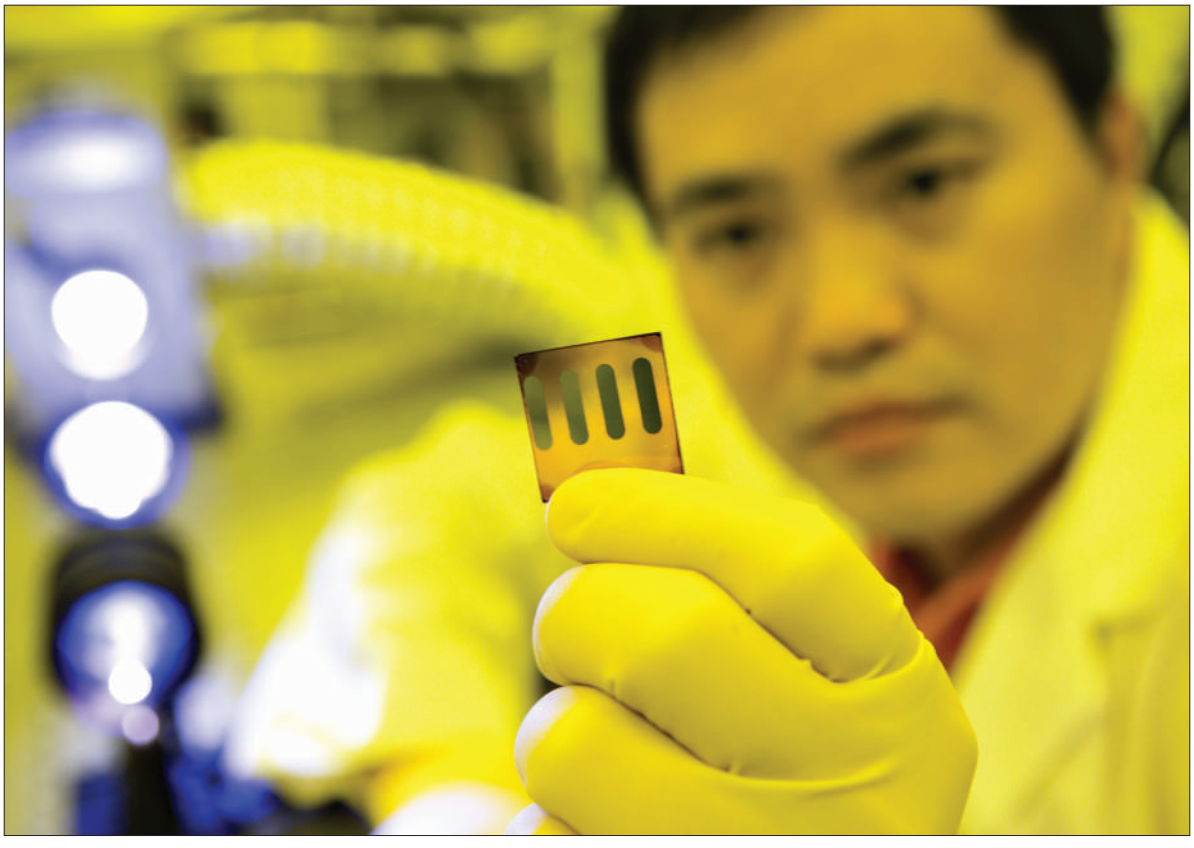

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Research Article
Efficacy of Neem Seed Extracts on Survival, Longevity of Housefly *Musca domestica* L. (Diptera: Muscidae)

A.E. Akinwale and W.A. Muse

Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract

Background and Objective: There is abundant information on the use of neem products, *Azadirachta indica* for the control of several species of insects. However, there is scanty information on studies of the comparative effects of ripe and unripe neem seed on insects. The objective of the study was to determine the effect of neem seed powder on biology of housefly, *Musca domestica*. **Materials and Methods:** Therefore, diet of ground rice and fish paste supplemented with 5, 10, 15 and 20% concentrations of ripe and unripe neem seed powder were fed on adult *Musca domestica* reared on a mixture of ground rice, ground fish and water in the ratio (1:1:1.5 w/v) and sugar to determined the survival and longevity of the housefly. **Results:** The survival of males and females exposed to ripe neem seed and unripe neem seed-treated diets followed similar trend from days 0-30 of exposure with no significant difference ($p>0.05$) in their survival. Survival of male and female flies significantly decreased ($p<0.05$) at 5-20% concentrations of ripe and unripe neem seed powder. There were significant differences in the mean longevities of males and females exposed to ripe and unripe neem seed powder at different concentrations and control ($p<0.05$). Longevity of male and female housefly exposed to the treated diets were not significantly affected ($p>0.05$). Mean weights of adult males and females maintained in unripe neem seed were higher than those maintained on ripe neem seed-treated diet at 5, 10, 15 and 20% concentrations. There was no significant difference between them but were significant when compared with the control diet. **Conclusion:** The study concluded that ripe and unripe neem seed treated diets at various concentrations were significantly toxic to *M. domestica* as they negatively affected the mean survival pattern, maximum survival, longevity and adult weights of *M. domestica* when compared with the control diet. Ripe and unripe neem seed powder have similar effects on the insect and both can be used to eliminate housefly at higher concentrations.

Key words: Efficacy, neem seed, survival, longevity, *Musca domestica*

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Corresponding Author: W.A. Muse, Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The housefly, *Musca domestica* Linnaeus, belongs to the order Diptera and family Muscidae. It is a cosmopolitan species, because of its close association with human settlements[1,2]. It is the most common species found in houses, refuse dumps, poultry farms, horse stables and ranches. They constitute a nuisance and also harbour disease-causing organisms. Excessive fly populations are irritating to farm workers and of public health problems when present near human habitations[3].

Neem (*Azadirachta indica*) belongs to the Meliaceae family. It is a fast-growing tree that can reach a height of between 15-20 and 35-40 m and grows well on a wide range of soils[4]. It produces ovoid-oblong fruits which are drupes and about 1-2 cm long, with one kernel in the seed. Fruits are green, turning yellow on ripening with aromatic garlic-like odour and bitter taste. The fruits only survive for three to four months annually depending on locality[5]. Neem tree can grow in tropical and subtropical regions with semi-arid to humid climates. It grows well in areas with annual rainfall of 400-1200 mm. Azadirachtin is one of the most promising natural compound extracted from the Neem seed whose antiviral, antifungal, antibacterial and insecticidal properties have been known for several years[6].

It was revealed that crude aqueous extracts obtained from fresh neem leaf against the larvae of *Anopheles* mosquito led to 100 and 63% mortality rate, respectively[7]. According to previous reports by Muse *et al.*[8], some Nigerian plants were reported to have effects on survival, oviposition and emergence of adult blowfly, *Chrysomya chloropyga*. They reported that all the plants affected the emergence of adults but *Erythrophleum guineense* significantly affected the survival of male and female blowflies. The effects on juvenile stages of *Culex quinquefasciatus* showed that the aqueous crude extracts and crude powder prepared from bark, fruits and leaves of neem slowly inhibited the growth and development of mosquitoes while the crude powder acted more as a barrier in which the mosquitoes probably died from suffocation[9]. Ripe neem seed (NS) and ripe neem seed kernel (NSK) powders incorporated in diets containing a mixture of ground rice and fish prolonged the development of larval instars and subsequent emergence of adults of blowfly, *Chrysomya chloropyga* but the ripe neem seed was more effective in reducing the survival, longevity and the fecundity of *C. chloropyga*[10].

The insect repellent and growth disrupting effects of neem seed extracts, particularly, Azadirachtin, are now well established and have been studied in many insect pest groups[11]. The products of *A. indica* are cheap, easy to prepare, eco-friendly and low-cost alternatives to agrochemicals. Extracts from various parts of *A. indica* have been compared with commercial pesticides on various pests of crops where they have been found to be efficacious and equally more cost effective[2].

There are few studies on the comparative effects of ripe and unripe neem fruit extracts. It was reported that acetone, ethanol and methanol extracts of *Azadirachta indica* obtained from the leaf, bark, ripe and unripe berries and tested for insecticidal activity on *Rastrococcus invadens* had effects on the survival at both high and low concentrations on the mealybugs[12]. *Melia azedarach* fruit extract reduced the population of Agromyzidae pea leaf miner *Liriomyza huidobrensis* on swiss chard and cucumber plants while *M. azedarach* immature fruit extracts resulted in fewer larval mines of the vegetable leaf miner *Liriomyza sativae* on cowpea plants[13].

The ripe neem seed powder has been used for the study of various aspects of the biology and control of several species of insect pests but there is scanty information on the use of unripe neem seed powder for the control the pests. The present study determines the comparative efficacy of ripe and unripe neem seed powder on the biology of the housefly, *Musca domestica*, a ubiquitous fly with world-wide distribution. This is with the view of ascertaining the effectiveness of the unripe neem seed for insect control.

MATERIALS AND METHODS

Rearing and maintenance of *Musca domestica*: The study was carried out between October, 2015 and July, 2016 in a well-lit insectary at temperature 28±2°C and 75±5% relative humidity in Obafemi Awolowo University, Ile-Ife, Nigeria. The adults of *M. domestica* collected in the field were reared in the laboratory to obtain self-sustaining colony. Mixture of ground rice, ground fish and water in the ratio (1:1:1.5 w/v) made into a paste[14] were provided for feeding and oviposition in cages (40×30×30 cm^3) with wire mesh (2×2 mm^2) dimension. Cotton wool soaked in water and also sugar were also placed in the cage. The maintenance medium was daily checked for eggs which were removed into separate cages and allowed to hatch. The food was changed every 48 h except for sugar cubes which were replaced when exhausted. The newly emerged adults were used for various experiments.

Processing of materials: The pulp of ripe and unripe neem seeds collected were removed and the seeds washed and air-dried for seven days. The ripe and unripe neem seeds were

separately blended into powdered form using Moulinex® kitchen blender. The rice and fish were also separately processed into powders accordingly. All the materials were kept in the deep-freezer at -10°C in the laboratory.

Bioassays

Survival and longevity of adult houseflies maintained on ripe and unripe neem seed powder-treated diets: Two sets of twenty pairs of newly emerged males and females each were put in two cages measuring (40×30×30 cm^3). They were provided with diets treated with 5, 10, 15 and 20% concentrations of ripe and unripe neem seed appropriately. The flies were also provided with sugar and water soaked in cotton wool. The flies were maintained on the treated diets for a period of 30 days. Two other sets of twenty pairs of newly emerged males and females were also maintained in two cages until the populations died out. Control experiment was also set up on diets without the neem seed powder. Diets were also replaced every 48 h except for sugar cubes which were replaced when exhausted. Percentage survival of the adult males and females and longevity of males and females in the treated diets and the control experiments were determined. Longevity was determined by the maximum number of days the flies lived. The experiments were replicated in quadruplicate.

Determination of growth of males and females on ripe and unripe neem seed powder extracts: Two sets of twenty pairs of newly emerged males and females were each placed in different cages with each provided with diets stated as stated earlier. Five flies were weighed individually on a Mettler balance every 5 days according to sex up till day 30 to determine the growth rates of the flies on each of the treated diets and determine the relationship between weight and age. The experiments were replicated four times.

RESULTS

Survival of *Musca domestica* exposed to diets treated with ripe and unripe neem seed powder: The mean percent survival of male and female *Musca domestica* exposed to different concentrations of ripe and unripe neem seed-treated diets for 30 days are shown in Fig. 1-2.

The survival of males and females exposed to ripe neem seed and unripe neem seed-treated diets followed similar trend from days 0-30 of exposure. Male populations at 5, 10 and 15% were stable at 90% up to day 10 and decreased progressively up to day 30 at 40% survival in 5 and 10% treated diet and 20 in 15% treated diet. This was in contrast with the control population which was stable at 100% for 14 days with 75% survival at day 30. Males exposed to 20% ripe neem seed-treated diet suffered continuous mortality from day 1 with 0% survival at day 30 of exposure. Survival of male in 5-20% concentrations of unripe neem seed treated diet also decreased continuously from day 0 terminating at 35 and 25% in 5 and 10% treated diet and 8% in 15 and 20% treated diet at day 30 (Fig. 1a, b).

Females exposed to 5, 10, 15 and 20% ripe neem seed-treated diets survived poorly from day 0, except those exposed to 15% treated diets, which survived at 100% for 6 days (Fig. 2a, b). Thereafter, female populations at various concentrations decreased continuously up to day 30, surviving at 25, 20, 10 and 0%. Female populations exposed to different concentrations of unripe neem seed-treated diets followed similar trend of survival with females in ripe neem seed. Females in 5, 10, 15 and 20% treated diets survived at 30, 15, 5 and 0% at day 30. Fifty percent survival for females exposed to ripe and unripe neem seed were between 17 and 24 and between 19 and 24 days respectively while those for males were 18 and 27 for unripe neem seed and 20 and 23 days for ripe neem seed.

Table 1 showed the mean maximum survival of male and female *M. domestica* exposed to diets treated with ripe and unripe neem seed powder at different concentrations. Mean maximum survival for males and females in ripe and unripe neem seed powder generally decreased with increase in percentage concentration of the extracts from 5-20% respectively. In males and females exposed to ripe seed, survival ranged from 73.87 and 59.09% and between 71.45 and 60.04 at 5-20% concentration respectively. Similarly, survival of males and females exposed to unripe neem seed ranged between 73.75 and 59.33% and between 72.14 and

Table 1: Mean maximum survival of males and females exposed to ripe and unripe neem seed powder

Concentration (%)	Ripe seed		Unripe neem seed	
	Male	Female	Male	Female
5	73.87±4.07[a]	71.45±3.96[a]	73.75±3.77[a]	72.14±3.84[a]
10	72.94±3.98[a]	69.03±4.85[a]	63.71±4.15[a]	68.19±4.96[a]
15	67.54±5.32[a]	62.29±6.20[a]	65.36±5.47[a]	65.69±6.05[a]
20	59.09±0.72[a]	60.04±6.23[a]	59.33±5.97[a]	53.99±7.35[a]
Control	91.57±1.84[b]	91.17±1.78[b]	91.57±1.84[b]	91.17±1.78[b]

Mean values followed by the same letter(s) along the same column are not significantly different ($p \leq 0.05$) by Scheffe test

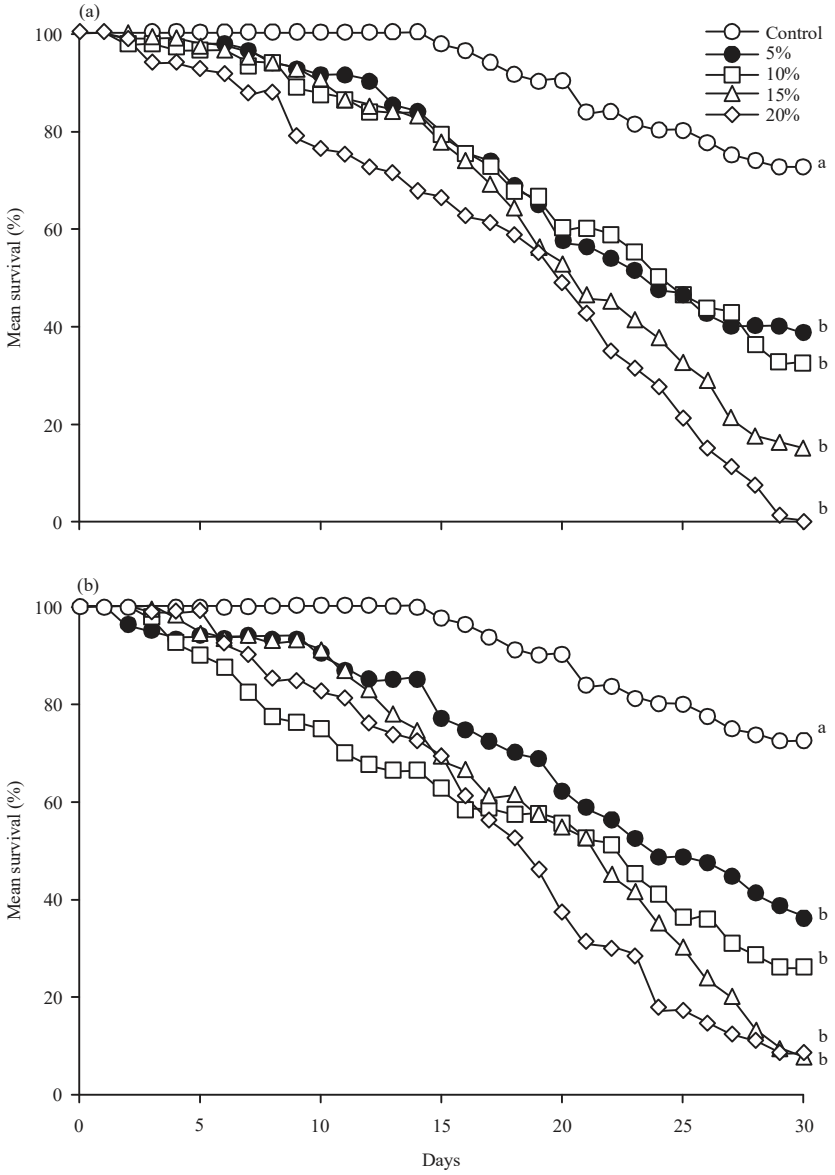

Fig. 1(a-b): Mean survival (%) of male *M. domestica* maintained on diet treated with (a) Ripe and (b) Unripe neem seed powder

53.99% at 5-20% concentration respectively. Mean survival in the control population was 91.17% in females and 91.57 in males. There was no significant difference in the mean survival of males and females exposed to ripe and unripe neem seed-treated diets at 5-20% concentrations. However, there was significant difference in all the treatments when compared with the control ($df = 4, 150, F = 7.35, p = 0.0000$; $df = 4, 150, F = 6.33, p = 0.0001$; $df = 4, 150, F = 8.12, p = 0.0000$; $df = 4, 150, F = 6.85, p = 0.0000$). There was no significant difference in the mean survival of male and female in ripe and unripe neem seed-treated diets. There was also no significant difference between the mean survival of males exposed to ripe and unripe neem seed-treated diets as well as females similarly exposed.

Mean longevity of male and female exposed to diets treated with different concentrations of ripe and unripe neem seed powder: Table 2 showed the mean longevity of male and female *M. domestica* exposed to diets treated with ripe and unripe neem seed powder at 5, 10, 15 and 20% concentrations. Mean longevity of males and females exposed to ripe neem seed was 48.00 days in 5% treated diet and

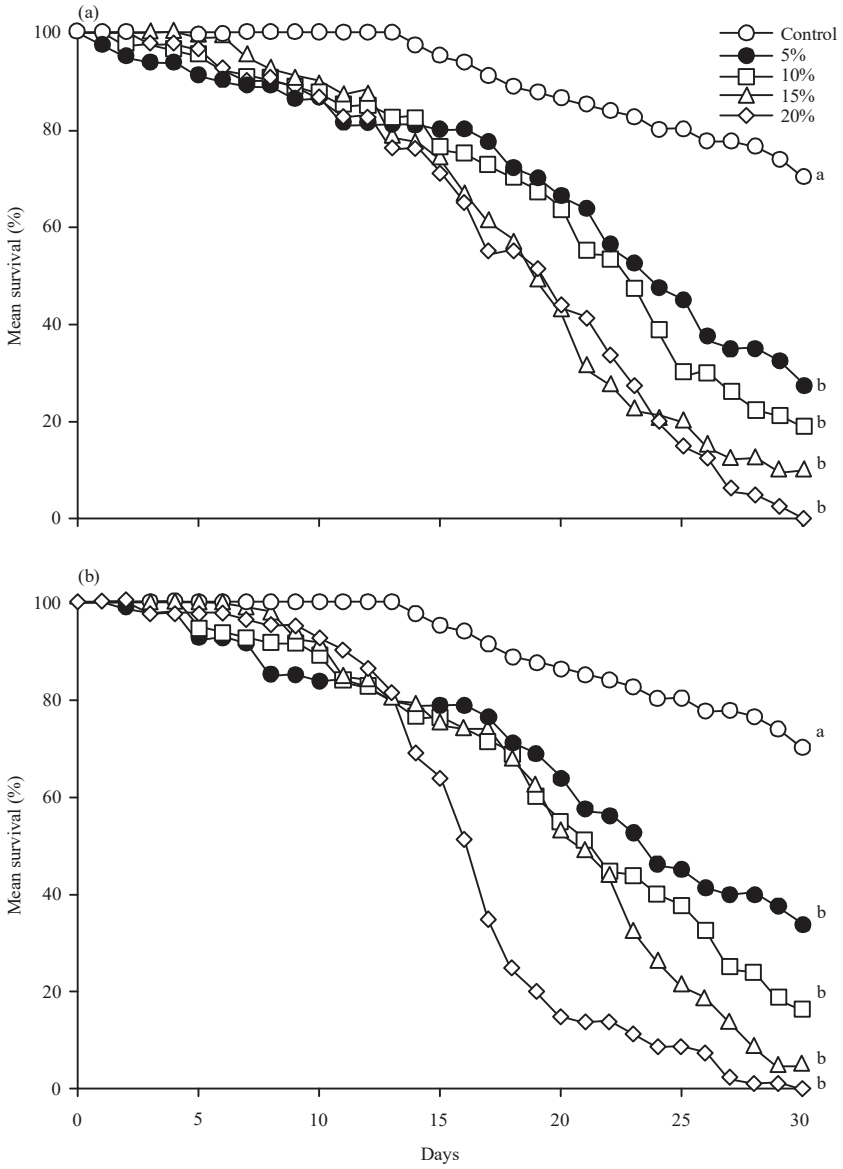

Fig. 2(a-b): Mean survival (%) of female *M. domestica* maintained on diet treated with (a) Ripe and (b) Unripe neem seed powder

Table 2: Mean longevity of male and female *M. domestica* exposed to diets treated with ripe and unripe neem seed powder at different concentrations

Concentration (%)	Ripe seed		Unripe neem seed	
	Male	Female	Male	Female
5	48.00±0.71[a]	43.00±1.47[a]	49.25±0.85[a]	44.50±0.65[a]
10	41.75±0.85[b]	36.75±1.65[b]	45.00±0.91[b]	36.75±1.55[b]
15	34.00±1.58[c]	32.00±0.71[b]	33.50±0.65[c]	30.25±1.11[c]
20	29.00±0.41[d]	27.50±1.32[b]	34.00±1.08[c]	28.00±0.71[c]
Control	58.50±1.04[e]	51.50±1.04[c]	58.50±1.04[d]	51.50±1.04[d]

Mean values followed by the same letter(s) along the same column are not significantly different ($p \leq 0.05$) by Scheffe test

decreased progressively to 29.00 in 20% treated diet. Similarly, female longevity decreased from 43.00-27.50 days in 5 and 20% concentrations respectively. There were significant differences in the mean longevity at various concentrations and the control for males and females ($df = 3,12$, $F = 135.82$, $p = 0.0000$; $df = 3,12$, $F = 53.92$, $p = 0.0000$).

Longevity of males and females exposed to diet treated with unripe neem seed powder similarly decreased with increase in concentrations of the treated diets. Longevity of males and females decreased from 49.25-34.00 days and from

Table 3: Mean weights (mg) of adult male and female *M. domestica* exposed to diets treated with ripe and unripe neem seed powder at different concentrations

Concentration (%)	Ripe neem seed		Unripe neem seed	
	Male	Female	Male	Female
5	13.50±0.57[a]	20.92±1.04[a]	12.71±0.40[a]	23.86±1.45[a]
10	11.28±0.43[a]	16.79±0.77[a]	12.79±0.49[a]	20.64±0.99[a]
15	10.93±0.32[a]	15.50±0.88[a]	12.57±0.48[a]	18.93±0.97[a]
20	10.57±0.17[a]	14.28±0.74[a]	12.29±0.43[a]	16.29±0.93[a]
Control	19.79±0.83[b]	29.21±0.78[b]	19.79±0.83[b]	29.21±0.78[b]

Mean values followed by the same letter(s) along the same column are not significantly different ($p \leq 0.05$) by Scheffe test

44.50-28.00 days in 5-20% respectively with significant difference between male and female longevity and the control (df = 3, 12, F = 132.72, p = 0.0000; df = 3, 12, F = 48.39, p = 0.0000).

There were no significant differences in the longevity of females exposed to diet treated with ripe and unripe neem seed at 5, 10, 15 and 20% concentrations. Similarly, there were no significant differences in the longevity of males similarly exposed at 5, 10 and 15% but was significant on exposure to 20% concentration (df = 6, t = 4.331, p = 0.005). There were significant differences in the longevity of males and females exposed to unripe neem seed at 5, 10, 15 and 20% concentrations respectively (df = 6, t = 4.6476, p = 0.004; df = 6, t = 4.5910, p = 0.004; df = 6, t = 4.6476, p = 0.004; df = 6, t = 4.6476, p = 0.004).

There were significant differences in the longevity of male and female exposed to ripe neem seed at 5 and 10% concentrations (df = 6, t = 3.0619, p = 0.02; df = 6, t = 2.6887, p = 0.04). However, there were no significant differences in the longevity of male compared female on diet treated with ripe neem seed at 15 and 20% concentrations (df = 6, t = 1.1457, p = 0.2921; df = 6, t = 1.0833, p = 0.3202).

Effects of diets treated with ripe and unripe neem seed powder on weights of adult males and females: Mean weights of adult males and females *M. domestica* on unripe neem seed-treated diets were higher than mean weights of adult males and females maintained on ripe neem seed-treated diets at 5, 10, 15 and 20% concentrations as shown in Table 3. There was no significant difference in the mean weights of male and female exposed to ripe and unripe neem seeds at 5-20% concentrations but there was significant difference in their weights when compared with the control respectively (df = 4, 135, F = 47.08, p = 0.0000; df = 4, 135, F = 29.66, p = 0.0000; df = 4, 135, F = 29.68, p = 0.0000; df = 4, 135, F = 15.29, p = 0.0000).

Adult weights of males and females on 10 and 20% concentrations of ripe neem seed-treated diets were significantly different from each other (t = 0.05). Similarly, adult weights of males and females maintained on 10 and 20% concentrations of unripe neem seed-treated diets were significantly different from each other (p = 0.05).

DISCUSSION

The daily survival of male population of *Musca domestica* exposed to different concentrations of ripe and unripe neem seed powder-treated diets followed similar trend throughout the survival period but there was no significant difference in their survival in the two diets. In spite of this, survival was consistently lower at day 30 of exposure in the unripe neem seed treated diet compared with the ripe neem seed. The unripe neem seed seems to be more toxic than the ripe neem seed considering the survival values at the end of the experiment. Generally, the diets affected more females than males throughout survival period. This demonstrated the possibility of decreased productivity of *M. domestica* since there will be drastic decrease in fecundity unlike females maintained in the control diet.

Azadirachtin is one of the most promising natural compounds obtained from neem tree, particularly from neem seeds[2]. It is a complex tetranortriterpenoid of the limonoid class that can be isolated in small amounts from all parts of the Neem tree with highest concentration in the mature seeds[15]. There is therefore a strong indication that the ripe and unripe neem seed powders used in the present investigation has some quantities of azadirachtins. The pronounced effect of the seed powders on the mean survival of male and female population indicates that they can be used in the control of the fly populations, particularly beyond the 20% concentration.

Mean longevity of female *M. domestica* was shorter than that of males at all the concentrations and were concentration-dependent. Owing to comparatively lower longevity, fecundity will obviously be reduced, therefore suppressing the survival of the housefly. A reduction in the survival, longevity and fecundity of *Chrysomya chloropyta* was reported when the blowfly was treated with ripe neem seed and ripe neem seed kernel powder-treated diets[10]. There was a high rate of larval mortality, reduction in adult longevity of *Anopheles* larvae treated with neem seed extracts[16]. A higher intake of Azadirachtin was correlated to a decrease in weight and an increase in mortality of the blowfly, *Protophormia terraenovae*[17].

The ripe and unripe neem seed-treated diets reduced the weights of males significantly more than the female weights. The weights of male and female insects affect the quality of male and female gametes, therefore, ripe neem seed affected the reproduction of *M. domestica* more than the unripe neem seed. Although ripe neem seed was more effective than the unripe neem seed, both diets were significantly different from the control diets. *Griffonias implicifolia* was reported to have stomach poison on *M. domestica*, affecting growth and development[18]. Baits impregnated with a commercial preparation of neem seed extract retarded growth of oriental, brown-banded and German cockroaches[19]. Crude powder extract from bark, fruit and leaves of neem inhibited the growth and development of *Culex quinquefasciatus*[9].

CONCLUSION

The powders of ripe and unripe neem seeds have similar insecticidal activity against the survival, longevity and weights of the adult male and female housefly, *Musca domestica*. The adult longevity, weights and survival patterns of males and females maintained on ripe and unripe neem seed powders-treated diets were less than those maintained in control diet. The reduction in adult weights of females is tantamount to reduction in fecundity which will therefore affects survival of the fly. Both ripe and unripe neem seed powders are safe for the control the houseflies.

SIGNIFICANCE STATEMENT

The study discovered the beneficial effect of the unripe neem seed in the control of *M. domestica*. This novel investigation will assist researchers exploit this critical area of neem research that might not have been previously explored. The new hypothesis of similarity in the effectiveness of ripe and unripe neem seeds is now arrived at.

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