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Inhibitory Activities of Ripe and Unripe Neem Seed Powders on the Life Cycle, Fecundity and Adult Emergence of Housefly *Musca Domestica* L. (Diptera: Muscidae)

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Abstract

This study evaluates the effects of ripe and unripe neem seed powder treated-diets on aspects of biology and reproduction of the housefly (*Musca domestica*) to determine the comparative efficacy of the neem seed powder on the housefly. Adult houseflies, were collected from the refuse dump site of Obafemi Awolowo University, Ile-Ife, Nigeria and were reared in a cage in the laboratory on a mixture of ground rice, ground fish and water in the ratio (1: 1: 1.5 w/v). Newly emerged adult houseflies were exposed to a paste of ground rice and fish supplemented with 5, 10, 15 and 20% concentrations of ripe and unripe neem seed powder respectively. Addition of neem seed power to housefly diet significantly decreased fecundity, ovary size and prolonged time to first egg laying when compared to the control. This effect was more severe with increasing concentrations of neem seed powder and varied with the source of neem powder (i.e. ripe or unripe seed). In general, ripe neem seed powder tends to have a more severe effect than the unripe neem seed powder. In addition, our study shows that neem seed powder disrupts the life cycle in *M. domestica*. Adult emergence of male and female from eggs deposited by females previously exposed to ripe and unripe neem seed-treated diets significantly decreased with the lowest emergence in the housefly fed with ripe neem seed treated diets. Results from this study presents potentials for the control of the housefly (*Musca domestica*) using extract from the neem plant.

Keywords: *Housefly; Neem; Fecundity; Ovary Size; Metamorphosis; Azadirachtin*

Introduction

The housefly, *Musca domestica* is a cosmopolitan pest which causes health problems in the environment [1]. It is a public health pest causing a serious threat to human and livestock by vectoring several pathogenic organisms such as protozoa cysts, helminth parasites, enteropathogenic bacteria, and enterovirus [2,3]. They are found in the tropics, warm temperate and cooler regions of the world [4]. Transmission takes place when the fly makes contact with people or their food. Most of the diseases can also be contacted directly through contamination of food, water, air, hands and person-to-person contact [5]. The body is covered with hair like projections and can mechanically transfer pathogens from the hairs of their body [6]. They can also regurgitate them in their vomit, and as well as transfer them in feces through their alimentary tract [5].

Plants have the richest source of natural pesticides and their extracts provide a safe and viable alternative to synthetic pesticides [7]. Botanicals are compatible with beneficial organisms, pest-resistant plants, thereby preserving a healthy environment in an effort to decrease reliance on synthetic pesticides. These multifarious biological activities against insects of agricultural and medical importance are primarily due to the presence of secondary metabolites [8]. Azadirachtin (AZA) is an active compound extracted from the Neem tree (*Azadiracta indica*) whose antiviral, antifungal, antibacterial and insecticidal properties have been known for several years [9]. For example [10], study on toxicity and residual effect of a topically applied crude extract of neem against second-instar larva of *Musca domestica* showed more deformed pupae and partial emergence of adult flies when compared with a pyrethroid (Coopex).

Azadirachtin structurally resembles insect ecdysones, which control the process of metamorphosis and exert a strong negative influence on behavior, postembryonic development and reproductive organs such as the ovary and testis and fecundity [11]. Fruit extracts of *M. azedarach* and *A. indica* showed feeding deterrent effects against the larvae of *Plutella xylostella* at higher doses [12] Similarly, NeemAzal-T/S (a commercial neem preparation) significantly inhibited larval growth and reduced feeding activity of pine moth, *Thaumetopoea pityocampa* [13] Aside being a feeding deterrent, azadirachtin has been shown to affect metamorphosis; for example injection of AZA into larvae of the blowfly led to a number of abnormal biological effects such as delay in pupation of larvae, reduction of pupal weight, and inhibition of adult emergence. Adults emerging from AZA-treated larvae were smaller and showed various malformations (Bidmon., *et al*.)**.** [14] also reported prolongation of larval duration, an inhibitory action on the larval development and with the halting of metamorphosis and morphogenesis which resulted in deleterious reduction of both pupation and adult eclosion in the *Muscina stabulans*. Similarly, boiled extract of the neem leaf of *Azadirachta indica* inhibited the growth of larvae and caused delay in pupation of *Anopheles gambiae* with a dose-dependent effect resulting in the death of larvae and formation of larval-pupal intermediates [15]. The third instar larvae of *Musca autumnalis* exposed to solutions of AZA resulted in the reduction of egg production egg hatchability and adult emergence [16]. Similar effects were also observed in third instar larvae of the cabbage moth, *Maestra brassicae* exposed to Neem EC in addition to failure of larval-larval and larval-pupal ecdysis [17].

With respect to nymph development [18], demonstrated that contact of neem seed oil on all the five nymphal instars of the gregarious phase of the desert locust, *Schistocerca gregaria* results in high mortality rate, prolonged nymphal development and distorted metamorphosis. Topically treated male and female nymphs of *Heteracris littoralis* with serial concentrations of azadirachtin showed dose-dependent adult mortality and death of the fourth and fifth instar nymphs about the time of ecdysis [19]. Injection of azadirachtin into last-instar nymphs of the American cockroach*, Periplaneta americana* delayed the molting process by a number of days [20].

Lethal activities of azadirachtin have been reported. Topical application of Jojoba oil and azadirachtin on the pre-pupae stage of *Rhynchophorus ferrugineus* increased lethal actions at all ages of pupae. Higher dose levels of each extract resulted in various adult deformities, shortened pupal durations and blocked adult emergence was blocked in different percent concentrations with (Bream., *et al*.) [21] also reported that azadirachtin effect on larval mortality was concentration dependent and caused different types of deformation in the larva, pupa and adult stages of mosquito.

Adult fecundity of *Culex pipiens* was reportedly decreased and sterility was increased by azadirachtin after treatment of the fourth instar and pupal stage with prolongation of duration of the larval stage [22]. Effects of different concentrations of neem extract and flufenoxuron, a synthetic insect growth regulator (IGR) on the histology of the ovary and testis of snout beetle, *Rhynchophorus ferrugineus* include growth and developmental disruptions which resulted in high mortality rate, reduction in body length, sex ratio, and morphological malformations in a dose-dependent manner [23]. This study further reveals that the IGR disrupted production of female gamete with the accumulation of yolk granules and follicular epithelial cells. Ovaries in treated adult females showed complete shrinkage with abolished oocyte growth, distortion of sperm tubes was observed in treated males and electron micrographs revealed disintegration and destruction in follicular cells and mitochondria in females.

Azadirachtin can be isolated in small amounts from all parts of the Neem tree, but it is present in highest concentration in the mature seeds [24]. In addition, potency of neem extract as pesticide varies with the part of plant that the extract was obtained. For example [25], demonstrated that the crude suspension of the neem seed had stronger larval mortality and anti-oviposition properties against noctuid moths than the pure azadirachtin. Onu and Baba [26] also reported that neem kernel oil and neem kernel powder suppressed oviposition and adult emergence in dermestid beetle, *Dermestes maculatus* while neem leaf powder caused the death to F_1 generation of the insect as well as disrupted developmental processes. Studies from [27] reported that neem seed kernel (NSK) incorporated in diets caused prolonged development of larval instars and subsequent emergence of adults in the blowfly (*Chrysomya chloropyga*), whereas, ripe neem seed (NS) led to reduction in survival, longevity and the fecundity in the same species.

The ripe neem seed powder has been used to study various aspects of the biology and control of several species of insect pests but there is little information on the use of unripe neem seed powder for the control of insect pests. The present study evaluates the comparative efficacy of ripe and unripe neem seed powder on the fecundity, ovary development and egg viability, life cycle and adult emergence of the housefly, *Musca domestica.*

Materials and Methods

Collection, Rearing and Maintenance of *Musca domestica*

The study was carried out in a well-lit insectary at temperature 28 ± 2°C and with 75 ± 5% relative humidity in Obafemi Awolowo University, Ile-Ife, Nigeria. Adult housefly, *Musca domestica* used were obtained from self-sustaining colony maintained on mixture of ground rice, ground fish and water in the ratio (1: 1: 1.5 w/v) and made into a paste [28]. Water and sugar were provided in the cage and the diet was checked regularly for eggs, which were removed in separate cages and allowed to hatch. The food was changed every 72 hours. Ripe and unripe neem seeds used for the experiment were collected from neem tree stands at different locations of Obafemi Awolowo University campus, Ile-Ife, Nigeria. They were de-pulped, washed, air-dried and separately blended into powdered form using Moulinex® blender in the laboratory. All the materials were kept at -10 °C in the laboratory before use.

Fecundity, Life cycle and sex ratio of newly-ecdysed adult housefly

Fecundity in treated and control flies was determined by daily picking and counting the number of eggs in each of the egg batches deposited by the females for up to 30 days of survival. The age at first egg laying by the females on each of the treated diets and control was also determined. The experiments were replicated four times. In order to evaluate life cycle and sex ratio of newly ecdysed flies, ripe and unripe neem seed powder were separately incorporated into the rice and fish diets at 5, 10, 15, and 20% concentrations in quadruplicate. Control experiments were set up without inclusion of the seed extracts in the diets. In each experiment, freshly laid egg batches obtained from the reservoir cages were exposed to the treated diets at different concentrations in a transparent plastic bowls. The bowls were covered with lids bearing muslin cloth at the center to provide aeration. Hatched eggs from control and treated diets were monitored daily at larva and pupa stages until adult emergence. The duration of development for each of the stages up to adult stage, as well as the sex ratios of adults were recorded accordingly. After four weeks, pupae that did not emerge into adults were considered dead and discarded.

Ovary development and egg viability in females maintained on ripe and unripe neem seed powder treated diets

The stages of the ovary development in females fed with treated diets at 5, 10, 15 and 20% concentrations of ripe and unripe neem seed powder and in control were determined. Randomly selected females from each of the treatment and control groups were dissected to expose the ovaries under illuminating Yashima Tokyo O.S.K. (No.750419) dissecting microscope at X2 magnification at days 0, 5, 10, 15 and 20 and photographed using the Sony Digital Camera 200α. The ovaries were measured with a meter ruler to determine the width and weighed using sensitive weighing balance. The experiment was replicated three times at each day of dissection.

Development of freshly laid egg batches from females maintained on the treated diets and control diets was observed to determine duration of stages of the life cycle up to adult emergence. Pupae that did not emerge into adults after four weeks were considered dead. Experiment was replicated four times.

Statistical analysis

We performed analysis of variance to evaluate the effects of different concentration of neem seed powder on fecundity, sex ratio, ovary length, ovary weight and egg viability. In addition, we used student T test to determine whether there is a significant difference between ripe and unripe neem seed powder. Post hoc test was conducted using Scheffe test.

Results

Fecundity, Life cycle and sex ratio of newly-ecdysed adult housefly

Figure 1 shows the mean number of eggs laid by females exposed to different concentrations of ripe and unripe neem seed-treated diets. Mean number of eggs decreased with increase in concentration of ripe and unripe neem seed from 78.26 ± 1.33 eggs (5%) to 34.83 ± 2.38 eggs (20%) in the ripe neem seed and 87.76 ± 1.64 eggs (5%) to 44.79 ± 0.61eggs (20%) in unripe neem seed) while mean number of eggs laid by the control females was 88.21 ± 1.77eggs. There was significant difference in the mean number of eggs at all concentrations including the control in ripe and unripe seeds respectively (df = 4, 95, F = 195.84, p = 0.0000; df = 4, 95, F = 270.73, p = 0.0001). In addition, there were significant differences in the mean fecundity in ripe and unripe neem seed extracts at all concentrations with more eggs laid on unripe neem seed-treated diet than on the ripe neem seed-treated diet (t = 0.05).

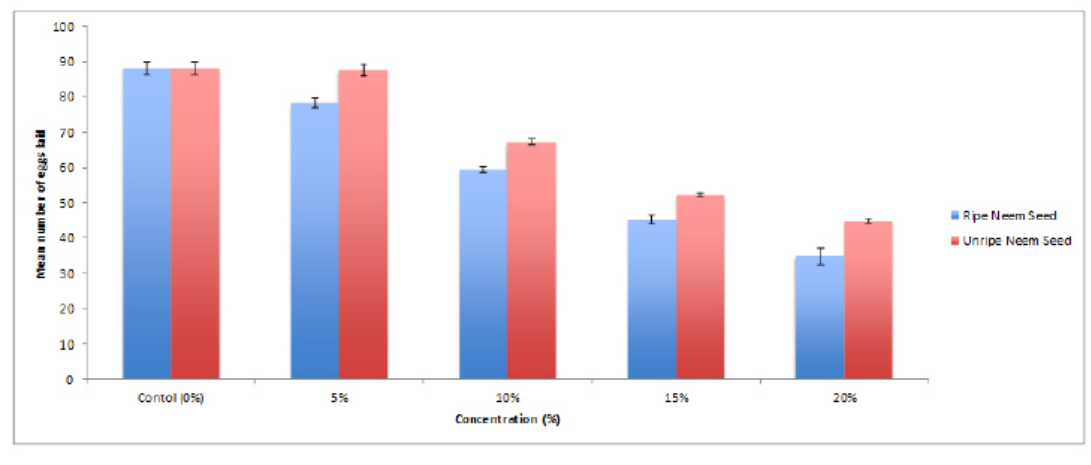

Figure 1: *Mean Fecundity of Female M. domestica Exposed to Diets Treated with Ripe and Unripe Neem Seed Powder.*

The mean day of first egg laying by females exposed to 5, 10, 15 and 20% concentrations of ripe and unripe neem seed-treated diets is shown in figure 2. Our results show that mean day of first egg laying increased with increase in concentration of ripe neem seed from day 3.50 ± 0.29 at 5% to 6.00 ± 0.001 at 20% concentration with a significant difference between the various concentrations and the control (df = 4, 15, F = 50.45, p < 0.0001). In the unripe neem seed-treated diet, mean day of egg laying was 3.25 ± 0.25 days at 5% concentration. It increased progressively with increase in concentration reaching 4.75 ± 0.25 days at 20% concentration. There was no significant difference in the mean day of egg laying at 5-20% concentrations but was significant when compared with the control (df = 4, 15, F = 50.45, p< 0.0001). Mean day to egg laying in females exposed to all concentrations of unripe neem seed were less females exposed to ripe neem seed-treated diet. There was no significant difference in the mean day of first egg laying in the ripe and unripe neem seed-treated diets at 5 and 10% concentrations but was significant at 15 and 20% concentrations (t = 0.05 and t = 0.003).

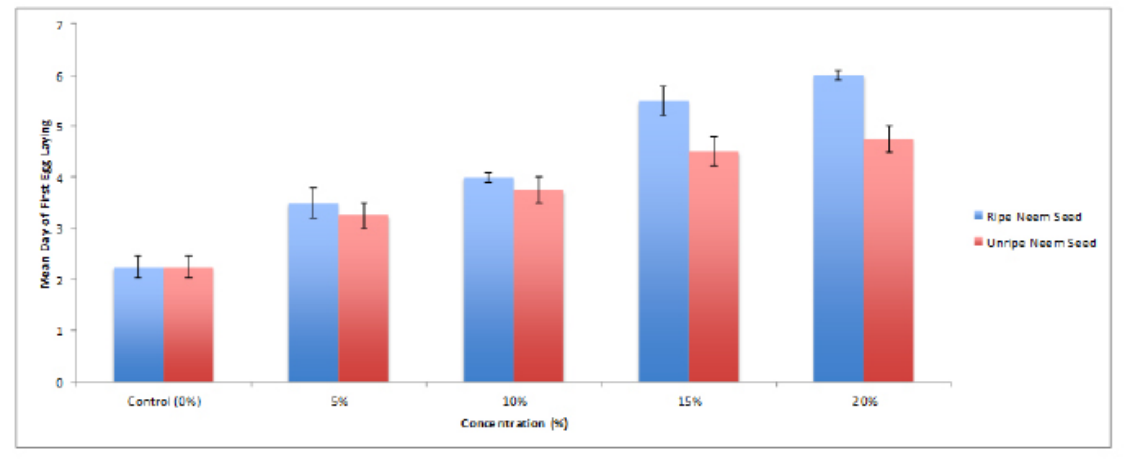

Figure 2: *Mean Day of First Egg Laying in Females of M. domestica Exposed to Ripe and Unripe Neem Seed Treated Diets.*

Table 1 presents the life cycle of *M. domestica* exposed to different concentrations of ripe neem seed-treated diets. Development was complete from egg to adult in eggs maintained on 5% ripe neem seed treated diet and control. Development of eggs on 10 and 15% treated diets terminated at the third instar larva while in the 20% treated diet, it did not develop beyond second instar larva. Total duration of development decreased with increase in concentration of the extract from 13.50 ± 0.29 days at 5% to 4.25 ± 0.48 days at 20% with 14.75 ± 0.25 days in the control. There was significant difference in the mean duration of development at all concentrations and the control (df = 4, 15, F = 84.605, p = 0.001).

Treatment	Egg	Larva			Pupa
		1st instar	2nd instar	3rd instar	
Control 0%	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	5.50 ± 0.29	7.25±0.25
5%	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	4.75 ± 0.25	14.75 ± 0.25[a]
10%	0.00 ± 0.00	1.25 ± 0.25	1.50 ± 0.29	3.75 ± 0.25	
15%	0.50 ± 0.29	1.50 ± 0.29	1.75 ± 0.25	4.00 ± 0.00	
20%	0.75 ± 0.25	1.50 ± 0.29	2.00 ± 0.00		

Table 1: *Duration of Life Cycle of M. domestica on Ripe Neem Seed-Treated Diets at Different Concentrations Mean values followed by the same letter(s) along the same column are not significantly different (p ≤ 0.05) by Scheffe test.*

We observed complete development from (i.e. egg to adult) in eggs maintained on 5% unripe neem seed-treated diet and control (Table 2) Development of eggs on 10, 15 and 20% unripe neem seed-treated diets was terminated at the third instar larva. There was significant difference in the mean duration of development at all concentrations and control (df = 4, 15, F = 72.109, p = 0.001). Total duration of development decreased with increase in concentration of the extract from 13.00 ± 0.001 days at 5% to 4.25 ± 1.11 days at 20% concentration. There was no significant difference in the mean total duration of development of the life stages at 5, 10, 15 and 20% ripe and unripe neem seed-treated diet and in the control.

Treatment	Egg	Larva			Pupa
		1st instar	2nd instar	3rd instar	
Control 0%	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	5.50 ± 0.29	7.25±0.25
5%	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	4.00 ± 0.00	7.00 ± 0.00[a]
10%	0.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	4.75 ± 0.25	
15%	0.00 ± 0.00	1.00 ± 0.29	1.50 ± 0.29	4.00 ± 0.00	
20%	0.25 ± 0.25	1.00 ± 0.00	1.50 ± 0.29	1.50 ± 0.87	

Table 2: *Duration of Life Cycle of M. domestica on Unripe Neem Seed-Treated Diets at Different Concentrations. Mean values followed by the same letter(s) along the same column are not significantly different ($p \leq 0.05$) by Scheffe test.*

Ovary development and egg viability in females maintained on ripe and unripe neem seed powder treated diets

Figure 3 shows the mean length of ovary obtained from dissected females exposed to different concentrations of ripe and unripe neem seed-treated diets. Mean length of ovary significantly decreased ($p < 0.001$) with increase in concentration in both ripe and unripe neem seed from 2.68 ± 0.23mg at 5% ripe neem seed and 3.21 ± 0.14mg at 5% unripe neem seed to 1.61 ± 0.21mg at 20% ripe neem seed and 2.17 ± 0.16mg at 20% unripe neem seed respectively. Although ovary length in females exposed to unripe neem seed were longer than those exposed to ripe neem seeds; this difference was not statistically significant (Figure 4).

Mean weights of ovary obtained from dissected females exposed to different concentrations of ripe and unripe neem seed-treated diets are shown in figure 5. As observed in ovary length, mean ovary weight decreased with increase in concentration of ripe and unripe neem seed from 0.41 ± 0.42 and 0.44 ± 0.34mg at 5% to 0.15 ± 0.46 and 0.19 ± 0.40mg at 20% respectively. (df = 3, 12, F = 6.78, p = 0.01 and df

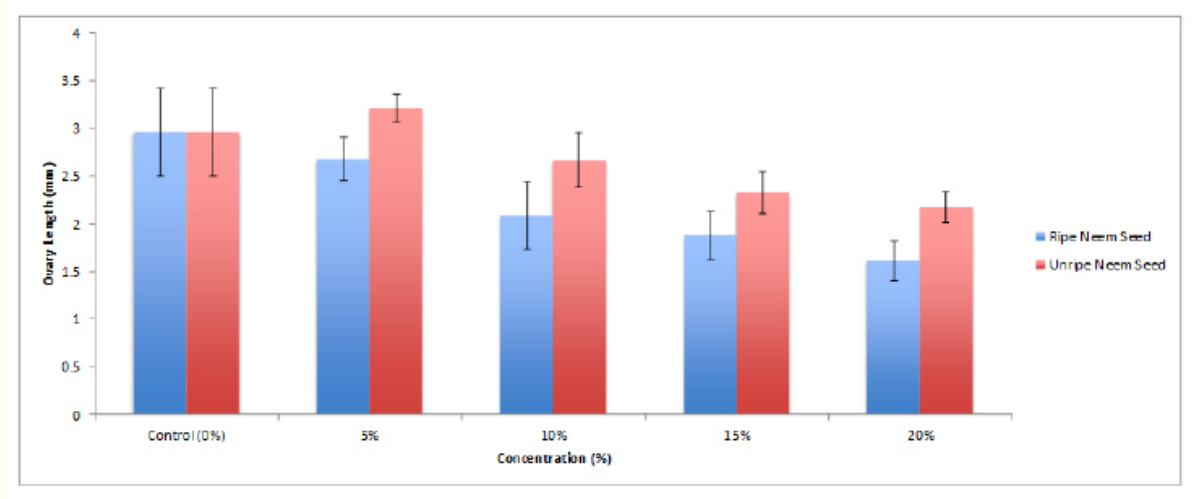

Figure 3: *Ovary Length in Female M. domestica Exposed to Different Concentrations of Ripe and Unripe Neem Seed Powder-Treated Diets.*

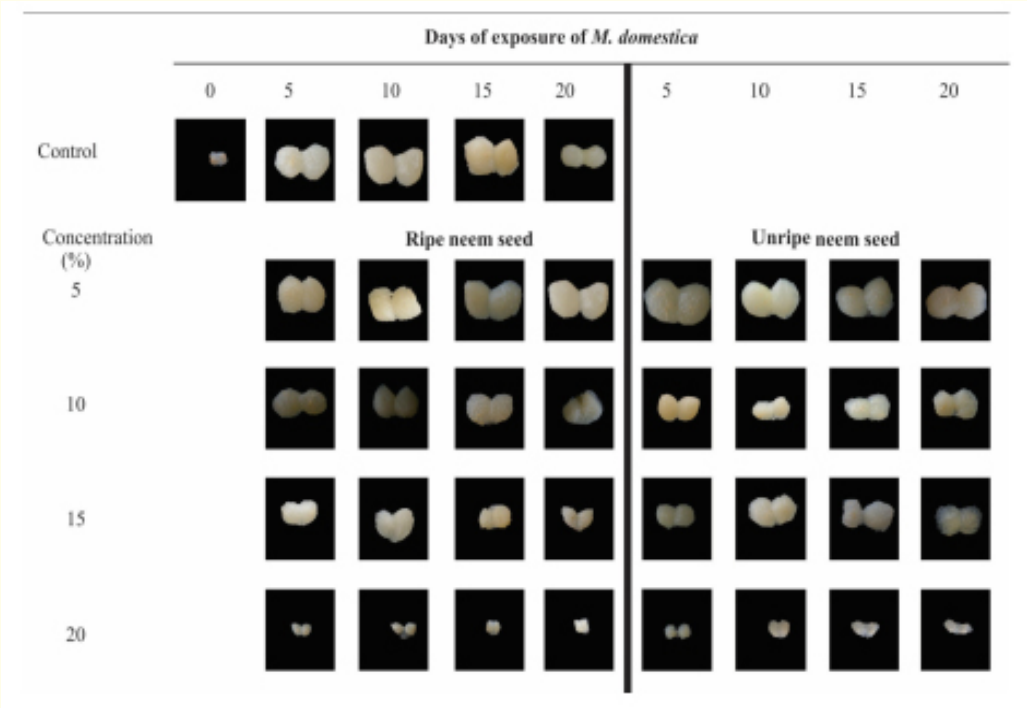

Figure 4: *Paired Ovaries from M. domestica Fed Diets Treated with Different Concentrations of Ripe Neem Seed and Unripe Neem Seed Powder Extract at Different Days of Exposure (Mag ×3).*

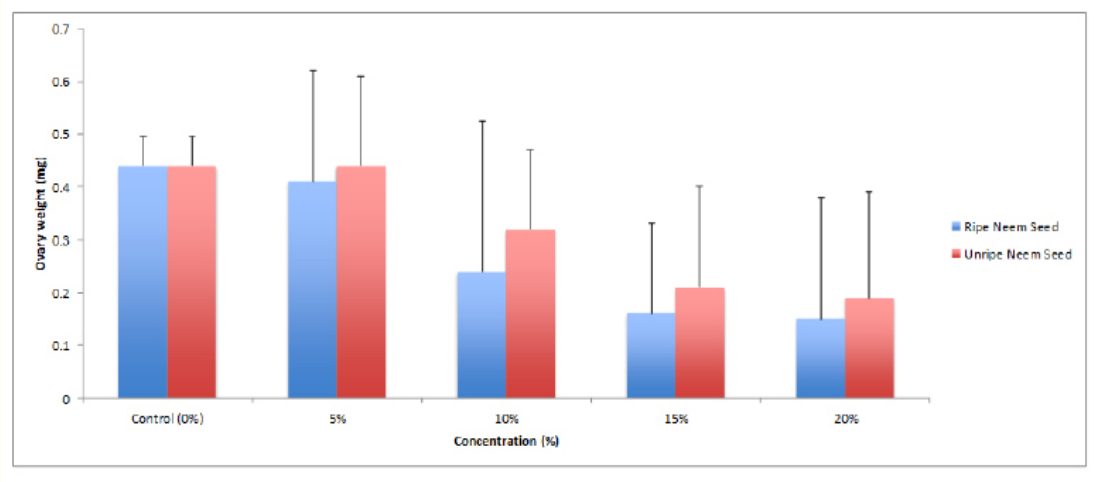

Figure 5: *Ovary Weight in Female M. domestica Exposed to Different Concentrations of Ripe and Unripe Neem Seed Powder-Treated Diets.*

= 3, 12, F = 10.90, p = 0.001). In general, we also observed larger ovaries in females fed with diets containing unripe neem extracts than those fed with diets containing ripe neem seed extracts, however this difference was not statistically significant. In addition, we were able to establish a strong correlation between the ovary weight and length at all concentrations of ripe neem seed (R = 0.89) and unripe neem seed (R = 0.92).

With respect to egg viability, average number of males that emerged from eggs maintained on 5% ripe neem seed-treated diet was 3.75 while 8.00 emerged from unripe neem seed-treated diet (Figure 6). There was significant difference in the emergence of males maintained on treated diets when compared with the control (df = 2, 9, F = 119.73, p < 0.00001). Similarly, females that emerged from eggs maintained on 5% ripe seed and 5% unripe seed were 4.25 and 5.25 respectively and were significantly different from each other (df = 2, 9, F = 192.44, p <0.00001). Eggs from females exposed to diet treated with 15% ripe and unripe neem seed hatched into larva but did not pupate. There was no significant difference in larva development for both ripe and unripe neem seed at this dose (df = 26, t = -1.4260, p = 0.1685). Overall, percentage of adults that emerged, irrespective of sex, from eggs laid by females exposed to 5% ripe and unripe neem seed-treated diets were 17.48 and 28.42% of the control diet.

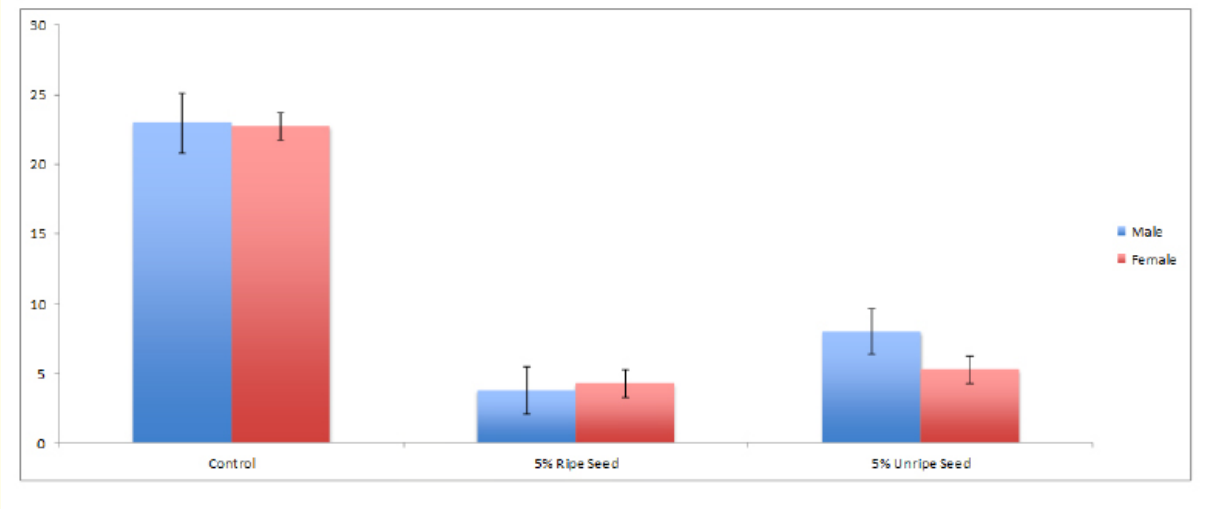

Figure 6: *Adult Emergence (Male and Female) from Eggs Exposed to Diets Treated with Ripe and Unripe Neem Seed Powder Extract.*

Discussion

Fecundity is a measure of fertility and it is the number of eggs laid within a particular period therefore a determinant of the population of an insect species. Fecundity of females exposed to ripe and unripe neem seed-treated diets decreased with increase in concentration of the diet, therefore a further increase in concentration of the diet will significantly decreased the fecundity of the housefly thus reducing the fertility of the females and possible extermination of the population within the exposed period in the laboratory.

Fecundity decreased at the same rate at 5 - 20% concentrations for ripe and unripe neem seed-treated diets. At 30% concentration, which is beyond the experimental schedule, fecundity will probably be 12 and 26 eggs at day 30 for ripe and unripe neem seed respectively. Ripe neem seed however was more effective in reducing fecundity than the unripe neem seed indicating the presence of a high concen-

tration of azadirachtin in the ripe neem seed [24]. Azadirachtin has a strong negative influence on fecundity [11]. Fecundity was reduced by about 50 percent in 20% ripe and unripe neem seed-treated diets when compared with the control diet demonstrating the effectiveness of the ripe and unripe neem seed in the control of housefly. The use of the powder extract has some merits since it allows the feeding of *M. domestica* on the treated diet rather than repelling them. Neem has direct causes on the oviposition deterrence [29]. Gaaboub and Hayes [16] reported the reduction in egg production when third instar larvae of *Musca autmnalis* was exposed to minimal concentration of azadirachtin. Adult fecundity of *Culex pipiens* was reportedly decreased by Azadirachtin [14]. Ghoneim., *et al*. [30] demonstrated that early third instar larvae of *M. domestica* treated with Margosan-O and Jojoba concentrations produced adult female flies which suffered fecundity inhibition with Margosan-O. Fertility was reduced and sterility index increased parallel to the concentration levels.

The earlier an insect lays its first eggs after adult eclosion, the more the number of egg batches that will be deposited in its lifetime. Ripe and unripe neem seed treated diets delay first egg laying and the delay increased with increase in concentration. The more the delay in concentration the lesser the fecundity of the female in per lifetime. First egg laying can therefore be further delayed or prevented out rightly when the females are exposed to concentrations above the 20% maximum. The different effects of the two extracts became significant at higher concentrations; therefore ripe neem seed was more effective in delaying first egg laying than the unripe neem seed. Botanical pesticides have been shown to interfere with reproduction of pests [31].

There was a strong positive relationship between the lengths and weights of ovary, both of which varied with the type of diet, with lower values in the ripe neem seed compared with the unripe neem seed. Decreased length and weight of the ovary in ripe neem seed suggests lower number of eggs, which may also affect their quality and quantity. The lengths and weights were drastically reduced when the values at 20% concentration of diet is compared with the control diet indicating that the quality and viability of eggs decreased as the concentrations increased. The ripe and unripe neem seed apparently reduced the size of eggs as the concentrations of treated diet increased. Ovaries in *Heteracris littoralis* treated with azadirachtin resulted in complete shrinkage [19].

There was complete development in the life cycle of *M. domestica,* from eggs to adult in 5% ripe and unripe neem seed-treated diets, which were not significantly different from the control diet. However, the ripe neem seed at 10 - 20% concentration prevented the completion of life cycle, as development could not go beyond second and third instar larvae, which ultimately prevented the emergence of adults. The ripe neem seed-treated diets were also toxic to the larvae thus obviating pupal formation from the third instar larvae. The ripe and unripe neem seed-treated diets apparently disrupted the reproduction of the housefly, therefore preventing the emergence of adult from 10% concentration.

The adults that emerged from eggs of females exposed to 5% ripe and unripe neem seed-treated diets and the control diet were significantly different from each other, indicating that the diets did not prevent egg maturation but made them highly unviable as a large percentage of the eggs from the treated females could not develop to adult stage. Gaaboub and Hayes [16] reported that azadirachtin caused a reduction of emerged *Musca auumnalis*. Progeny emergence was inhibited in *C. maculatus* [32]. (Bidmon., *et al.*) reported that the injection of azadirachtin into the larvae of blowfly, *Calliphora vivina* causes an inhibition of adult emergence. Pathak and Krishan [33] demonstrated a significant reduction in reproduction potential particularly egg viability and egg yield in *Corcyra cephalonica* exposed to neem oil vapor. Neem has direct effect on sterility [29,34,35].

Conclusion

In conclusion, based on the results obtained from this study, the powders of ripe and unripe neem seed have insecticidal effects against all the reproductive stages and as well as the adult male and female housefly, *Musca domestica*. When compared with the control diet, the neem powders significantly reduced the female fecundity and delayed first egg laying. At higher concentrations, neem powder prevented significant number of eggs deposited by females from developing indicating that most of the eggs laid were not viable. In addition, the few that developed did not reach adult stage. The sizes of eggs laid by females were concentration dependent as females exposed to 20%

treated diet produced significantly smaller eggs. Thus, the use of relatively cheap and non-poisonous ripe and unripe neem seed powder may have prospects in controlling the population of the insect vector (*Musca domestica*) from the environment.

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