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Efficacy of Neem Seed and Neem Seed Kernel Powders onthe Survival, Longevity and Fecundity of Blowfly*Chrysomya chloropyga* (Wied.) (Diptera:Calliphoridae).

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Abstract:*First, second and third instar larvae of the blowfly, Chysomya chloropyga collected from laboratory stock were exposed to a mixture of ground rice and fish supplemented with 1, 2, 3, 4, 6 and 10 % concentrations of neem seed (NS) and neem seed kernel(NSK) powders respectively.Twenty pairs of laboratory-reared adult males and females were also maintained separately on diets treated with 1, 2, 4, 6 and 10% NS and NSK for survival, longevity, fecundity and progressive weights of adult male and female at 5-day interval. The NS-as well as NSK- treated diets prolonged the development of second instar larvae but not significantly different ($p > 0.05$) from the duration of first and third instar larvae as well the pupal period in different concentrations of treated diets. Percent adult emergence decreased significantly with increase in concentration NS treated-diets from 1-6 % but there was no emergence of adults in 10 % treated diets. There was no significant difference in the emergence of adults from first, second and third instar larvae treated with NSK. Percent survival of male and female populations in NS-treated diets were 45 and 60% at day 40 of exposure but female in 10%-treated diet was 30%. Control male and female were at 80 and 75%. There was no significant difference ($p>0.05$) in the survival of male and female exposed to diffrent concentrations of NSK-treated diets throughout the 40-day exposure with percentage survival of male and female at 66 and 79% respectively. Females survived better than males at all concentrations of NS- and NSK-treated diets.Maximum longevity of male and female was 45 and 47 days respectively in 10% NS-treated diet and significantly different ($p<0.05$) from longevity of male and female at 62 and 70 days respectively in 10% NSK-treated diet. Mean fecundity and total number of eggs laid decreased with increase in concentration of powders of NS and NSK. Females exposed to control diets deposited significantly more eggs than eggs laid by females exposed to different concentrations of NS- and NSK- treated diets ($p < 0.05$). Mean weights of males and females in different concentrations of NS- and NSK-treated-diets generally increased from days 0 to 10 and fluctuated thereafter but there was no significant difference in the weights of males and females exposed to different concentrations throughout the 40 days of exposure.*
In conclusion, NS and NSK powders incorporated in diets prolonged the develpoment of larval instars and subsequent emergence of adults and their weights but the NS was more effective in reducing the survival, longevity and the fecundity of C. chloropyga.

Keywords: Blowfly, *Chrysomya chloropyga,* Neem Seed, Neem Seed Kernel, Larval development, Adult Survival and Fecundity.

I. Introduction

*Chrysomya*is a genus of blowflyclosely related to the housefly, *Musca domestica*, particularly with regard to habitat and behaviour. Members of the family Calliphoridaeare carrion feeders. The larvacauses Myiasis in sheep and cattle resulting in economic loss of livestock. The adults often visit toilets and transmit pathogen when they have access to human foods. Adult *Chrysomya*are a nuisance in meat and fish markets in different parts of Nigeria. It has also been used to predict a more accurate time of death (Anderson, 2001; Von Zuben, 2001; Williams &Villet, 2006; Joseph *et al*., 2011).

Numerous laboratory and field trials using the leaf extracts, seed kernel extracts, and powdered seeds applied to crops as a foliar spray or powder, have demonstrated the efficacy of neem's natural pesticides as insect-repellent and feeding deterrent agents. Reduction in fecundity, ovicidial activity, larval mortality, and developmental abnormalities among a variety of Coleopteran, Hemipteran, including Heteropteran, Homopteran, Hymenopteran, Lepidopteran, Orthopteran, and Thysanopteran pests exposed to neem extracts, particularly azadirachtin, have been reported (Copping and Menn, 2000; Wandscheer*et al*., 2004; Koul and Wahab, 2004; Isman, 2006).According to Metspalu*et al*. (2010) third instar larvae of the cabbage moth,*Maestrabrassicae* exposed to Neem EC showed significant prolongation in the development. Neem EC also caused failure of larval-larval and larval-pupalecdysis as well as inhibited larval development, increased mortality, antifeedant / deterrent effects and growth regulatory activity for*M. brassicae*larvae and pupae.

Onu and Baba (2003) reported that neem kernel oil and neem kernel powder suppressed oviposition and adult emergence in *Dermestesmaculatus* on dried fish. Khan *et al*. (2007) demonstrated a decrease in fecundity of*Bactroceracucurbitae*and *Bactroceradorsalis* exposed to neem compound owing to its effect on ovarian development. Medina *etal*. (2004) reported that azadirachtin affected the ovarioles of *Chrysoperlacarnea*and eggs laid in treated females were significantly smaller in number than those in the control. Ghazawi*et al*. (2007) topically treated male and female nymphs of*Heteracrislittoralis*with serial concentrations of azadirachtin and reporteddose-dependent mortality of theinsects.

War *et al*. (2011) examined the synergistic activity of a neem oil formulation with endosulfan against *Spodopteralitura*. Antifeedant activity was significantly greater in Neem oil formulation plus endosulfan treatment than in individual treatments. Amirmohammadi*et al*. (2012) reported neem to prevent mortality within 48 hours of third instar larvae of *Hyphantriacunea* treated with a commercial product of Neem extract, Achook®. Al-Fifi (2009) reported increased mortality against resting and flying*Schistocercagregaria* when treated with various concentrations of neem products. The treatment also reduced the fitness of the locusts in terms of their flight performance, as well as their adipokinetic potency.

In spite of the abundant information on the use of neem in the control of insects, there is scanty information on the insecticidal effect of the neem products in the Diptera, particularlytheblowfly, *Chrysomya chloropyga*, an ubiquitous blowfly in Nigeria. The present study was designed as a preliminary investigation on the efficacy of Neem Seed and Neem Seed Kernel on the survival, longevity and fecundity of the*Chrysomya chloropyga.*

II. Materials And Methods

The study was carried out between December 2011 and August 2012 in a well-lit laboratory at temperature 27 ± 2 °C and with 70 ± 5 % relative humidity in ObafemiAwolowo University, Ile-Ife, Nigeria. Adult blowflies *Chrysomya chloropyga* were collected with sweep net from the refuse dump site of ObafemiAwolowo University, Ile-Ife, Nigeria (7°30´47´´N, 4°31´9´´E).

Maintenance of flies.

Adult blowflies collected were maintained in a reservoir cage on a mixture of ground rice and fish (1: 1: 1.5 w/v) made into a paste (Anantiko*et al*., 1982). The flies were provided with sugar and water soaked in cotton wool. The maintenance medium was daily checked for eggs which were removed in separate cages and allowed to hatch. The food medium was changed every 72 hr except for sugar cubes which were replaced when exhausted.

Bioassay.

Effect of treated diets on the survival of larvae.

Neem Seed (NS) and Neem Seed Kernel (NSK) were separately incorporated into diets at 1, 2, 3, 4, 6 and 10% concentrations and were used to determine larval survival up to adult stage fromfirst, second and third instars respectively. A control experiment was set up without the inclusion(NS) and (NSK)in the diet. In each experiment, 20 larvae were exposed to the treated diets in 3 replicates at each concentration in transparent plastic cup (4 cm high, 10cm diameter and 30 ml capacity). The bowls were covered with muslin cloth to provide aeration. The larvae were provided with a treated diet of rice and fish and water in ratio 1:1:1.5 w/v (Anantiko *et al*., 1982).

Larval development and pupal formation in the treated and control diets were monitored daily. Dead larvae, pupa formation and adult emergence were recorded accordingly. After 4 weeks, pupae that did not emerge into adults were considered dead and also recorded accordingly.

Effect of treated diets on survival and longevity of adult blowflies.

Twenty pairs of adult males and females were put in cages measuring ($40 \times 30 \times 30$ cm^3). They were provided with mixture of ground fish and rice and water and mixed separately with NS and NSK at 1, 2, 4, 6 and 10% concentrations. Each of the 20 pairs of flies in different concentrations of treated diets were also provided with sugar and water soaked in cotton wool and maitained until the death of each of the population, while taking mortality records.Control experiment was also set up. Diets were replaced every 72hr except for sugar cubes which were replaced when exhausted.All experiments were replicated in triplicate.

Mean percent survival of the adult males and females in the treated and the control dietswere determinedbynumber remaining as theflies die dailydivided by numberintroduced at thebegining of theexperimentmultiplied by 100. Longevity was determined by the maximum number of days the flies lived.

Effect of treated diets on fecundity.

Lifespan fecundity in each of the treated diets and in the control was determined by counting the number of eggs in each egg batches depositedby the females and the mean number of eggs calculated in each case.

Effect of treated diets on adult weights.

Weight of males and females were measured using a Mettler balance at five days intervals to determine a correlation between weight and age; and weight and NS and NSK concentrations.

Statistical Analyses.

Data obtained were analyzed using descriptive and inferential statistics in SPSS® version 20 statistical package.

III. Results

Larval Development.

The effects of the various concentrations of neem seed and neem seed kernel powders onlarval development, survival and adult emergence of first, second and third instars of *Chrysomya chloropyga* are presented in Tables 1 -3.

At 1, 2, 3 and 4 % concentrations of neem seed treated diet; duration of first instar larval stage was 1.33 ± 0.33 days while 6 and 10 % recorded 1.67 ± 0.33 days. The second instar larval stadium was 2.00 ± 0.58 days at 1 % treatment, and 2.67 ± 0.33 days at 2 and 3 %. Larvae exposed to 10 % treated diet survived for 3.33 ± 0.33 days while the control was 1.33 ± 0.33 days. The third instar larval stadium at 1, 2, 3, 6 and 10 % was2.67 days. Pupae formed from the third instar larvae developed for 6.67 ± 0.88, 6.33 ± 0.67, 7.00 ± 0.00, 7.00 ± 0.00 and 8.00 ± 0.58 days in 1, 2, 3, 4 and 6 % respectively before emerging as adults. There was no emergence of adults from pupae that developed from third instar exposed to 10 % treated diets. There was no significant difference in the pupal duration of 6.67 ± 0.88 in the control and 1 % treated diet, ($p > 0.05$). There was no significant difference between days of development of the first and third instars and the pupal stage at various concentrations of treated diets, ($F = 0.238$, $F = 0.256$, $F = 1.122$, $p > 0.05$) but there was significant difference in the days of development of second instar larvae in different concentrations of treated diets, ($F = 6.238$; $df = 6, 14$; $p < 0.05$).

At all concentrations of neem seed kernel-treated diet and the control, duration of first instar larval stage was 1.33 ± 0.33 days ($F = 0.000$; $df = 6, 14$; $p > 0.05$). There was no significant difference in the days of development of second and third instar larvae exposed to different concentrations of treated diet and the control ($F = 0.117$;$df=6,14$;$p>0.05$), ($F = 0.238$; $df=6,14$ $p > 0.05$). Pupae formed from previously exposed third instar in neem seed kernel diet turned to adults at different days of development, but there was no significant difference in day of development of pupae between different concentrations of treated diet and the control ($F = 2.103$; $df=6,14$;$p > 0.05$). Days of development of pupae emerging from 10 % treated diet was 7.67 ± 1.20 days and was longer than those that emerged from other concentrations treated diets and the control.

Percent adult emergence of *C. chloropyga*larvae reared on different concentrations of neem seed treated diets were obtained for first, second and third larval instars (Table 3). Emergence of adults was between 100 % in the control, 98.30 % in 1 % and 21.65 % in 6 % neem seed treated diet. There was no significant difference in the emergence between 1 and 4 % and the control ($F = 18.893$; $df = 6, 14$; $p = 0.05$). Similarly, there was no significant difference in the percentage of adult emerging from second and third instar larvae at 1 and 4 % and the control ($F = 111.667$, $F = 92.035$, $p = 0.05$). There was no emergence of adult from first, second and third instar larvae separately exposed to 10 % concentrations of neem seed diet.

Mean percent emergence of adults of*C. chloropyga* larvae from first instar larvae exposed to 1, 2, 3, 4 and 6 % concentrations of neem seed kernel-treated diet were not significantly different from each other ($F = 3.042$: $df=6,14$; $p = 0.05$). There was 100 % emergence of adults from second instar larvae exposed to the different concentrations of neem seed kernel-treated diets. Percent emergence of adults ranged between 100 and 93.33% from third instar larvae exposed to different concentrations of treated diets but were not significantly different from each other, including the control, ($F = 0.500$; $df=6,14$; $p > 0.05$).

Table 1. Meanof days of development of first instar larvae exposed to diets treated with different concentrations of neem seed (NS) powder.

Larval Instars				
Treatment (%)	First	Second	Third	Pupae
1	1.33 ± 0.333^{a}	2.00 ± 0.577^{ab}	2.67 ± 0.333^{a}	6.67 ± 0.882^{a}
2	1.33 ± 0.333^{a}	2.67 ± 0.333^{b}	2.67 ± 0.667^{a}	6.33 ± 0.667^{a}
3	1.33 ± 0.333^{a}	2.67 ± 0.333^{b}	2.67 ± 0.667^{a}	7.00 ± 0.000^{a}

4	1.33±0.333[a]	3.33±0.333[b]	2.00±0.577[a]	7.00±0.000[a]
6	1.67±0.333[a]	3.00±0.000[b]	2.67±0.882[a]	8.00±0.577[a]
10	1.67±0.333[a]	3.33±0.333[b]	2.67±0.882[a]	No emergence of adults
Control	1.33±0.333[a]	1.33±0.333[a]	2.67±0.667[a]	6.67±0.882[a]

Means followed by the same letter along the same column are not significantly different ($p \leq 0.05$) by Tukey HSD test.

Table 2. Mean of days of development of first instar larvae exposed to diet treated with different concentrations of neem seed kernel(NSK) powder.

	Larval Instars			
Treatment (%)	First	Second	Third	Pupae
1	1.33±0.333[a]	1.67±0.333[a]	2.67±0.333[a]	5.00±0.000[a]
2	1.33±0.333[a]	2.00±0.577[a]	2.67±0.333[a]	5.33±0.333[a]
3	1.33±0.333[a]	2.00±0.577[a]	2.67±0.333[a]	5.67±0.667[a]
4	1.33±0.333[a]	2.00±0.577[a]	2.67±0.333[a]	6.00±0.577[a]
6	1.33±0.333[a]	2.00±0.577[a]	2.67±0.333[a]	6.67±0.667[a]
10	1.33±0.333[a]	2.33±0.667[a]	2.33±0.333[a]	7.67 ±1.200[a]
Control	1.33±0.333[a]	1.67±0.333[a]	2.33±0.333[a]	5.33±0.333[a]

Means followed by the same letter along the same column are not significantly different ($p \leq 0.05$) by Tukey HSD test.

Table 3. Mean percent adult emergence of*C. chloropyga*larvae maintained with diet treated with neem seed - and neem seed kernel powder.

Treatment (%)	Neem Seed(NS)			Neem Seed Kernel(NSK)		
	1st Instar	2nd Instar	3rd Instar	1st Instar	2nd Instar	3rd Instar
Control	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00	100.00[a]
1	98.30[a]	100.00[a]	95.00[a]	100.00[a]	100.00	96.67[a]
2	90.00[a]	100.00[a]	100.00[a]	98.30[a]	100.00	90.00[a]
3	75.00[a]	100.00[a]	95.00[a]	100.00[a]	100.00	96.67[a]
4	71.66[a]	83.31[a]	83.30[a]	100.00[a]	100.00	100.00[a]
6	21.68[b]	41.70[b]	23.32[b]	100.00[a]	100.00	93.33[a]
10	0.00[b]	0.00[c]	0.00[c]	91.67[b]	100.00	96.67[a]

Means followed by the same letter along the same column are not significantly different ($p \leq 0.05$) by Tukey HSD test.

Adult Survival and Longevity.

The mean percent survival rate of male and female adult *Chrysomyachloropyga* exposed to different concentrations of neem seed and neem seed kernel treated diets up to day 40 are shown in Figures 1 - 4.

Male populations in the control and in 1, 2, 4 and 6 % concentrations of neem seed treated diets were stable up to 10 days of exposure (Fig.1). Male exposed to 10 % neem seed treated diet was stable at 100% up to day 6. Male populations maintained in the different concentrations of diet and in control thereafter decreased progressively up to day 40 at 78.3, 42.5, 36.7, 50.0, 42.5 and 36.7% for the control, 1, 2, 4, 6 and 10 % neem seed-treated diets respectively.Female population exposed to control diet was stable at 100% up to 28 days and thereafter progressively decreased up to 75 % at day 40 (Fig.2). Female populations exposed to different percentage concentrations of neem seed-treated diet were stable up to day 8 with females exposed to 4 % neem seed-treated diet surviving at 100 % up to day 18. The populations decreased progressively up to day 40 with survival at over 50 % and 31.7 % for females exposed to 10% neem seed-treated diet. Females outlived and survived better than males in all the treated diets and the control.

Males maintained inneem seed kernel-treated diet and those exposed to control diet were stable at 100% up to day 10. The male populations generally decreased progressively thereafter surviving above 50% at 40 days of exposure (Fig.3). Female population maintained in different concentrations of neem seed kernel-treated diet and in control diet survived at 100 % longer than the male population (Fig.4). Pattern of survival was similar in both sexes, maintained in neem seed kernel treated diet, with population above 60 % survival at day 40.

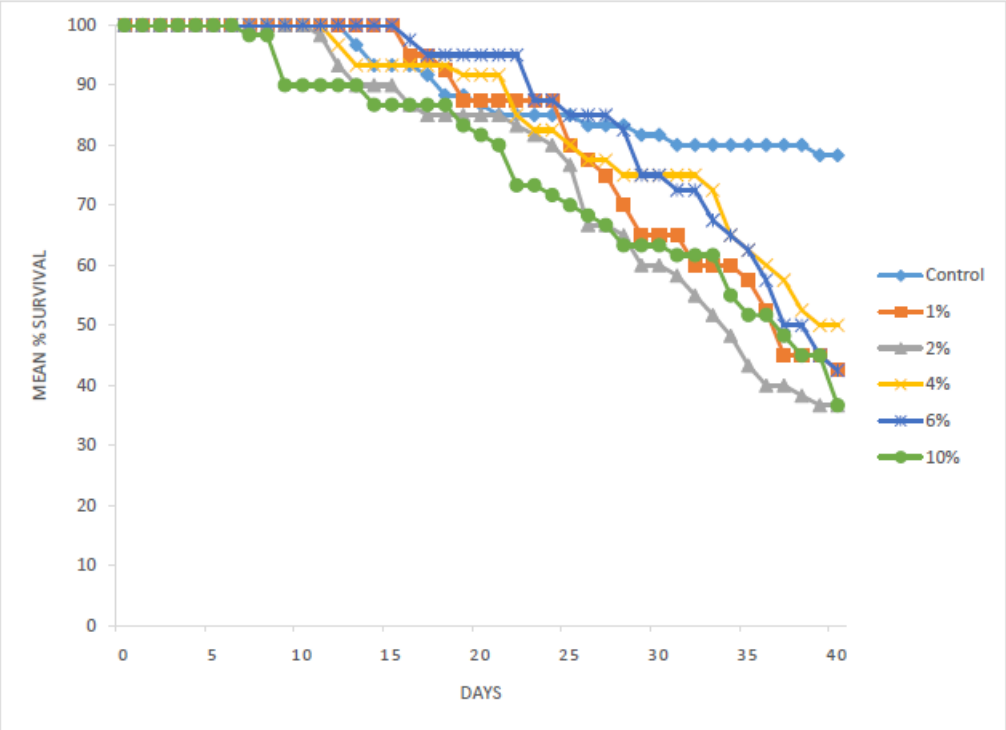

Fig 1. Mean percent survival of male *C. chloropyga* maintained on neem seed-treated diet.

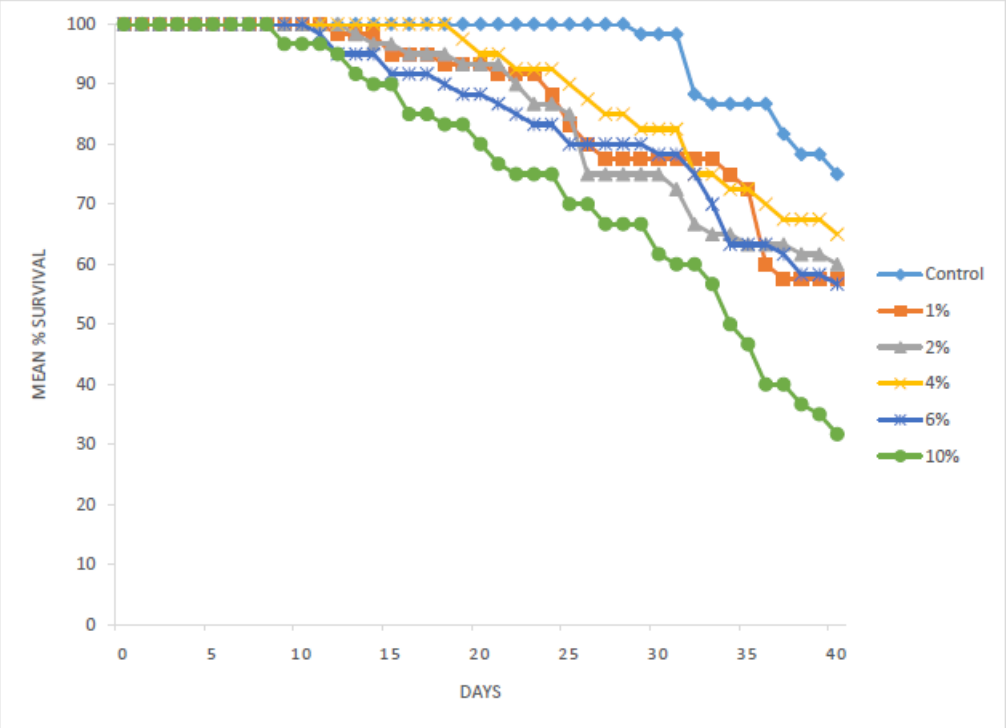

Fig 2. Mean percent survival of female *C. chloropyga* maintained on neem seed-treated diet.

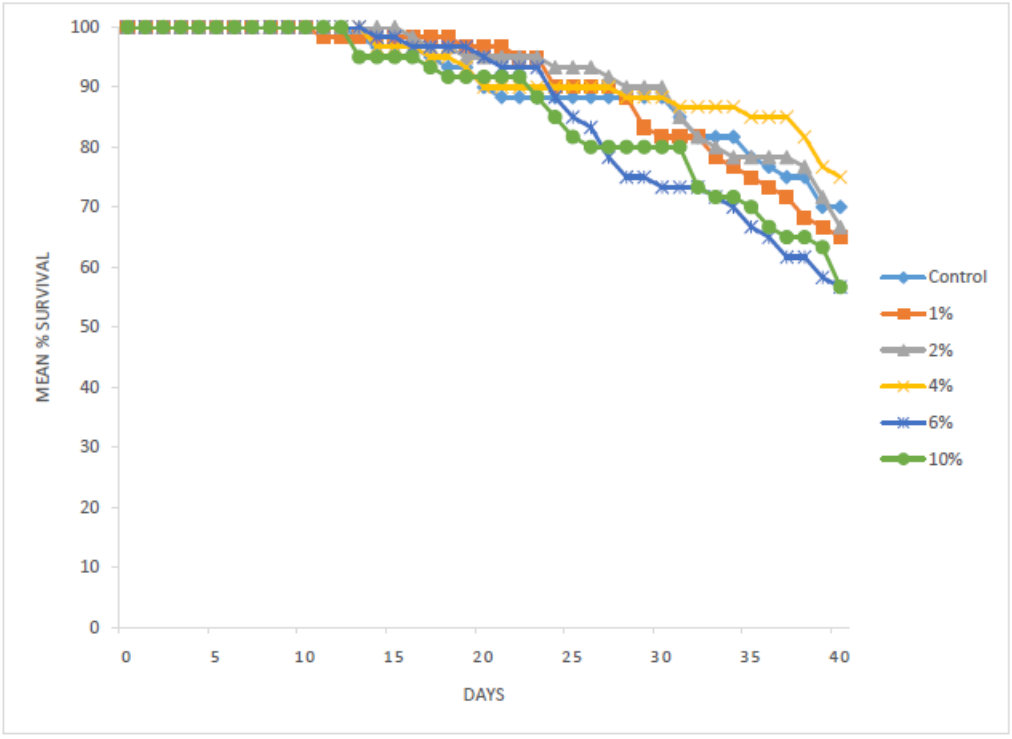

Fig 3. Mean percent survival of male *C. chloropyga* maintained on neem seed kernel-treated diet.

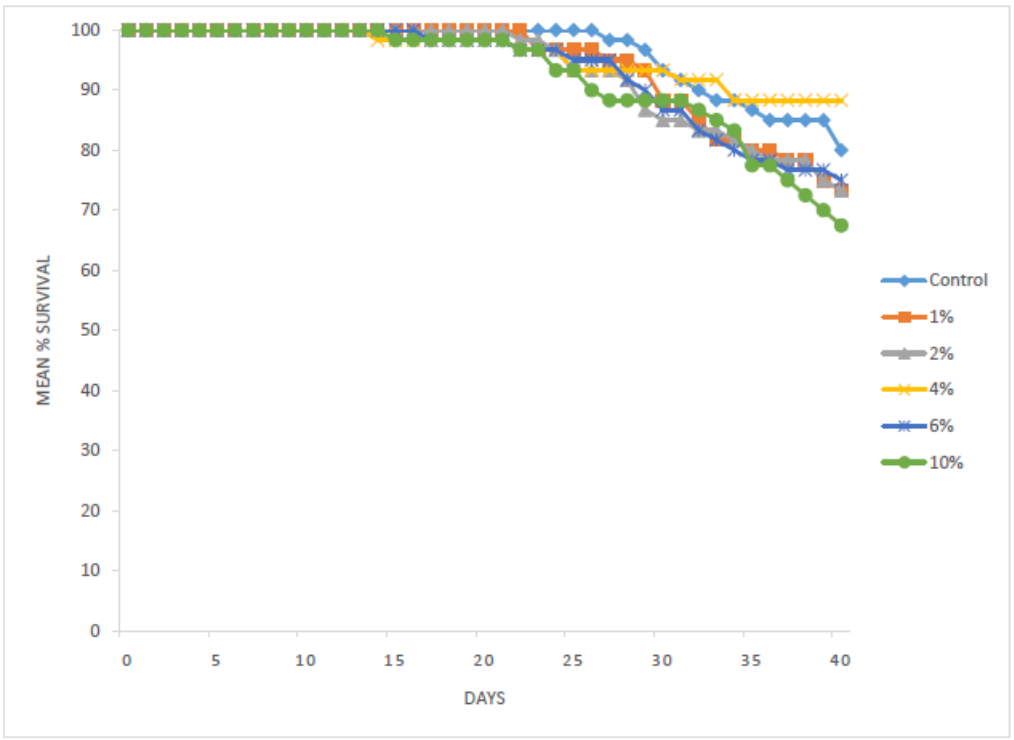

Fig 4. Mean percent survival of female *C. chloropyga* maintained on neem seed kernel-treated diet.

Maximum longevity of treated males and females.

Maximum longevity in days of *C. chloropyga*fed diet containing various concentrations of the neem seed and neem seed kernel powders are shown in Table 4. Male blowflies fed on 1 % neem seed and neem seed kernel diets lived a maximum of 54 days and 72 days compared with females which lived 69 days and 76 days, respectively. At 2 % neem seed and neem seed kernel concentration diets, males lived for 57 days and 76 days while females lived for 60 days and 78 days. Males lived for 60 days and 71 days at 4 % treated neem seed and neem seed kernel diets while the females lived for 62 days and 76 days. Male and female flies maintained on 6 %

neem seed containing diet lived 57 days and 59 days while female counterpart maintained on diets containing 6 % neem seed kernel powder lived 71 days and 76 days. At 10 % neem seed-treated diet, males lived 45 days and females 47 days while in the neem seed kernel diet-treated male and female lived 62 days and 70 days, respectively. Males and females blowflies in the control diet lived 72 days and 73 days, respectively.

There was significant difference in the maximum longevity of males ($F = 9.358$; $df = 5, 12$; $p = 0.001$) and females ($F = 7.286$; $df = 5, 12$; $p = 0.002$) maitained on neem-treated diets. Males and females reared in the control diet lived longer than males and females exposed to diets containing 1, 2, 4, 6 and 10 % neem seed diets. Males and females similarly exposed to different concentrations of neem seed kernel-treated diets showed no significant difference in their longevities ($F = 1.173$; $df = 5, 12$; $p = 0.377$), ($F = 2.808$; $df = 5, 12$; $p = 0.66$) respectively. There was no significant difference between male and female maximum longevity on neem seed-treated diet ($t = 1.677$, $df = 2$, $p = 0.235$) and similarly no significant difference between male and female maximum longevity on neem seed kernel-treated diets($t = 2.000$, $df = 2$, $p = 0.184$). Males significantly lived longer in neem seed kernel than in neem seed treated diets ($t = 10.250$, $df = 2$, $p = 0.009$) while females significantly lived longer in neem seed kernel- than in neem seed-treated diets ($t = 3.928$, $df = 2$, $p = 0.053$).

Table 4. Maximum longevity (days) of male and female*C. chloropyga* exposed to different concentrations of neem seed- and neem seed kernel-treated diets.

Treatment (%)	Neem Seed(NS)		Neem Seed Kernel(NSK)	
	Male	Female	Male	Female
1	54^{ab}	69^{bc}	72^{a}	76^{a}
2	57^{ab}	60^{bc}	76^{a}	78^{a}
4	60^{b}	62^{b}	71^{a}	76^{a}
6	57^{ab}	59^{b}	71^{a}	76^{a}
10	45^{a}	47^{a}	62^{a}	70^{a}
Control	72^{c}	73^{c}	72^{a}	73^{a}

Means followed by the same letter along the same column are not significantly different ($p \leq 0.05$) by Tukey HSD test.

Fecundity of females exposed to neem seed- and neem seed kernel-treated diets.

The total number of eggs laid by females exposed to different concentrations of neem seed and neem seed kernel-treated dietsis shown in Table 5. Total number of eggs decreased with increase in concentration of neem seed from 1854.00 ± 204.73 eggs (1 % neem seed) to 701.67 ± 325.31 (10 % neem seed). The total number of eggs laid (2824.33 ± 97.35 eggs) by females exposed to control diet was significantly higher thanthe number of eggs laid by females exposed to different concentrations of neem seed-treated diet ($F = 15.927$; $df = 5, 12$; $p = 0.00$).Highest mean fecundity of 141.72 ± 6.72 eggs obtained in the control female was followed by female exposed to 1 % neem seed-treated diet and minimum of 35.10 ± 3.07 eggs obtained in 10% treated diet ($F = 15.927$; $df = 5, 12$; $p = 0.00$).

Total number of eggs laid by females exposed to 1, 2 and 4% neem seed kernel-treated diets were 1921.00 ± 463.09, 1707.33 ± 434.91 and 1473.67 ± 242.09 eggs respectively. Females exposed to 10% treated diet laid 1144.67 ± 374.36 eggs but there was no significant difference in the total number of eggs laid by females exposed to the different diets, including the control ($F = 1.067$; $df = 5, 12$; $p = 0.425$). Similarly, fecundity decreased with increased in concentration of neem seed kernel treated-diets from 96.05 ± 3.45 at 1% to 57. 23 ± 2.86 in 10% treated diets ($F = 2.961$; $df = 5, 12$; $p = 0.057$).

Table 5. Mean fecundity and total number of eggs produced by females exposed to different concentrations of neem seed-and neem seed kernel-treated diets.

Treatment (%)	Neem seed(NS)		Neem seed kernel(NSK)	
	Total number of eggs (N = 20)	Fecundity/♀	Total number of eggs (N = 20)	Fecundity/♀
1	1854.00 ± 204.73^{c}	92.70 ± 4.72^{c}	1921.00 ± 463.09^{a}	96.05 ± 3.45^{ab}
2	1705.33 ± 57.72^{bc}	85.27 ± 5.37^{bc}	1707.33 ± 439.91^{a}	85.37 ± 3.82^{ab}
4	1269.33 ± 245.44^{abc}	63.47 ± 4.08^{abc}	1473.67 ± 242.09^{a}	73.69 ± 3.35^{ab}
6	802.67 ± 111.33^{ab}	40.13 ± 2.90^{ab}	1421.33 ± 302.86^{a}	71.02 ± 2.86^{ab}
10	701.67 ± 325.31^{a}	35.10 ± 3.07^{a}	1144.67 ± 374.36^{a}	57.23 ± 2.86^{a}
Control	2824.33 ± 97.35^{d}	141.72 ± 6.72^{d}	2824.33 ± 97.35^{a}	141.72 ± 6.72^{b}

Means followed by different letter(s) are significantly different from each other ($p \leq 0.05$) by Tukey HSD test.

Weights of male and female *C. chloropyga* exposed to different concentrations of neem seed and neem seed kernel-treated diets.

There was significant difference in the mean weight of males exposed to different concentrations of diet at ages 0, 5, 10 up to 40 days of adults (Table 6). At different percentage concentrations of diets, there was

significant difference in the weight of males and in the control diet**.**There was significant difference in the mean weight of females for day 0, day 5, day 10 up to day 40 at 1, 2, 6 and 10 % concentration of diets. There was no significant difference in weights of females in the control diet and at 4 % treated diets ($F = 2.075$, $df = 8, 18$, $p = 0.095$; $F = 1.462$, $df = 8, 18$, $p = 0.239$) respectively, (Table 7).

The mean weight of male *C. chloropyga*in different concentrations of neem seed kernel treated-diets showed that there was no significant difference at different ages of the adult but there was significant difference in the mean weight of males exposed to 1, 2, 4 and 6 % treated diets, (Table 8). Weights of females exposed to different concentrations of treated diets at ages 0 to 40 days revealed the presence of significant difference in the weight of females and in the control diet, (Table 9).

There were significant differences in the mean weights of males and females in the control diet at different ages of the adults ($t = 2.485$, $df = 8$, $p = 0.038$; $t = 2.768$, $df = 8$, $p = 0.024$) respectively. There was no significant difference in the weights of males and females exposed to 1, 2, 4, 6 and 10 % concentration of diets at 0 to 40 days.

Table 6: Mean weight (g) of male *C. chloropyga* in different concentrations of neem seed treated diets.

	Treatment					
Age (days)	**Control**	**1 %**	**2 %**	**4 %**	**6 %**	**10 %**
0	0.026 ± 0.0025^{a}	0.018 ± 0.0020^{a}	0.020 ± 0.0000^{a}	0.020 ± 0.0000^{a}	0.018 ± 0.0020^{a}	0.020 ± 0.0000^{a}
5	0.024 ± 0.0025^{a}	0.034 ± 0.0025^{b}	0.026 ± 0.0040^{a}	0.036 ± 0.0040^{b}	0.028 ± 0.0020^{ab}	0.032 ± 0.0020^{b}
10	0.032 ± 0.0020^{b}	0.032 ± 0.0020^{b}	0.032 ± 0.0020^{ab}	0.034 ± 0.0025^{ab}	0.032 ± 0.0020^{b}	0.030 ± 0.0000^{b}
15	0.032 ± 0.0020^{b}	0.032 ± 0.0020^{b}	0.040 ± 0.0032^{b}	0.036 ± 0.0025^{b}	0.030 ± 0.0000^{b}	0.032 ± 0.0020^{b}
20	0.036 ± 0.0025^{b}	0.032 ± 0.0020^{b}	0.034 ± 0.0025^{b}	0.030 ± 0.0000^{ab}	0.034 ± 0.0025^{b}	0.032 ± 0.0020^{b}
25	0.034 ± 0.0025^{b}	0.032 ± 0.0020^{ab}	0.038 ± 0.0020^{b}	0.036 ± 0.0025^{ab}	0.033 ± 0.0025^{b}	0.030 ± 0.0000^{b}
30	0.034 ± 0.0025^{b}	0.035 ± 0.0029^{b}	0.034 ± 0.0025^{b}	0.034 ± 0.0025^{ab}	0.030 ± 0.0000^{b}	0.030 ± 0.0000^{b}
35	0.032 ± 0.0020^{b}	0.035 ± 0.0029^{b}	0.034 ± 0.0025^{ab}	0.034 ± 0.0025^{ab}	0.030 ± 0.0000^{b}	0.030 ± 0.0000^{b}
40	0.024 ± 0.0025^{a}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{b}	0.030 ± 0.0000^{b}

Means followed by the same letter along the same column are not significantly different ($p \leq 0.05$) by Tukey HSD test.

Table 7: Mean weight (g) of female *C. chloropyga* in different concentrations of neem seed treated diets.

	Treatment					
Age (days)	**Control**	**1 %**	**2 %**	**4 %**	**6 %**	**10 %**
0	0.026 ± 0.0068^{a}	0.022 ± 0.0020^{a}	0.020 ± 0.0045^{a}	0.030 ± 0.0000^{a}	0.022 ± 0.0020^{a}	0.022 ± 0.0020^{a}
5	0.030 ± 0.0045^{a}	0.030 ± 0.0000^{ab}	0.036 ± 0.0068^{b}	0.034 ± 0.0040^{a}	0.030 ± 0.0000^{ab}	0.032 ± 0.0037^{bc}
10	0.034 ± 0.0025^{a}	0.034 ± 0.0040^{abc}	0.036 ± 0.0025^{b}	0.032 ± 0.0020^{a}	0.030 ± 0.0032^{ab}	0.030 ± 0.0000^{ab}
15	0.032 ± 0.0020^{a}	0.034 ± 0.0025^{abc}	0.032 ± 0.0020^{ab}	0.030 ± 0.0000^{a}	0.032 ± 0.0020^{bc}	0.032 ± 0.0020^{abc}
20	0.038 ± 0.0020^{a}	0.040 ± 0.0000^{bc}	0.034 ± 0.0025^{ab}	0.036 ± 0.0025^{a}	0.040 ± 0.0000^{c}	0.040 ± 0.0000^{c}
25	0.044 ± 0.0040^{a}	0.048 ± 0.0020^{c}	0.036 ± 0.0025^{b}	0.034 ± 0.0025^{a}	0.030 ± 0.0000^{ab}	0.032 ± 0.0020^{bc}
30	0.048 ± 0.0020^{a}	0.048 ± 0.0020^{c}	0.036 ± 0.0025^{b}	0.034 ± 0.0025^{a}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{ab}
35	0.030 ± 0.0000^{a}	0.036 ± 0.0025^{abc}	0.030 ± 0.0000^{ab}	0.034 ± 0.0025^{a}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{ab}
40	0.032 ± 0.0037^{a}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{a}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{ab}

Means followed by the same letter along the same column are not significantly different ($p \leq 0.05$) by Tukey HSD test.

Table 8: Mean weight (g) of male *C. chloropyga* in different concentrations of neem seed kernel treated diets.

	Treatments					
Age (days)	**Control**	**1 %**	**2 %**	**4 %**	**6 %**	**10 %**
0	0.022 ± 0.0020^{a}	0.022 ± 0.0020^{a}	0.020 ± 0.0000^{a}	0.022 ± 0.0020^{a}	0.022 ± 0.0020^{a}	0.022 ± 0.0020^{a}
5	0.032 ± 0.0020^{a}	0.030 ± 0.0000^{b}	0.030 ± 0.0000^{b}	0.030 ± 0.0000^{ab}	0.030 ± 0.0000^{abc}	0.028 ± 0.0020^{a}
10	0.030 ± 0.0000^{a}	0.030 ± 0.0000^{b}	0.030 ± 0.0000^{b}	0.030 ± 0.0000^{ab}	0.028 ± 0.0020^{ab}	0.030 ± 0.0000^{a}
15	0.032 ± 0.0020^{a}	0.032 ± 0.0020^{b}	0.032 ± 0.0000^{bc}	0.032 ± 0.0020^{abc}	0.030 ± 0.0000^{abc}	0.030 ± 0.0000^{a}
20	0.032 ± 0.0020^{a}	0.038 ± 0.0020^{b}	0.040 ± 0.0000^{c}	0.036 ± 0.0025^{bc}	0.036 ± 0.0025^{bc}	0.032 ± 0.0020^{a}
25	0.030 ± 0.0000^{a}	0.040 ± 0.0000^{ab}	0.040 ± 0.0000^{c}	0.038 ± 0.0020^{bc}	0.038 ± 0.0020^{c}	0.034 ± 0.0025^{a}
30	0.030 ± 0.0000^{a}	0.036 ± 0.0025^{b}	0.038 ± 0.0020^{c}	0.040 ± 0.0000^{bc}	0.040 ± 0.0000^{c}	0.034 ± 0.0025^{a}
35	0.030 ± 0.0000^{a}	0.030 ± 0.0000^{b}	0.038 ± 0.0020^{c}	0.040 ± 0.0000^{bc}	0.040 ± 0.0000^{c}	0.032 ± 0.0020^{a}
40	0.034 ± 0.0025^{a}	0.032 ± 0.0020^{ab}	0.032 ± 0.0020^{bc}	0.042 ± 0.0020^{c}	0.040 ± 0.0000^{c}	0.032 ± 0.0020^{a}

Means followed by the same letter along the same column are not significantly different ($p \leq 0.05$) by Tukey HSD test.

Table 9: Mean weight (g) of female *C. chloropyga* in different concentrations of neem seed kernel treated diets.

	Treatments					
Age (days)	**Control**	**1 %**	**2 %**	**4 %**	**6 %**	**10 %**
0	0.022 ± 0.0020^{a}	0.020 ± 0.0032^{a}	0.022 ± 0.0020^{a}	0.022 ± 0.0020^{a}	0.018 ± 0.0020^{a}	0.024 ± 0.0025^{a}
5	0.028 ± 0.0020^{a}	0.026 ± 0.0025^{ab}	0.028 ± 0.0020^{a}	0.030 ± 0.0032^{ab}	0.028 ± 0.0020^{ab}	0.026 ± 0.0025^{ab}
10	0.032 ± 0.0020^{ab}	0.030 ± 0.0000^{abc}	0.032 ± 0.0020^{abc}	0.030 ± 0.0000^{ab}	0.028 ± 0.0020^{ab}	0.030 ± 0.0000^{abc}
15	0.034 ± 0.0025^{ab}	0.032 ± 0.0020^{bcd}	0.030 ± 0.0000^{ab}	0.032 ± 0.0020^{ab}	0.028 ± 0.0020^{ab}	0.030 ± 0.0000^{abc}
20	0.048 ± 0.0020^{c}	0.042 ± 0.0020^{de}	0.050 ± 0.0000^{d}	0.042 ± 0.0020^{cd}	0.040 ± 0.0000^{c}	0.034 ± 0.0025^{bc}
25	0.050 ± 0.0000^{c}	0.046 ± 0.0025^{e}	0.048 ± 0.0020^{d}	0.050 ± 0.0000^{d}	0.044 ± 0.0025^{c}	0.038 ± 0.0020^{c}
30	0.050 ± 0.0000^{c}	0.048 ± 0.0020^{e}	0.042 ± 0.0020^{cd}	0.050 ± 0.0000^{d}	0.042 ± 0.0020^{c}	0.034 ± 0.0025^{bc}
35	0.044 ± 0.0025^{bc}	0.044 ± 0.0025^{de}	0.040 ± 0.0000^{bcd}	0.048 ± 0.0020^{cd}	0.040 ± 0.0000^{c}	0.030 ± 0.0000^{abc}
40	0.040 ± 0.0000^{bc}	0.040 ± 0.0032^{cde}	0.040 ± 0.0000^{bcd}	0.038 ± 0.0020^{bc}	0.038 ± 0.0020^{bc}	0.030 ± 0.0000^{abc}

Means followed by the same letter along the same column are not significantly different ($p \leq 0.05$) by Tukey HSD test.

IV. Discussion

The first, second and third instar larvae and adults of *C. chloropyga* treated with neem seed and neem seed kernel powder displayed dose - dependent effect. Neem treatment seems to inhibit molting in larvae *C. chloropyga* owing to the prolonged larval-pupal period, suggesting the presence of growth regulating substance. When first, second and third instar larvae were treated with 4 and 6 % concentrations of neem seed powder, few adults emerged while larvae died at about the time of ecdysis and in 10 % neem seed treated – diet, there was no emergence of adults from pupa. The seed powder exerts a toxic effect on the larvae since the larval period was prolonged before pupation with a significant kill of larvae at higher concentrations.

Pupal periods of previously exposed larvae in different concentrations of treated diets were simultaneous prolonged before adult emergence. According to Hussien (1995), the failure of adult emergence could occur as a result of insufficient pressure in the ptilinum and hardening of the opercular suture. Plant extracts that accelerate larval development or prolonged it probably caused hormonal imbalance in the organism (Muse *et al*., 2002) which probably affected the normal development of structures that facilitate adult ecdysis.

There was no significant difference in the developmental periods of first, second and third instar larvae, including the pupal stadia at various concentrations of diets. Emergence of adults progressively decreased in

number with increase in concentration of neem seed and with no emergence in a 10 % neem seed – treated diet. There was no significant difference in the number of adult emerging at concentrations 1 to 10 % of neem seed kernel. Neem seed powder at 10 % concentration prevented the emergence of adult but there was adult emergence in 10 % neem seed kernel treated diet suggesting the presence of effective components in the neem seed powder that prevented formation of adult structures. It appears the neem seed kernel powder would be effective at concentrations above 10 % since the pupal period increased with increase in concentration of the neem seed kernel treatment at 5.00 ± 0.00, 6.00 ± 0.58 and 7.67 ± 1.20 days in 1, 4 and 10 % neem seed kernel - treated diet respectively. The extended periods of development is beneficial for the control of *C. chloropyga* because it would expose the larvae and the pupae to predators for a longer period, even if the extract did not kill them outrightly. The present result is in agreement with Ghazawi*et al.* (2007) who reported that a 10 % concentration of neem seed prevented the emergence of grasshopper*Heteracrislittoralis*. Kraus *et al.* (1986) reported that larvae *Epilachnaverivestis* fed azadirachtin from neem seed demonstrated feeding inhibition resulting in suspended development and subsequently death of the organism. Olaifa and Akingbohugbe (1986) reported that the antifeedant effect of *Azadirachtaindica* was enhanced by *Petiveriaalliacea* while the combination of *A. indica* and *Piper guineneense* produced significantly high mortality in *Zonocerusvariegatus*. According to Pener*et al*. (1989), treatment of*Locustamigratoria*with azadirachtin prevented fifth instar male nymphs to undergo the metamorphic moult to adult. Al-Fifi (2006) reported that azadirachtin treatment of instar nymphs of*Schistocercagregaria* caused delay in the next molt and death in delayed molt. Naqvi *et al*. (2007) reported a similar result for not only the partial emergence of adult*Muscadomestica*, but also for abnormalities in the development of second instar and deformation of pupae after administering neem extract. In this investigation, neem seed caused the emergence of malformed adults of *C. chloropyga.*

In the survival experiment, male population generally trailed behind the female population in all the treated and untreated diets. The female population in the control was stable at 100 % up to 26 days compared to those in the treated diets which was up to 13 days particularly at 4 % treated diet. The mortality effect of neem treated diets was more pronounced in the male flies than the females. Females feed better than males probably because the males were more active. Females possess abundant fat cells which detoxifies some of the toxic components. Male population therefore declined faster than the female population in the various concentrations of neem seed and neem seed kernel - treated diets. Muse *et al*. (2003) reported that*Chromolaenaodorata, Erythophleumguineense, Capsicum frutescens* and *Piper guineense* incorporated into larval food reduced the survival of *C. chloropyga* indicating the existence of mortality agents in several species of the plants.

Males and females maintained on neem seed kernel - treated diets outlived those maintained on diets incorporated with neem seed. Maximum life span recorded at 10 % neem seed - treated diet was 45 and 47 days for male and female; and 62 and 70 days for male and female exposed to neem seed kernel - treated diet. Female population in the control diet survived better than male similarly exposed with a stable population at 100 percent for up to 28 days compared with 12 days for males. There was a significant difference in the survival between *C. chloropyga* maintained on neem seed and neem seed kernel powder treated diets. When the flies were exposed to different concentrations of the neem seed, it was observed that as the concentration was increased, survival and longevity simultaneously decreased. Neem seed seems to reduce the longevity of the blowflies than neem seed kernel. Reproducing females that survived for longer periods produce several generations of flies that will be widely dispersed;therefore, the reduction in the survival of females in treated diet is a means of control for the blowflies. Siriwattanarungsee*et al*. (2008) reported that neem - treated flies had reduced longevity when compared with untreated control and more pronounced in males than females. Di Illio*et al*. (1999) also showed that longevity of Mediterranean fruit fly*Ceratitiscapitata*was decreased after exposure with neem compound.

Success of individuals contributes to the success of the species and this is in relation with the number of viable offspring it produces during its lifetime (Pekkala*et al.*, 2011). Thus, factor affecting fecundity affects the survival and continuity of the species. In this study, there was progressive decrease in mean fecundity with increase in concentration of treated diets from 1 to 10 % neem seed and neem seed kernel – treated diets. There was a significant difference in the total number of eggs laid at the various concentrations of neem treated diets. Since the lowest number of eggs was recorded at 10 % neem seed and neem seed kernel. Both powders have potential to prevent egg laying at higher concentrations. Similarly, fecundity decreased with increase in concentration of neem seed kernel diets from 96.05 ± 3.45 at 1 % to 57. 23 ± 2.86 at 10 % treated diets. Khan *et al*. (2007) demonstrated a decrease in fecundity of *Bactroceracucurbitae*and *Bactroceradorsalis* exposed to neem compound owing to its effect on ovarian development. Medina *et al*. (2004) reported that azadirachtin affected the ovarioles of *Chrysoperlacarnea*and eggs laid in treated females were significantly smaller in number than those in the control.

Male and female weights increased from 0 to day 5 in neem seed and neem seed kernel – treated diets. It seems the newly ecdysed adults fed significantly for five days immediately after emergence to strengthen body parts for subsequent adult activities, including mating and egg development. Since male and female weights fluctuated from day 10 at five days interval, up to day 40, it seems that the incorporation of neem seed and neem

seed kernel powders in the diet of the flies fed *ad libitum* did not deter feeding at the concentration used in this investigation. From the results, the slight weight gain which accompanies consumption of higher concentrations of treated diet may be responsible a reduction in food intake. Wilps (1986) reported that the ratio between the amount of food ingested and that portion of it converted in body weight decreases in*Phormia terrae-novae*, depending on the azadirachtin concentration consumed. According to Martinez and Van Emden (1999), sublethal concentrations of azadirachtin treated diet offered to larvae of *Spodopteralittoralis* reduced food intake and azadirachtin did not influence digestion efficiency but diminished the ability of the larvae to convert both ingested and digested nutrients into growth. Senthil*et al*. (2007) reported that extracts of neem caused a reduction in weight of treated third and fourth nymphal instars of brown planthopper, *Nilaparvatalugens* (Stål). Neem seed extract, Neemix, was reported to reduce weight gain among treated root weevils *Diaprepesabbreviatus* (L.) (Weathersbee and Tang, 2002). Since weight of treated adult *C. chloropyga* neither increased nor decreased but fluctuated throughout the test period, it seems that concentrations of neem treatment in the diets were not high enough to prevent a significant weight loss in adult *C. chloropyga*, but the neem seed and neem seed kernel powder treated diet prevented the expected exponential increase in the weights of females between day 0 to 20.

In summary, this study shows that the neem seed powder prolonged the development of second instar larvae and prevented the emergence of adults in 10 % treated diets. Neem seed kernel powder did not affect the development of larva, pupa and adult emergence at the various concentrations of treated diets. Neem seed and neem seed kernel treated diets progressively decreased the number of males and females from days 0 to 40 when compared with the control diets, with neem seed being more effective than the neem seed kernel. Neem seed treated diets effectively decreased the maximum longevity of males and females in 10 % concentration in comparison with neem seed kernel treated diets similarly exposed. Neem seed powder at 6 and 10 % significantly reduced fecundity when compared with neem seed kernel powder at the same percent concentrations.

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