 4) and provided daily with fresh beef (10 g cut into two small pieces) as a food source. Then, the boxes were tightly sealed with a lid. The lids were cut to ≈3/4 the total size of the box top, with the remaining area covered by a fine silk screen cloth (100 meshes/mm^2) for ventilation.

Once the larvae had reached early third instar, neem extract was added to their food source, while the controls (group 1) in this experiment were fed only 10 g of fresh minced beef. In the treated groups, the commercial neem extracts were prepared at 0.025%, 0.05%, 0.1%, and 0.2% concentration, with distilled water used as a solvent. These suspensions were incorporated with 10 g of fresh minced beef for groups 2, 3, 4, and 5, respectively. The beef with neem extract was fed to the larvae only on the first day of feeding. On the following day, the larvae of all treated groups were fed on a fresh change of 10 g of fresh beef

without neem, and unadulterated fresh beef was replaced daily thereafter until the beginning of pupation. This experiment was replicated three times.

Experiment 2: feeding bioassay with multiple doses of neem extract for C. megacephala and M. domestica

One hundred and fifty newly hatched larvae (the first instar), obtained from the same batch of eggs, were divided into five groups of 30 larvae each. The larvae in each group were introduced into separate transparent plastic boxes using a new camel-haired brush (No. 4) and provided daily with fresh beef (10 g cut into two small pieces) as a food source. Then, the boxes were tightly sealed with a lid. The lids were cut to ≈3/4 the total size of the box top, with the remaining area covered by a fine silk screen cloth (100 meshes/mm^2) for ventilation.

Once the larvae had reached early third instar, neem extract was added to their food source, while the controls (group 1) in this experiment were fed only 10 g of fresh minced beef. In the treated groups, the commercial neem extracts were prepared at 0.025%, 0.05%, 0.1%, and 0.2% concentration, with distilled water used as a solvent. These solutions were incorporated with 10 g of fresh minced beef for groups 2, 3, 4, and 5, respectively. The neem extract was added to the fresh beef, and this combination was replaced daily thereafter until the beginning of pupation. Mortality of the larvae was observed and recorded from the start of the experiment, and the developmental time of larvae in each group was noted. The larvae were counted until the beginning of pupation. This experiment was replicated three times.

Parameters

The effects of neem extract on development were evaluated by the following eight parameters: (1) larval mortality, (2) pupal mortality, (3) pupal weight (average weight per pupa), (4) percentage of adult emergence, (5) longevity of adults, (6) survival rate of adults, (7) wing length, and (8) fecundity.

Larval mortality was recorded daily at the beginning of the feeding bioassay until the end of the study. Pupal mortality was also observed daily during the pupation period. Pupal weight, one of the indicators for evaluating development (Armbruster and Hutchinson 2002), was examined. As for the weighing procedure, all pupae were individually transferred onto three digital decimal scales (Shinko Denshi, Japan) using a piece of paper to prevent trauma to the puparia. Only the dark brown puparia (the third day of pupation) were weighed from each group, and the percentage of adult emergence was recorded daily.

To determine the longevity of adults, the flies were fed with 10% glucose mixed with 1.5% multivitamin syrup and fresh beef once they had emerged within the rearing cage. This food was changed daily. On the fifth day of emergence, the males were transferred into a new cage with a food source, and they were observed daily for longevity.

Regarding the survival rate of adults, flies that emerged from the puparia were examined daily for their survival. The dead males were recorded by number and then processed to evaluate the adult size by measuring their wing length—one of the indicators of insect growth (Kitthawee et al. 1992; Armbruster and Hutchinson 2002). The right wing of each fly was dissected using a sharp blade under a dissecting microscope (Olympus, Japan) and transferred onto a glass slide using fine forceps. Another glass slide was placed to brace the wing. The wing length was measured from the axial incision to the R_{4+5} vein, excluding the fringe setae, using a vernier caliper under the dissecting microscope.

As for oviposition by females, each fly was transferred into a small cage (16×16×16 cm) for individual observation. Glucose and fresh beef were provided as food and an oviposition site. When oviposition occurred (batch of eggs on the beef), flies in the second generation were reared using fresh beef as food, without adding the neem extract. All females were recorded for their longevity. Once they had died, their wing lengths were measured as previously described for males.

The parameter of fecundity was monitored from the second generation of the flies. Since the egg and first instar of the flies are delicate, fecundity was assessed by counting the number of second instar.

Data analysis

Mortality data are shown as mean ± SE. Difference of the mean among treatment and control groups was determined by the chi-square test. All analyses were performed using SPSS version 10.1, and the level of $\propto=0.05$ was deemed significantly different.

Results

The effects of neem extract, given once only and in multiple doses, on *C. megacephala* are summarized in Table 1 in the form of percent larval and pupal mortalities and adult emergence. The average of larval and pupal mortalities was dose dependent and in agreement with the adult emergence (Table 1). The average larval mortalities in the treated groups decreased significantly in the first generation of flies assessed, but higher mortalities were

Table 1 Bioactivity of *C. megacephala* after feeding third instar with neem extract (0.24% azadirachtin A) in the first generation

Treatment[a]	% Larval mortality (range, %)[b]			% Pupal mortality (range, %)[b]			% Adult emergence (range, %)[b]		
	First gen.	Second gen.	Third gen.	First gen.	Second gen.	Third gen.	First gen.	Second gen.	Third gen.
Single dose									
Control	1.11a (0.00–3.33)	1.11a (0.00–3.33)	2.22a (0.00–3.33)	1.11a (0.00–3.33)	1.11a (0.00–3.33)	0.00a (0.00–0.00)	97.78a (96.67–100.00)	97.78a (93.33–100.00)	97.78a (93.33–96.67)
0.025	5.56a,b (3.33–6.67)	2.22a (0.00–3.33)	3.33a (0.00–3.33)	5.56a,b (3.33–6.67)	1.11a (0.00–3.33)	0.00a (0.00–0.00)	88.89b (86.67–90.00)	96.67a (93.33–100.00)	96.67a (93.33–96.67)
0.05	7.78a,b,c (6.67–10.00)	2.22a (0.00–3.33)	2.22a (0.00–3.33)	13.33b,c (10.00–16.67)	2.22a (0.00–3.33)	2.22a (0.00–3.33)	78.89b,c (76.67–80.00)	95.56a (93.33–100.00)	95.56a (93.33–96.67)
0.1	13.33b,c (10.00–16.67)	4.44a (3.33–6.67)	3.33a (3.33–6.67)	14.44b,c (13.33–16.67)	3.33a (3.33–3.33)	1.11a (0.00–3.33)	72.22c (70.00–73.33)	92.22a (90.00–93.33)	95.56a (96.67–96.67)
0.2	16.67c (13.33–20.00)	4.44a (3.33–6.67)	3.33a (3.33–6.67)	17.78c (16.67–20.00)	3.33a (3.33–3.33)	2.22a (0.00–3.33)	65.56c (63.33–70.00)	92.22a (90.00–93.33)	94.44a (96.67–100.00)
Multiple dose									
Control	0.00a (0.00–0.00)	2.22a (0.00–3.33)	3.33a (3.33–3.33)	2.22a (0.00–3.33)	1.11a (0.00–3.33)	1.11a (0.00–3.33)	97.78a (96.67–100.00)	96.67a (93.33–100.00)	95.56a (93.33–96.67)
0.025	11.11b (10.00–13.33)	2.22a (0.00–3.33)	4.44a (3.33–6.67)	15.56b (13.33–16.67)	4.44a (3.33–6.67)	2.22a (0.00–3.33)	73.33b (73.33–73.33)	93.33a (90.00–96.67)	93.33a (90.00–96.67)
0.05	18.89b (16.67–20.00)	4.44a (3.33–6.67)	3.33a (3.33–3.33)	13.33b (13.33–13.33)	4.44a (3.33–6.67)	2.22a (0.00–3.33)	67.78b (66.67–70.00)	91.11a (90.00–93.33)	94.44a (93.33–96.67)
0.1	21.11b,c (20.00–23.33)	3.33a (0.00–6.67)	3.33a (3.33–3.33)	18.89b,c (16.67–20.00)	5.56a (3.33–6.67)	3.33a (3.33–3.33)	60.00b,c (56.67–63.33)	91.11a (86.67–93.33)	93.33a (93.33–93.33)
0.2	24.44c (23.33–26.67)	4.44a (3.33–6.67)	3.33a (3.33–3.33)	25.56c (23.33–30.00)	5.56a (3.33–6.67)	3.33a (3.33–3.33)	50.00c (46.67–53.33)	90.00a (86.67–93.33)	93.33a (93.33–93.33)

Gen. Generation

[a] Each treatment involved 30 larvae and was replicated three times.

[b] Averages followed by the same lowercase letters within a column are not significantly different ($P>0.05$; chi-square test).

detected in the multiple dose group. Among the concentrations tested, the 0.2% neem extract caused 16.67% and 24.44% larval mortality in the once only and multiple dose groups, respectively. However, neem extract did not interfere with the developmental rate of the surviving larvae in the treated groups. In the case of both once only and multiple doses, the larvae's growth rate was similar to that of the controls, with their reaching pupal stage by days 5 to 7 after hatching. As for *C. megacephala* pupal mortality and adult emergence, the neem extract seemed to only affect these parameters in the first generation of flies (Table 1).

In the case of *M. domestica*, the effects of neem on the biological activities of this species were similar to those for *C. megacephala* in that larval and pupal mortalities increased when applied with high doses of neem extract (Table 2). However, *M. domestica* showed higher susceptibility to neem extract than *C. megacephala*, by having higher larval mortalities (Table 2). Female *M. domestica* that survived to adulthood produced no eggs after feeding on neem concentrations at or above 0.1% and 0.2% (Table 2).

The pupal weight assessment after the third instar of *C. megacephala* and *M. domestica* had been fed with neem extract demonstrated a median pupal weight that statistically decreased in the first and second generations ($P>0.05$; Mann–Whitney *U* test; Tables 3 and 4, respectively), with a greater decrease observed in *M. domestica* (Table 4).

There was no significant difference in adult longevity between *C. megacephala* and *M. domestica* after being treated with a single dose of neem extract, but a slight decrease was noticed after multiple doses (Table 5). Weekly observations of *C. megacephala* after feeding on neem extract in experiment 1 showed no significant difference in the survival rate between male and female after third instar being fed once only. Adult survival decreased sharply from beginning to end from the fourth to the seventh week. In contrast, multiple doses of neem extract resulted in earlier mortality (the third week) in both male and female *C. megacephala*. Assessment in *M. domestica* exhibited a similar trend (data not shown).

Reduction in adult size, as determined by wing length, after the third instar were fed with neem extract, was similar for both *C. megacephala* (Table 6) and *M. domestica* (Table 7), particularly in the case of females of the first generation ($P>0.05$; Mann–Whitney *U* test). Likewise, reduction in fecundity, expressed as the number of progeny in *C. megacephala* (Table 8) and *M. domestica* (Table 9) in two subsequent generations, was found, with *M. domestica* showing more effect by laying no eggs in the second generation (Table 9). There was no effect on fecundity in the third generation of *C. megacephala* (Table 8) and *M. domestica* (Table 9) that had been fed once only on neem extract.

Table 2 Bioactivity of *M. domestica* after feeding the third instar with neem extract (0.24% azadirachtin A) in the first generation

Treatment[a]	% Larval mortality (range, %)[b]			% Pupal mortality (range, %)[b]			% Adult emergence (range, %)[b]		
	First gen.	Second gen.	Third gen.	First gen.	Second gen.	Third gen.	First gen.	Second gen.	Third gen.
Single dose									
Control	1.11a (0.00–3.33)	1.11a (0.00–3.33)	1.11a (0.00–3.33)	2.22a (0.00–3.33)	2.22a (0.00–3.33)	1.11a (0.00–3.33)	96.67a (93.33–100.00)	96.67a (96.67–96.67)	97.78a (96.67–100.00)
0.025	6.67a,b (3.33–10.00)	3.33a (3.33–3.33)	3.33a (3.33–3.33)	12.22b (10.00–13.33)	2.22a (0.00–3.33)	2.22a (0.00–3.33)	81.11b (76.67–83.33)	94.44a (93.33–96.67)	94.44a,b (93.33–96.67)
0.05	11.11b,c (10.00–13.33)	3.33a (3.33–3.33)	3.33a (3.33–3.33)	15.56b,c (13.33–16.67)	4.44a (3.33–6.67)	3.33a (3.33–3.33)	73.33b,c (70.00–76.67)	92.22a (90.00–93.33)	93.33a,b (93.33–93.33)
0.1	16.67b,c (13.33–20.00)	5.56a (3.33–6.67)	4.44a (3.33–6.67)	18.89b,c (16.67–20.00)	5.56a (3.33–6.67)	3.33a (3.33–3.33)	64.44c,d (60.00–70.00)	88.89a (86.67–93.33)	92.22a,b (90.00–93.33)
0.2	21.11c (16.67–23.33)	6.67a (6.67–6.67)	5.56a (3.33–6.67)	24.44c (23.33–26.67)	5.56a (3.33–6.67)	5.56a (3.33–6.67)	54.44d (53.33–56.67)	87.78a (86.67–90.00)	88.89b (86.67–93.33)
Multiple dose									
Control	2.22a (0.00–3.33)	2.22a (0.00–3.33)	1.11a (0.00–3.33)	3.33a (3.33–3.33)	2.22a (0.00–3.33)	2.22a (0.00–3.33)	94.44a (93.33–96.67)	95.56a (93.33–100.00)	96.67a (93.33–100.00)
0.025	16.67b (13.33–20.00)	6.67a (6.67–6.67)	3.33a (3.33–3.33)	16.67b (13.33–20.00)	4.44a (3.33–6.67)	2.22a (0.00–3.33)	66.67b (63.33–73.33)	88.89a,b (86.67–90.00)	94.44a (93.33–96.67)
0.05	20b,c (20.00–20.00)	8.89a (6.67–10.00)	4.44a (3.33–6.67)	17.78b (16.67–20.00)	5.56a (3.33–6.67)	3.33a (3.33–3.33)	62.22b,c (60.00–63.33)	85.56b (83.33–86.67)	92.22a (90.00–93.33)
0.1	24.44b,c (23.33–26.67)	ND	ND	26.67b,c (23.33–30.00)	ND	ND	48.89c (43.33–53.33)	ND	ND
0.2	33.33c (23.33–43.33)	ND	ND	35.56c (30.00–40.00)	ND	ND	31.11d (20.00–46.67)	ND	ND

Gen. Generation, *ND* not determined, as adults in the first generation did not lay eggs

[a] Each treatment involved 30 larvae and was replicated three times.

[b] Values followed by the same lowercase letters within a column are not significantly different ($P>0.05$; chi-square test).

Table 3 Pupal weight of *C. megacephala* after feeding the third instar with neem extract (0.24% azadirachtin A) in the first generation

Treatment[a]	Median of pupal weight (range, mg)[b] (*n*)		
	First generation	Second generation	Third generation
Single dose			
Control	0.055 (0.032–0.069)a (89)	0.055 (0.032–0.066)a (89)	0.056 (0.035–0.068)a (88)
0.025	0.054 (0.031–0.062)a (85)	0.051 (0.031–0.063)b (88)	0.055 (0.031–0.065)a (87)
0.05	0.052 (0.030–0.063)b (83)	0.052 (0.029–0.064)b (88)	0.055 (0.031–0.067)a (88)
0.1	0.052 (0.028–0.060)b (78)	0.051 (0.035–0.062)b (86)	0.052 (0.028–0.064)a (87)
0.2	0.051 (0.027–0.065)c (75)	0.050 (0.029–0.067)b (86)	0.052 (0.032–0.065)a (87)
Multiple dose			
Control	0.054 (0.034–0.067)a (90)	0.055 (0.032–0.069)a (88)	0.055 (0.033–0.065)a (87)
0.025	0.051 (0.032–0.062)b (80)	0.053 (0.035–0.064)a,b (88)	0.056 (0.033–0.067)a (86)
0.05	0.049 (0.028–0.057)b,c (73)	0.051 (0.032–0.061)b (86)	0.052 (0.030–0.064)a (87)
0.1	0.047 (0.026–0.059)c (71)	0.052 (0.029–0.065)b (87)	0.054 (0.035–0.065)a (87)
0.2	0.044 (0.025–0.053)d (68)	0.050 (0.032–0.064)b (86)	0.053 (0.032–0.066)a (87)

[a] Each treatment involved 30 larvae and was replicated three times.
[b] Medians followed by the same lowercase letters within a column are not significantly different ($P>0.05$; Mann–Whitney *U* test).

Discussion

Laboratory bioassay evaluations of the neem extract containing 0.24% azadirachtin A performed in this study demonstrated increased larval and pupal mortalities in both *C. megacephala* and *M. domestica*, with the dose-dependent response having low to moderate effects (see Tables 1 and 2). More mortality was noticed in *M. domestica* than in *C. megacephala*, which may lead to lower pupal weight and adult emergence and smaller sized adults, and then, cause no egg laying in the second generation of *M. domestica*. The reason for more susceptibility to neem extract on the part of *M. domestica* as compared to *C. megacephala* in this study is unknown. However, the larvicidal effect of azadirachtin in our investigation is in line with the results obtained by Miller and Chamberlain (1989), who investigated the horn fly, *H. irritans*, and stable fly, *S. calcitrans* and *M. domestica*. On the other hand, failure to kill the immature stages of *C. megacephala* when continuously in contact with neem extract has been reported by Kumarasinghe et al. (2000), who observed this in larvae exposed to the homogenized fresh leaf extract of *A. indica*. To enhance toxicity, a combination of neem-based insecticide with other substances may be applied, as

Table 4 Pupal weight of *M. domestica* after feeding the third instar with neem extract (0.24% azadirachtin A) in the first generation

Treatment[a]	Median of pupal weight (range, mg)[b] (*n*)		
	First generation	Second generation	Third generation
Single dose			
Control	0.035 (0.021–0.049)a (89)	0.037 (0.021–0.048)a (89)	0.035 (0.021–0.048)a (89)
0.025	0.034 (0.020–0.047)b (84)	0.035 (0.021–0.045)b (87)	0.035 (0.021–0.045)a (87)
0.05	0.034 (0.019–0.047)b,c (80)	0.035 (0.018–0.046)b (87)	0.036 (0.019–0.049)a (87)
0.1	0.033 (0.019–0.045)c (75)	0.034 (0.020–0.045)b (85)	0.034 (0.018–0.046)a (86)
0.2	0.033 (0.017–0.046)c (71)	0.035 (0.019–0.047)b (84)	0.034 (0.020–0.047)a (85)
Multiple dose			
Control	0.034 (0.021–0.048)a (88)	0.035 (0.019–0.047)a (88)	0.035 (0.020–0.049)a (89)
0.025	0.030 (0.018–0.045)b,c (75)	0.033 (0.018–0.045)b (84)	0.034 (0.018–0.047)a (87)
0.05	0.030 (0.016–0.043)b,c (72)	0.034 (0.020–0.048)b (82)	0.035 (0.020–0.049)a (86)
0.1	0.028 (0.016–0.041)c (68)	ND	ND
0.2	0.025 (0.015–0.040)d (60)	ND	ND

ND Not determined, as adults in the first generation did not lay eggs
[a] Each treatment involved 30 larvae and was replicated three times.
[b] Medians followed by the same lowercase letters within a column are not significantly different ($P>0.05$; Mann–Whitney *U* test).

Table 5 Longevity of adult *C. megacephala* and *M. domestica* after feeding the third instar with neem extract (0.24% azadirachtin A) in the first generation

Treatment[a]	Median of longevity (range, day)[b] (*n*)			
	C. megacephala		*M. domestica*	
	Male	Female	Male	Female
Single dose				
Control	27 (4–41)a (45)	27 (3–40)a (43)	23 (3–37)a (40)	24 (3–39)a (47)
0.025	26 (3–42)a (38)	28 (4–38)a (42)	24 (4–38)a (35)	23 (4–37)a (38)
0.05	26 (5–39)a (34)	26 (4–37)a (37)	23 (3–37)a (32)	24 (3–40)a (34)
0.1	27 (4–41)a (26)	26 (3–39)a (39)	24 (3–36)a (28)	24 (4–38)a (30)
0.2	26 (3–39)a (32)	27 (3–38)a (27)	23 (3–36)a (28)	24 (3–39)a (21)
Multiple dose				
Control	26 (3–40)a (41)	27 (4–43)a (47)	23 (4–38)a (38)	24 (3–40)a (47)
0.025	25 (3–37)b (30)	27 (4–37)b (36)	21 (3–38)b (29)	21 (3–39)b (31)
0.05	24 (4–33)b (29)	26 (3–37)b (32)	20 (3–34)b (27)	20 (3–37)b (29)
0.1	25 (3–34)b (24)	26 (3–35)b (30)	20 (2–32)b (22)	21 (3–34)b (22)
0.2	21 (3–32)c (21)	27 (3–33)b (24)	19 (2–30)c (12)	20 (2–33)b (16)

[a] Each treatment involved 30 larvae and was replicated three times.
[b] Medians followed by the same lowercase letters within a column are not significantly different ($P>0.05$; Mann–Whitney *U* test).

previously reported by Singh et al. (2007), who combined neem with the bacteria, *Bacillus thuringiensis* Berliner, against the moth larvae, *H. armigera* (Hübner). In like manner, combining azadirachtin with the fungus, *Paecilomyces fumosoroseus* (Wize), increased mortality of the whitefly, *Bemisia argentifolii* Bellows & Perring (James 2003).

Suppression of adult emergence by feeding neem extract in the larval diet gave more long-term efficacy than immediate toxicity in both fly species in this study, particularly in the first generation (see Tables 1 and 2), suggesting the disruption of fly metamorphosis. The extent of growth disruption observed is comparable to that seen in other reports dealing with azadirachtin, which acts as a potent insect growth inhibitor (Miller and Chamberlain 1989; Annadurai and Rembold 1993). Naqvi et al. (2007) reported a similar condition for not only the partial emergence of adult *M. domestica*, but also for abnormalities in the development of second instar and deformation of pupae after administering neem extract. In addition,

Table 6 Wing length of *C. megacephala* after feeding the third instar with neem extract (0.24% azadirachtin A) in the first generation

Treatment[a]	Median of wing length (range, mm)[b] (*n*)					
	First generation		Second generation		Third generation	
	Male	Female	Male	Female	Male	Female
Single dose						
Control	8.2 (7.1–8.8)a (45)	8.4 (7.5–8.9)a (43)	8.2 (7.7–8.7)a (42)	8.4 (7.7–8.7)a (46)	8.2 (7.5–8.7)a (42)	8.3 (7.5–8.7)a (46)
0.025	8.2 (7.2–8.7)a (38)	8.3 (7.4–8.8)a (42)	8.1 (7.5–8.6)a (46)	8.2 (7.5–8.6)a (41)	8.2 (7.6–8.6)a (40)	8.3 (7.5–8.7)a (47)
0.05	8.2 (6.7–8.7)a (34)	8.4 (7.1–8.8)a (37)	8.2 (7.6–8.6)a (38)	8.2 (7.6–8.6)a (48)	8.2 (7.5–8.6)a (39)	8.2 (7.5–8.7)a (47)
0.1	8.2 (6.8–8.7)a (26)	8.2 (7.3–8.8)a (39)	8.2 (7.6–8.5)a (38)	8.3 (7.7–8.6)a (45)	8.2 (7.5–8.6)a (43)	8.3 (7.4–8.6)a (43)
0.2	8.1 (6.7–8.6)a (32)	8.3 (7.4–8.8)a (27)	8.1 (7.5–8.5)a (40)	8.2 (7.6–8.6)a (43)	8.1 (7.5–8.6)a (51)	8.2 (7.6–8.6)a (34)
Multiple dose						
Control	8.3 (7.1–8.7)a (41)	8.4 (7.5–8.9)a (47)	8.3 (7.3–8.7)a (39)	8.4 (7.5–8.7)a (48)	8.3 (7.6–8.6)a (41)	8.3 (7.5–8.7)a (45)
0.025	8.2 (7.2–8.6)a (30)	8.3 (7.2–8.8)a,b (36)	8.2 (7.5–8.6)a (45)	8.3 (7.5–8.7)a,b (39)	8.2 (7.6–8.6)a (39)	8.3 (7.6–8.6)a (45)
0.05	8.1 (7.4–8.5)a (29)	8.2 (7.5–8.7)b,c (32)	8.2 (7.4–8.6)a (38)	8.2 (7.4–8.7)b (44)	8.2 (7.5–8.6)a (40)	8.2 (7.5–8.6)a (45)
0.1	8.1 (7.1–8.6)a (24)	8.1 (7.4–8.6)b,c (30)	8.2 (7.4–8.6)a (41)	8.2 (7.3–8.6)b (41)	8.2 (7.4–8.6)a (38)	8.2 (7.5–8.6)a (46)
0.2	8.0 (6.5–8.4)a (21)	8.1 (7.3–8.7)c (24)	8.2 (7.4–8.6)a (35)	8.2 (7.5–8.6)b (46)	8.1 (7.5–8.6)a (39)	8.2 (7.7–8.6)a (45)

[a] Each treatment involved 30 larvae and was replicated three times.
[b] Medians followed by the same lowercase letters within a column are not significantly different ($P>0.05$; Mann–Whitney *U* test).

Table 7 Wing length of *M. domestica* after feeding the third instar with neem extract (0.24% azadirachtin A) in the first generation

Treatment[a]	Median of the wing length (range, mm)[b] (*n*)					
	First generation		Second generation		Third generation	
	Male	Female	Male	Female	Male	Female
Single dose						
Control	5.8 (5.2–6.4)a (40)	5.9 (5.2–6.5)a (47)	5.9 (5.3–6.4)a (45)	5.9 (5.3–6.6)a (42)	5.8 (5.2–6.4)a (40)	5.9 (5.2–6.5)a (48)
0.025	5.8 (5.1–6.3)a (35)	5.9 (5.2–6.3)a (38)	5.8 (5.2–5.3)a (41)	5.85 (5.3–6.4)a (44)	5.8 (5.1–6.3)a (36)	5.8 (5.2–6.5)a (49)
0.05	5.8 (5.0–6.2)a (32)	5.8 (5.3–6.4)a (34)	5.9 (5.2–5.3)a (43)	5.9 (5.3–6.4)a (40)	5.8 (5.2–6.3)a (37)	5.9 (5.1–6.4)a (47)
0.1	5.8 (5.2–6.1)a (28)	5.8 (5.1–6.4)a (30)	5.9 (5.0–6.4)a (38)	5.8 (5.3–6.4)a (42)	5.8 (5.3–6.2)a (39)	5.8 (5.2–6.4)a (44)
0.2	5.8 (5.2–6.2)a (28)	5.8 (5.2–6.4)a (21)	5.8 (5.1–6.3)a (37)	5.9 (5.2–6.4)a (42)	5.8 (5.3–6.3)a (34)	5.8 (5.2–6.3)a (48)
Multiple dose						
Control	5.8 (5.5–6.3)a (38)	5.9 (5.4–6.4)a (47)	5.8 (5.3–6.4)a (38)	5.9 (5.3–6.4)a (48)	5.9 (5.1–6.4)a (45)	5.9 (5.4–6.4)a (42)
0.025	5.6 (5.1–6.0)b (29)	5.7 (5.2–6.1)b (31)	5.8 (5.2–6.4)a (34)	5.8 (5.3–6.4)a (46)	5.9 (5.1–6.3)a (42)	5.9 (5.2–6.3)a (43)
0.05	5.6 (5.1–5.9)b (27)	5.7 (5.2–5.9)b (29)	5.8 (5.2–6.3)a (36)	5.8 (5.2–6.4)a (41)	5.8 (5.2–6.3)a (37)	5.8 (5.2–6.4)a (46)
0.1	5.3 (5.1–5.8)c (22)	5.3 (5.1–5.9)c (22)	ND	ND	ND	ND
0.2	5.3 (5.0–5.7)c (12)	5.4 (5.0–5.7)c (16)	ND	ND	ND	ND

ND Not determined, as adults in the first generation did not lay eggs
[a] Each treatment involved 30 larvae and was replicated three times.
[b] Medians followed by the same lowercase letters within a column are not significantly different ($P>0.05$; Mann–Whitney *U* test).

deformation of the mouthparts was recently detected in the nymph of the Southern green stink bug, *Nezara viridula* (L.), after exposure to neem extract, thereby rendering the insects incapable of feeding, which led to death (Singha et al. 2007). Aggarwal and Brar (2006) indicated that a low concentration of neem-based product (NeemAzal T/S 1.0% administered at 200 mg/l) on the whitefly parasitoid, *Encarsia sophia* (Gennadius), caused no effect on adult emergence, but a higher dose (800 mg/l) yielded a significant reduction of emergence.

Adult flies emerging from our experiments were assessed for their longevity, and our results showed that neem-treated flies demonstrated slightly reduced longevity when compared to the untreated controls, with males being more evident in this than females (see Table 5). The effect on longevity was more demonstrative in *M. domestica* than

Table 8 Fecundity of *C. megacephala* after feeding the third instar with neem extract (0.24% azadirachtin A) in the first generation, expressed as the number of second instar in the second, third, and fourth generations

Treatment[a]	Mean ± SE of second instar[b]		
	Second generation	Third generation	Fourth generation
Single dose			
Control	481.52±3.34a	497.42±2.75a	489.46±2.91a
0.025	355.04±3.48b	495.52±2.32a	487.53±2.35a
0.05	285.99±3.71c	482.84±1.66b	495.10±3.57a
0.1	272.15±3.17c	493.85±2.21a	490.11±3.59a
0.2	272.55±3.62c	481.36±1.76b	488.43±2.47a
Multiple dose			
Control	488.32±2.28a	499.83±2.02a	491.24±2.08a
0.025	273.64±3.06b	495.55±2.68a	485.95±3.86a
0.05	236.21±2.86c	474.86±1.83b	490.12±2.84a
0.1	205.44±3.00d	463.22±1.79c	478.74±1.97b
0.2	143.81±4.68e	465.96±1.83c	479.33±3.64b

[a] Each treatment involved 30 larvae and was replicated three times.
[b] Mean followed by the same lowercase letters within a column is not significantly different ($P>0.05$; Student *t*-test).

Table 9 Fecundity of *M. domestica* after feeding the third instar with neem extract (0.24% azadirachtin A) in the first generation, expressed as the number of second instar in the second, third, and fourth generations

Treatment[a]	Mean ± SE of second instar[b]		
	Second generation	Third generation	Fourth generation
Single dose			
Control	503.77±1.76a	486.26±2.18a	495.82±3.52a
0.025	498.81±1.87a,b	486.11±2.45a	491.17±3.67a
0.05	496.09±3.80b	493.68±1.83b	493.32±3.14a
0.1	475.79±1.79c	481.50±1.72a	488.91±1.86a
0.2	473.26±2.58c	481.63±1.90a	490.06±2.57a
Multiple dose			
Control	507.86±2.59a	497.38±2.16a	487.15±3.62a
0.025	201.67±2.67b	467.64±1.92b	492.72±2.83a
0.05	159.02±3.16c	435.37±2.05c	458.47±3.30b
0.1	ND	ND	ND
0.2	ND	ND	ND

ND Not determined, as adults in the first generation did not lay eggs
[a] Each treatment involved 30 larvae and was replicated three times.
[b] Mean followed by the same lowercase letters within a column is not significantly different ($P>0.05$; Student *t*-test).

in *C. megacephala*. This finding was in accordance with the data of Di Ilio et al. (1999), which showed that the longevity of *C. capitata* was slightly decreased after exposure to neem compound. In our study, the survival rates of adult *M. domestica* were barely observed in the first 3 weeks, but they, including the control flies, abruptly decreased at the beginning of the fourth week. Flies in the treated groups exhibited lower survival rates than those in the control group, in which mortality may be due to natural factors that are similar in other experiments (Weathersbee III and Tang 2002).

When the neem extract was provided with the larval diet, we observed a significant reduction in pupal weight and adult wing length in both *C. megacephala* and *M. domestica*, which indicated the smaller size of adult obtained. Multiple doses of neem extract confirmed the damage to both parameters. A similar finding on the reduction of weight in insects treated with neem extract was observed in *D. abbreviatus* larvae (Weathersbee III and Tang 2002), when mixing neem extract with larval food.

Our data provided evidence of the effect neem extract has against the fecundity of *C. megacephala* and *M. domestica*, as assessed by the second instar of the subsequent generation (see Tables 8 and 9). This phenomenon is in agreement with previously reported decreases in oviposition for insects treated with neem products (Musabyimana et al. 2001; Bruce et al. 2004). Large alterations in the fecundity of insects exposed to neem have been extensively reported, such as those in the fly, *C. capitata* (Steffens and Schmutterer 1982); banana root borer, *Cosmopolites sordidus* (Germar) (Musabyimana et al. 2001); and mosquitoes, *A. stephensi* and *A. culicifacies* (Dhar et al. 1996). The work published by Khan et al. (2007) microscopically demonstrated that the decrease in fecundity of *B. cucurbitae* and *B. dorsalis* exposed to neem compound was due to the block of ovarian development. Likewise, mixing of a commercial formulation of neem in the adult diet caused reduction in the fecundity of *C. capitata* by interfering with oogenesis (Di Ilio et al. 1999). The block in the ovarian activity of *C. capitata*, resulting from neem compound, was verified by histological observation (Di Ilio et al. 1999). Results from the study of Lucantoni et al. (2006) clearly indicated that the neem-treated female mosquito, *A. stephensi*, displayed a delay in oocyte development in the vitellogenesis and choriogenesis phases. Ultrastructural investigation of that study also revealed the complete block of oogenesis, impairment of vitellogenesis and vitelline envelope formation, and severe degeneration of follicle cells. As discussed by Weathersbee III and Tang (2002), the disruption of reproductive capability could lead to substantial population decline over time. Furthermore, Dhar et al. (1996) revealed that exposure to neem extract suppressed rather than inhibited oviposition in mosquitoes. This might, in part, explain our result that flies in the third generation showed normal bioavailability after exposure to neem. Otherwise, it is plausible that our neem-based product contained minimal azadirachin (0.24%) or the administration to flies was of too low of a dose.

The results of our experiments showed that the incorporation of neem extract in larval food of *C. megacephala* and *M. domestica* could lead to greater inhibition of growth and fecundity in subsequent generations than in larval and pupal mortalities. Regarding this, it could be implied that application in the field might delay fly development, and multiple applications would increase potential effects to prolong development for a more effective control strategy. An evaluation of the effects of neem-based products in the field is worth investigating.

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