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EVALUATION OF COW DUNG AND NEEM IN THE CONTROL OF ANOPHELES SPP

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ABSTRACT

The control of malaria is complex and has previously focused on the use of drugs and chemicals targeting the parasite and the vectors respectively. However, this has been a challenge because the parasite and the vectors have developed resistance. However, integrated approaches that incorporate use of cow dung and plants products could increase the chances of malaria control. Deliberate organic contamination as a measure against mosquitoes has been shown to have a larvicidal effect but the findings have not been tested in semi-field conditions. For a long time, neem has been reported to possess larvicidal properties against mosquitoes. However, the combined effect of cow dung and neem on immature stages of Anopheles has not been investigated. This research was therefore designed to investigate the effect of cow dung alone and a combination of cow dung and neem on the immature stages of Anopheles mosquitoes. In the laboratory experiments, 10 individuals of each immature stage were placed in bowls containing either cow dung, neem alone or neem and cow dung combined. Mortality was recorded after 24 hours. Semi-field experiments were conducted in a screen house where sub-plots measuring 1.5 x 0.5 m were created. Cow dung, neem alone or cow dung combined with neem was added. 50 individuals of each immature stage were then introduced in each sub- plot and then mortality was recorded. Analysis of variance tests was performed to determine the effect of cow dung, neem alone and a combination of neem with cow dung on the immature stages of Anopheles species in both the laboratory and the semi-field conditions. Results for laboratory experiments indicated that cow dung and neem individually caused significant mortality of immature stages of mosquitoes. Also, a combination of cow dung and neem caused higher mortality than cow dung, neem alone but the difference was not significant. In semi-field experiments, there was a significant difference in mortality between all the immature stages in sub-plots containing cow dung alone. The mortality was higher in sub-plots applied with a combination of cow dung and neem but as reported in the laboratory experiments the difference was not significant. The findings of this study have demonstrated that cow dung, neem alone and a combination of neem and cow dung caused mortality of the immature stages and can thus be used in management of larval stages in the mosquito breeding sites.

Key Words: Cow dung, Neem, Anopheles, Larval stage

1.0 INTRODUCTION

Deliberate water pollution using organic matter is a naturalistic method of malaria vector control ([1-3]. The technique has been used successfully to control *Anopheles fluviatilis* and *An. maculatus* in India, Malaysia and Singapore [3]. *Culicine* mosquitoes such as *Culex vishnui, Cx. pseudovishnui and Cx tritaeniorynchus* mosquitoes have previously been controlled using pig droppings and horse manure in India [1]. On decomposition, the dung of most herbivores may deter species that prefer breeding in fresh water such as *An. gambiae* from ovipositing. It has been reported that, eggs laid in polluted water usually tend to keep together in clumps and thus their viability is reduced [4]. It is thought that an olfactory response causes the gravid mosquitoes to avoid the polluted water or the lowering of the surface tension caused by organic pollution may have a negative effect on the aquatic stages of mosquitoes by preventing them from attaching to the surface film from where they breathe atmospheric oxygen [5]. Additionally, on decomposition organic materials produce toxic substances, which if consumed by mosquito larvae are likely to cause death [6]. Such harmful substances have been identified as ammonium salts and nitrite [7].

The success of this method mainly depends on the type of the material used to pollute mosquito breeding habitats. Knowledge on environmental management and mosquito breeding has been used in the past to control malaria in irrigation systems. On the other hand Neem (*Azadirachta indica*) products have been shown to exhibit a wide range of effects that are potentially useful for mosquito control [8] . These effects are frequently attributed to the azadirachtin contents of the products. However, there are no

studies to done to test the efficacy of both the cow dung and neem in the control of immature stages of *Anopheles* mosquitoes. Furthermore this study has not been done in semi field conditions. This study therefore investigated the effects of cow dung, neem separately and in combination with neem on immature stages of *Anopheles* species both in the lab and semi field conditions in Ahero rice irrigation scheme.

2. MATERIALS AND METHODS

2.1 Description of the study area

The studies were conducted in the laboratories, insectary and semi-field conditions within National Irrigation Board (NIB) Ahero. Ahero Rice Irrigation Scheme is located in Nyando District, Nyanza province, approximately 400km west of Nairobi and 24km East of Kisumu. Ahero lies between latitude 0^o 10'S and longitude 34^o 55'E. The area experiences a mean annual temperature of 23^oC, mean annual rainfall 1300mm and mean relative humidity of 76%. These conditions render the area highly vulnerable to malaria transmission. According to 1999 census, the population of the district is 61,556 [9]. Majority of the inhabitants belong to the Luo ethnic group. *Anopheles gambiae* s.l and *An. arabiensis* are the most important malaria vectors in this area. Mosquito populations rapidly increase during the rainy season while rice irrigation fields act as mosquito breeding grounds during the dry season. A high malaria incidence in the area makes it an important target for studies on the vector control.

2.2 Mosquito rearing for experiments

Anopheles mosquitoes were used in all the experiments. Mosquito rearing was done in an insectary within NIB Ahero. Climatic conditions within the insectary were maintained at 24 - 30ºC temperature, 60 -80% relative humidity and 12L: 12D photoperiod. Wild, indoor-resting, blood fed *Anopheles* mosquitoes were collected from 0600hrs to 0800hrs from houses in Ahero irrigation scheme, Nyando District, Western Kenya, by means of aspiration. They were immediately transported to the field laboratory at Ahero NIB for sorting out. Identification was done by the use of naked eye, magnifying lens or microscope where necessary in order to distinguish gravid female *Anopheles* spp. from males and other species of no medical importance. Males and species of no medical importance were discarded while the selected gravid females were transferred into standard mosquito cages measuring each 30 x 30 x 30 cm.

The gravid female mosquitoes from the field were provided with oviposition cups to lay eggs. The laid eggs were dispensed in larval rearing trays (measuring 31 x 21 x 8 cm) each containing 1 litre of clean distilled water in which they hatched and underwent development from the first instar larval stage (L1) all through to pupation. The emerged larvae were fed on approximately 0.04mg of Vipan® fish food per tray containing 100 larvae. The water in which larvae were bred was replaced after every 2 days. To accomplish this, larvae were sieved using a plastic sieve and transferred into trays containing fresh distilled water. This process was continued until development from the first instar larvae (L1) through to pupae was completed. Pupae were collected using plastic droppers and transferred into clean bowls containing distilled water. Bowls containing pupae were put in adult standard mosquito holding cages (30 x 30 x 30 cm) and covered with mosquito netting. The bowls were left to stay in the cages until adult mosquitoes emerged. After emergence, adults were fed on 6% sucrose solution for 3 days using folded paper towel in form of wicks while water was provided using soaked cotton wool placed on top of the cages. Adult female mosquitoes aged between 3 - 4 days were starved for 6 hours and then allowed to feed on the fore-arm of a human volunteer as a source of blood meal for egg laying. Feeding was done under complete darkness between 0700hrs and 0800hrs continuously for the 3 days. The feeding process lasted for 10 – 15 minutes. Gravid female mosquitoes were provided with clean tap water in half full Petri dishes in which to lay eggs. Laid eggs were collected, dispensed in larval rearing trays and the process continued. The resultant larvae were used in the subsequent experiments.

2.3 Cow dung

Fresh cow dung was collected from Freshian cows. The cows were kept in a zero grazing unit in NIB Ahero. The cow dung was green and coarse in texture at the time of collection. The main diet of the cow was mainly Napier grass and commercial feeds. The cow dung was collected from the same cows every day between 0800 and 0900hrs. It was placed in plastic paper bags and immediately transported to the laboratory at NIB Ahero. This was used in the laboratory and semi-field experiments to test the effects of cow dung and a combination of neem and cow dung on the immature stages of *Anopheles* mosquitoes.

2.4 Laboratory experimental procedures.

Experiments aimed at determining the effect of cow dung and cow dung combined with neem on immature stages of *Anopheles* mosquitoes were carried out for a period of 4 weeks. The experiments were undertaken in the laboratory at NIB Ahero using bowls. The bowls measured 19 cm x 9 cm each and were orange in colour Fig 2.1.

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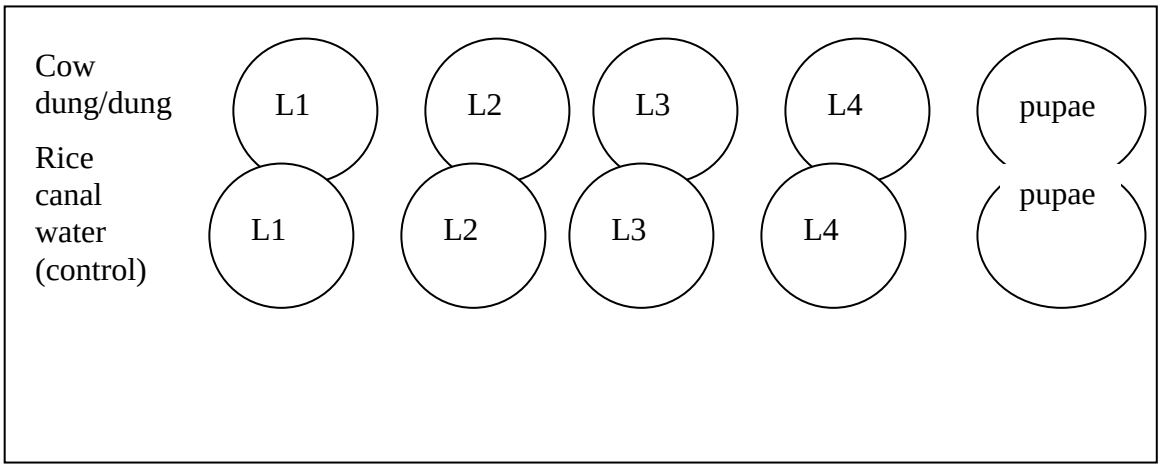

Figure 2.1: Experimental layout of bowls in the laboratory.

2.4.1 Experimental procedure on effect of cow dung on immature stages of *Anopheles* mosquitoes.

The effect of cow dung on the immature stages of *Anopheles* mosquitoes was carried out in the laboratory for a period of 4 weeks. The larvae and pupae of *Anopheles* spp. mosquitoes used in this experiment were reared as described in section 2.2. The term 'immature stages' as used in this section refers to early instar larvae (L1 and L2), late instar larvae (L3 and L4) or pupae. Ten grams of fresh cow dung was put in each of five bowls each measuring 19 cm x 9 cm. Thirty milliliters of water from rice canal was added and the mixture was stirred using a glass rod and then placed on the laboratory benches for 2 hours to allow the floating debris to settle down. Ten individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced into the bowls (each bowl contained a separate immature stage). Rice canal water without addition of cow dung served as the control. The experiment was replicated 30 times. Each bowl containing the pupae was put inside an adult mosquito holding cage to prevent the emerging adults from escaping. Larvae in each bowl were fed on approximately 0.04 mg of Vipan® fish food per day. Each day individuals in each bowl were removed using plastic pipettes. They were then placed in petri dishes containing distilled water. Counting was done and their number recorded separately according to whether they were dead or alive. Dead individuals were discarded after counting and the emerged adults transferred to the adult mosquito cages. Similar experiments were done using 1g of neem cake powder as the treatment.

2.4.2 Experimental procedure on effects of cow dung and neem combined on immature stages of *Anopheles* mosquitoes in the laboratory.

Experiments were carried out to investigate the larvicidal activity of cow dung and neem against immature stages of *Anopheles* spp of mosquitoes. *Anopheles* spp larvae and pupae used in this experiment were reared as described in section 2.2. One gram of neem was mixed with 1 litre of water from rice canal. The mixture was stirred and left for 12 hours. Thirty milliliters of this solution was mixed with 10 g of fresh cow dung in bowls measuring each 19 cm x 9 cm. The mixture was stirred using a glass rod and placed on the laboratory benches for 2 hours for the floating debris to settle. Ten individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced. Rice canal water in bowls served as the control. This experiment was replicated 30 times. Each day individuals in each bowl were removed using a plastic pipette and placed in petri dishes. They were counted and recorded on whether they are dead or alive using the procedure explained for cow dung experiments conducted in the laboratory (section 2.4.1).

2.5 Experimental sub-plots

Similar experiments were carried out in semi-field conditions conducted in sub-plots that were inside a screen house at NIB, Ahero. The screen house measured 4.5 m x 1.5 m, was wooden and covered with mesh wire. The land inside the screen house was sub- divided into 5 sub-plots. Each sub-plot measured 0.5 m x 1.5 m. Between any two sub-plots, a 0.5 m wide and 5 cm high mud walled band was created to prevent mixing of treatments (Figs. 2.2 and 2.3).

Figure 2.2: A screen house which was used for semi-field experiments

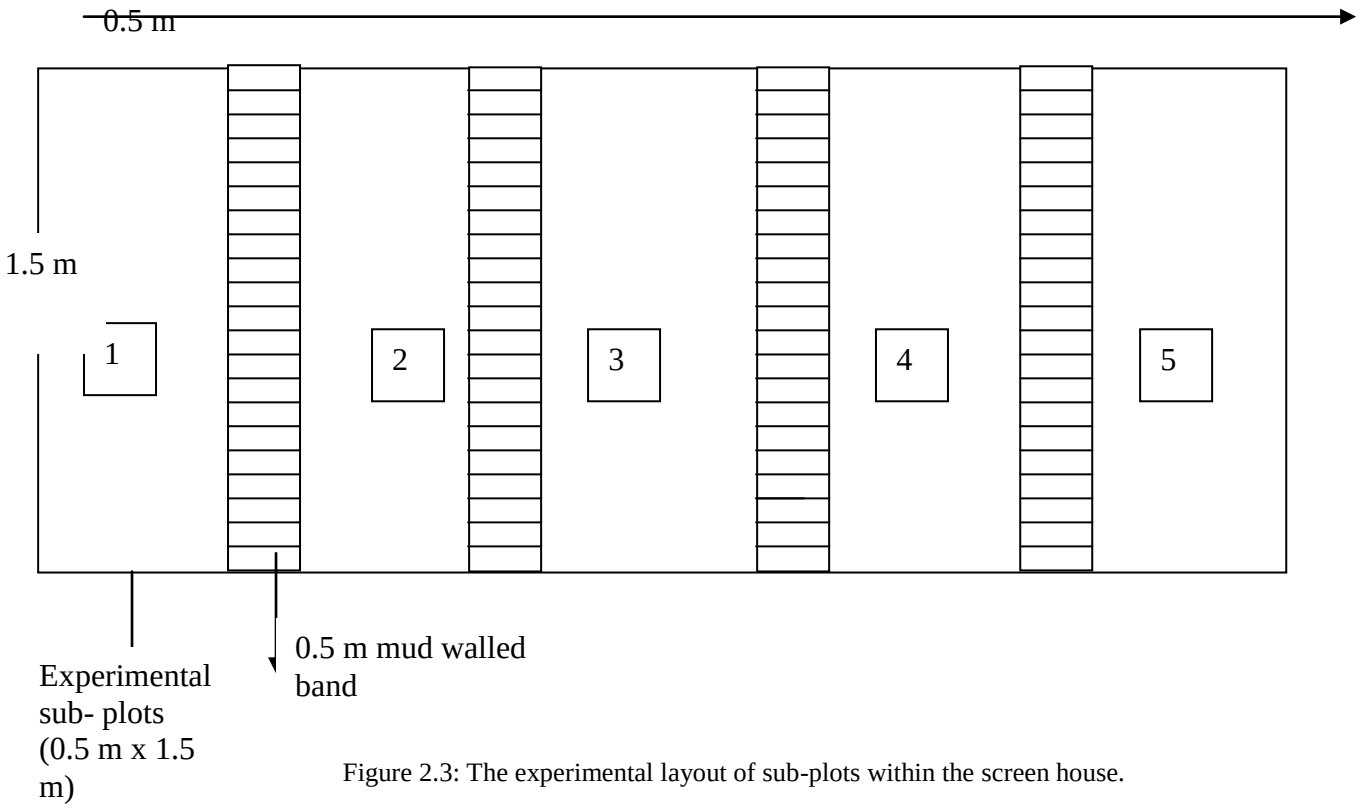

Figure 2.3: The experimental layout of sub-plots within the screen house.

2.5.1. Experimental procedure on effect of cow dung on Anopheles mosquitoes in semi-field conditions.

The effect of cow dung on immature stages of *Anopheles* spp. mosquitoes in semi-field was investigated. *Anopheles* spp larvae and pupae used in this experiment were reared as described in section 2.2. Each of the 5 sub-plots measuring 1.5m x 0.5m (Fig. 2.3) was filled with 6 litres of water from rice canal and two hundred grams of fresh cow dung added. The mixture was stirred using a glass rod and left for 2 hours for the floating debris to settle. In each of the 5 sub-plots, 50 individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced. Rice canal water (6 litres) alone was used as the control. The experiment was replicated 30 times. In sub-plots containing pupae, a mosquito cage was mounted on top to prevent emerging adults from escaping. Larvae in each sub-plot were fed with approximately 200 mg of Vipan® fish food daily. Individual immature stages were removed using a pipette and placed in petri dishes. They were counted and those that were dead or alive corded. Similar experiments were done using 10g of neem cake powder as the treatment.

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2.5.2 Experimental procedure on effects of cow dung combined with neem, on Anopheles mosquitoes in semi-field conditions.

To determine the effect of combining cow dung and neem, each sub-plot was filled with 6 liters of water from rice canal. *Anopheles* spp. larvae and pupae used in this experiment were reared as described in section 2.2. Two hundred grams of fresh cow dung and 20 g of neem powder were added. The mixture was stirred thoroughly and left for 2 hours for the floating debris to settle. In each sub-plot, 50 individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced. Rice canal water (6 litres) alone was used as the control. The experiment was replicated 30 times. In sub-plots containing pupae, a mosquito cage was mounted on top to prevent emerging adults from escaping. Larvae in each sub-plot were fed with approximately 200 mg of Vipan® fish food daily. Individual immature stages were removed, they were counted and those that were dead or alive recorded.

2.5.3. Control experiments in semi-field conditions

Control experiments were carried out in plastic troughs measuring 42 x 16 cm and orange in colour. They were half filled with soil from rice field and 10 litres of rice canal was added. The mixture was stirred using a glass rod and the mixture left for 2 hours for the floating debris to settle. In each trough, 50 individuals of each immature stage (L1, L2, L3, L4 and pupae) were introduced. The experiment was replicated 30 minutes. Feeding of larvae and monitoring of survival was done as earlier described (Section 2.4.1).

2.7 Data management and analysis

All data obtained from this study were statistically analyzed using Statistical Analysis System® (2002) statistical package. Analysis of Variance (ANOVA) tests was used on data collected to evaluate the effects of cow dung alone and a combination of neem and cow dung on the immature stages of *Anopheles* mosquitoes both in laboratory and semi-field experiments. Significantly different means (<0.05) were separated using Student-Newman-Keuls (SNK).

3.0 RESULTS

3.1 Effects of cow dung on immature stages of *Anopheles* mosquitoes in the laboratory.

Out of the 1200 larvae and 300 pupae used to test the effect of cow dung, it was observed that all the immature stages reared in rice canal water containing cow dung had the highest mortality in the first instar larval stage (mean mortality was 7.23 ± 0.23). This was followed by the second instar larvae with mean mortality of 6.65 ± 0.05. Third and fourth instar larvae had a lower mortality of 6.00 ± 0.04 and 5.53 ± 0.04 respectively while the lowest mortality was in the pupal stage (1.37 ± 12; Fig 3.1). In the controls (rice canal water) the mortality was low in all the stages when compared to mortality in cow dung. The highest mortality was recorded in the 4th instar stage (1.67 ± 0.06) and the lowest in pupae stage (1.37 ± 0.05) Fig. 3.1

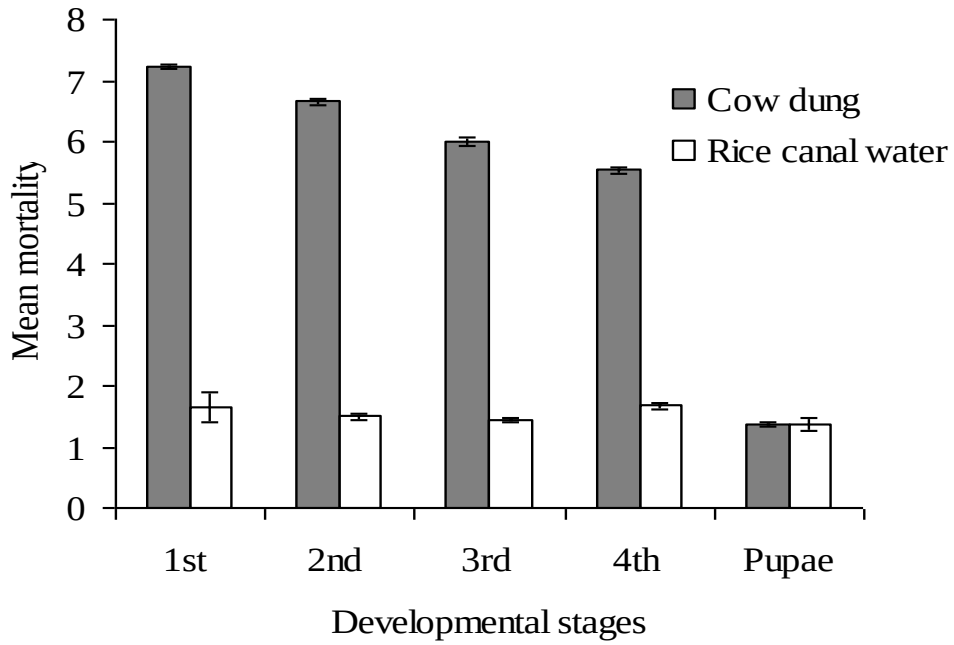

Figure 3.1: Mean mortality of immature stages of *Anopheles* species after being placed in bowls containing cow dung and rice canal water. Standard error bars of the mean number of dead individuals are shown.

A significant difference in mortality was recorded between all the immature stages (F= 88.22; P<0.0001) that were in bowls containing fresh cow dung (Fig 3.1). It was also observed that there was a significant difference in mortality of the first instar larval stage that were in bowls containing cow dung compared to those placed in the control (rice canal water ; F= 215.27; P<0.0001). Likewise for L2, L3 and L4 instar stages that were in cow dung, there was a significant difference in mortality in comparison to those placed in rice canal water (F=88.22; P<0.0001). It was also observed that there was a significant difference in mortality between the early instar larvae (L1 and L2) and the fourth instar larvae placed in bowls containing cow dung (F= 88.22; P, <0.0001; Fig. 3.1). There was no significant difference in mortality of the pupal stage between those placed in bowls containing cow dung and those in bowls containing rice canal water (control; F= 0.74; P=0.529).

3.2 Effects of cow dung on *Anopheles* mosquitoes in semi-field conditions.

Mortality of 1st instar larvae was observed to be the highest (35.87 ± 0.01) for the larvae placed in sub-ploys containing cow dung. The late instar larvae (L3 and L4) had a lower mortality (29.17 ± 0.01 and 28.00 ± 0.03 respectively) than the early instar larvae (L1 and L2) (Fig 3.2). The lowest mortality was observed in the pupal stage with a mean mortality of 1.97 ± 0.10. All immature stages that were in sub-plots containing rice canal water (control) had a lower mortality compared to those in cow dung (Fig. 3.2). In these sub-plots the highest mortality was in 2nd instar larvae (2.93 ± 0.06) while the lowest (2.03 ± 0.06) was noted in the 3rd instar larvae (Fig. 3.2).

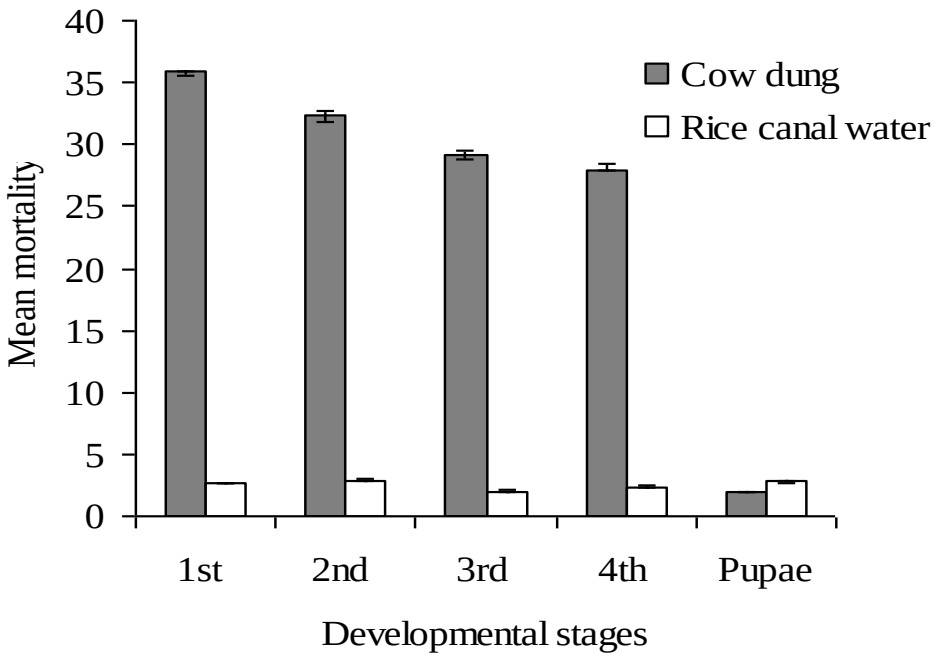

Figure 3.2: Mean mortality of immature stages of *Anopheles* species after being placed in sub-plots containing cow dung and rice canal water. Standard error bars of the mean number of dead individuals are shown

In sub-plots with cow dung there was a significant difference in mortality between all the immature stages (F= 560.09; P<0.0001). A significantly higher mortality (F, 560.09; P, <0.0001; Fig 3.2) was noted in the early instar with a mean mortality of 35.87 ±0.01 and 32.30 ± 0.01 respectively. Among the experimental groups there was no significant difference (F= 560.09; P> 0.05) in mortality between 3rd (mean mortality 29.17 ± 0.01) and 4th instar larvae (28.00 ± 0.03). It was observed that, in all the immature stages in cow dung there was a higher mortality compared to those in rice canal water (F=535.16; P<0.0001; Fig. 3.2).

3.3 Effects of neem on immature stages of *Anopheles* mosquitoes in the laboratory.

A significant difference in mortality was recorded between all the immature stages (F= 45.54; P<0.0001) that were in bowls containing neem (Fig 3.3). The highest mortality was observed in the first instar (7.43 ± 0.03) followed by the second instar (7.00 ± 0.04). However the difference in mortality between L1 and L2 was not significant (F= 45.54; P, >0.05; Fig. 3.3). It was also observed that there was no significant difference in mortality of the first and second instar larval stage compared to the third instar stage which had a mean mortality of 6.00 ± 0.05 (F= 45.54; P, >0.05; Fig. 3.3). A significant difference was observed in mortality of the 4th instar (3.88 ± 0.08) compared to 1st ,2nd and 3rd instars in bowls containing neem. Pupae that were reared in

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bowls containing neem alone had the lowest mortality (mean mortality 1.50 ± 0.11) the difference was significant (*F*= 45.54; *P*, < 0.0001; Fig. 3.3).

In comparing the mortality in the experimental and the control, a significant difference (*F*= 215. 27; *P*, <0.0001) in the first instar (7.43 ± 0.03) was noted compared to the control which had a mean mortality of 1.66 ± 0.05. Similarly there was a significant difference in mortality between the second instar placed in neem compared to those in rice canal water (*F*= 99.33; *P*, <0.0001; Fig. 3.3). In the third instar placed in neem, there was a significant difference in mortality compared to those in the rice canal water only *F*= 140.41; *P*, < 0.0001; Fig. 3.3. A similar mortality was noted in the fourth instar whereby the experimental had significantly higher mortality than the control (*F*= 55.72; *P*, 0.0001; Fig. 3.3) There was no significant difference (*F*= 0.74; *P*, 0.529) in mortality of the pupal stage between those placed in bowls containing neem (mean mortality 1.50 ± 0.11) and those containing rice canal water (1.37 ± 0.05).

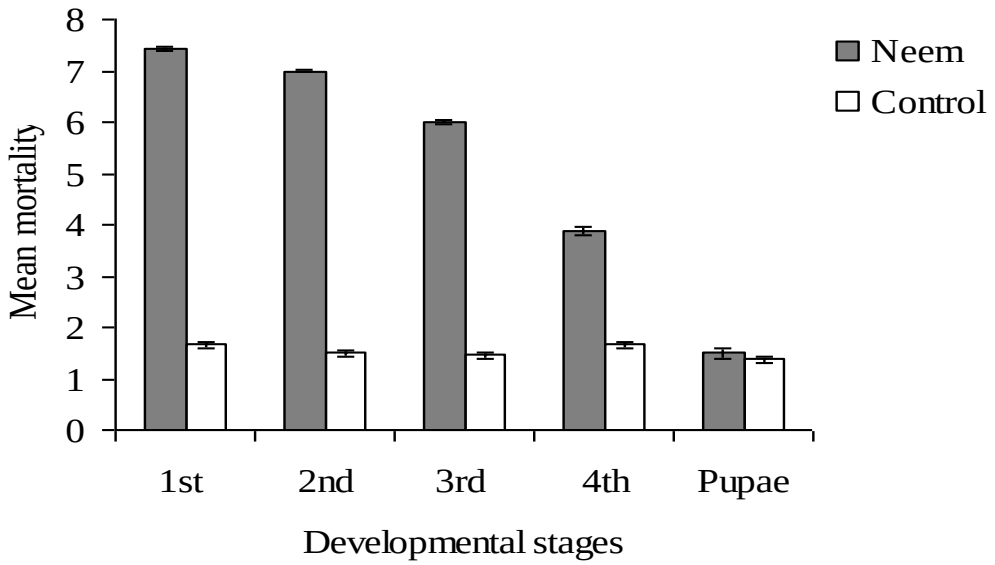

Figure 3.3: Mean mortality of immature stages of *Anopheles* species after being placed in bowls containing neem and rice canal water. Standard error bars of the mean number of dead individuals are shown.

3.4 Effects of neem on immature stages of *Anopheles* mosquitoes in the semi-field.

The highest mortality was noted in the first instar placed in sub-plots containing neem (37.03 ± 0.02) this was followed by the second instar larvae with a mean mortality of 36.03 ± 0.02. The difference in mortality between the L1 and L2 was not significant (*F*, 757.19; *P*, > 0.05; Fig. 3.4). The mortality was lower in the late instar stages (mean mortality of 31.13 ± 0.03 and 29.13 ± 0.03 respectively) compared to the first and second instars. The difference in their mortality was significant (*F*= 757.19; *P* <0.0001; Fig 3.4).

In comparing the experimental and the control, a significant higher mortality was noted in the first instar placed in sub-plots containing neem than those in the rice canal water. (*F*= 615.97; *P*, 0.0001). In second instar, the mortality was significantly higher (*F*= 535.15; *P*, < 0.0001) in the experimental (37.03 ± 0.02) than in the control (2.67 ± 0.06). In the late instar (L3 and L4), a similar significantly higher mortality was noted (*F*= 535.1; *P*, <0.0001 and *F*= 567.39, *P*, <0.0001 respectively). In the pupal stage the difference in mortality was not significant between the experimental and the control (*F*= 10.25 ; *P*, > 0.05).

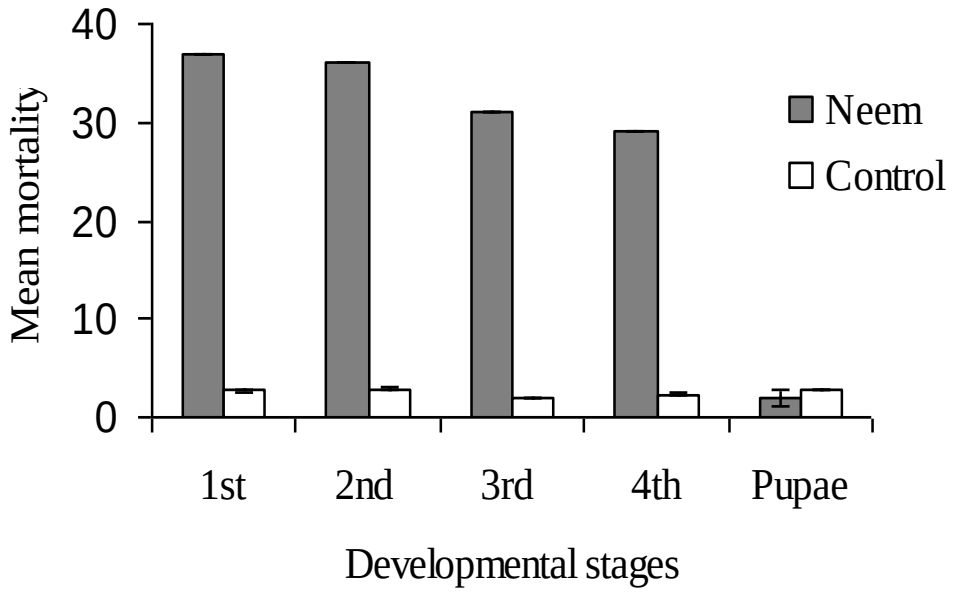

Figure 3.4: Mean mortality of immature stages of *Anopheles* species after being placed in sub-plots containing neem and rice canal water. Standard error bars of the mean number of dead individuals are shown.

3.5 Effects of cow dung combined with neem on immature stages of *Anopheles* mosquitoes in the laboratory.

The highest mortality was observed in the early instar larvae (L1 and L2) reared in cow dung and neem (mean mortality 8.11 ± 0.03 and 7.82 ± 0.03 for L1 and L2 respectively) while the lowest mortality was observed at pupal stages in a similar treatment (1.04 ± 0.07; Fig. 3.5). In the control (rice canal water) the mortality of all immature stages was lower compared to that of those in cow dung and neem. The highest mortality in the control (rice canal water) was in the 4th instar stage (1.67 ± 0.06) while the lowest was also in pupal stage 1.37 ± 0.05 (Fig 3.5).

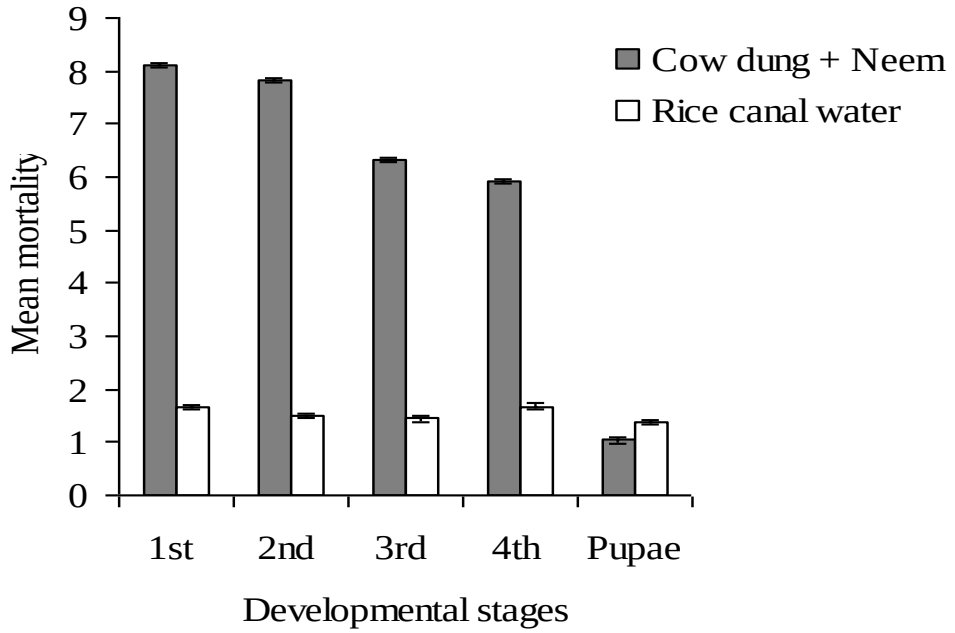

Figure 3.5: Mean mortality of immature stages of *Anopheles* species after being placed in bowls containing a combination of cow dung with neem and rice canal water. Standard error bars of the mean number of dead individuals are shown.

In all the immature stages placed in bowls containing neem combined with cow dung a significant difference in mortality was observed (F= 252.68; P<0.0001; Fig. 3.5). For all immature stages (L1 - L4 and pupae) that were placed in bowls containing a combination of neem and cow dung a significantly higher mortality was observed than in those placed in bowls containing rice

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canal water (*F*= 252.68; *P*<0.0001; Fig.3.5). The late instar larvae (L3 and L4) placed in bowls containing cow dung combined with neem revealed a significantly lower mortality than the early instars (L1 and L2) placed in a similar treatment (*F*=55.72; *P*<0.0001). However, there was no significant difference in mortality between the pupae that were in bowls containing cow dung and neem and those in bowls containing rice canal water (*F*= 0.74; *P*>0.529; Fig.3.5).

3.6 Effects of cow dung combined with neem, on *Anopheles* mosquitoes in semi-field conditions.

A higher mortality (mean mortality 38.97 ± 0.01 and 37.30 ± 0.02) was observed in the early instar larvae (L1 and L2 respectively) compared to the late instar larvae (mean mortality of 34.00 ± 0.02 and 32.00 ± 0.02 respectively). Pupal stage showed the lowest mortality 4.63 ± 0.06 (Fig. 3.6). Among the immature stages in the control, 4th instar larvae had the lowest mortality 2.03 ± 0.06.

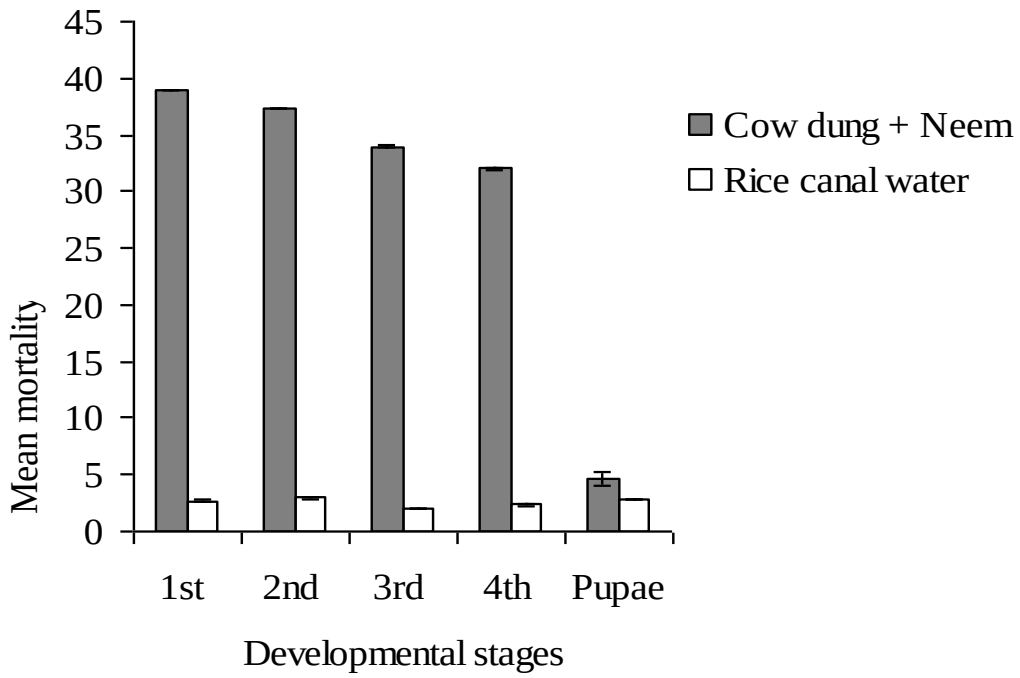

Figure 3.6: Mean mortality of immature stages of *Anopheles* species after being placed in sub-plots containing a combination of cow dung and neem or rice canal water. Standard error bars of the mean number of dead individuals are shown.

In sub-plots containing a combination of neem and cow dung, there was no significant difference in mortality between 1^{st,} 2nd and 3rd instars. However, a significant difference was observed between 1st and 4th instars stage (*F*= 701.97; *P*<0.0001; Fig. 3.6). The pupae in cow dung combined with neem had a significantly low mortality (*F*=10.25; *P*<0.0001; Fig 3.6) than those in the control. There was a significant difference in mortality between immature stages placed in sub-plots containing cow dung and neem combined compared with those in rice canal water (*F*=701.97; *P*<0.0001; Fig 3.6). Pupae in cow dung combined with neem had significantly higher mortality than pupae in the rice canal water (*F*, 10.25; *P*, <0.0001)

3.7 Comparison of the effects of cow dung and neem combined compared to cow dung alone in the laboratory

A comparison between the effect of cow dung combined with neem on the immature stages and cow dung alone was done using the data from the laboratory experiments. In the first instar larvae higher mortality (8.11 ± 0.03) was recorded in cow dung and neem than in cow dung alone (7. 23 ± 0.24; Fig. 3.7). A similar trend was recorded in the second instar larvae, higher mortality was recorded in larvae placed in a combination of cow dung and neem (7.82 ± 0.03) while mortality of larvae in cow dung had a lower mean mortality of 6.65 ± 0.05 (Fig. 3.7). Third instars larvae placed in a combination of cow dung and neem also had a higher mortality compared to those placed in cow dung alone (6.33 ± 0.03 and 6.00 ± 0.04 respectively; Fig. 3.7). Similarly in the fourth instar larvae in a combination of cow dung and neem, mortality was higher (5.91 ± 0.04) than in cow dung alone (5.53 ± 0.04; Fig. 3.7). However, there was no significant difference in mortality between all the larval instars placed in a combination of cow dung and neem in comparison to those that were placed in cow dung alone (*F*,140.44; *F*>0.05; Fig. 3.7).

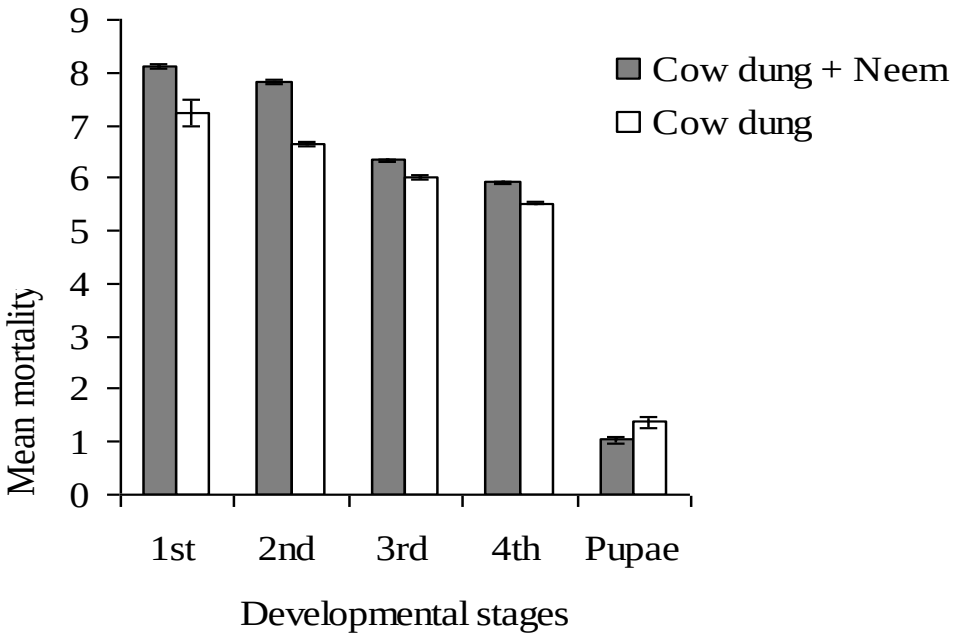

Figure 3.7: Mean mortality of immature stages of Anopheles species after being placed in cow dung combined with neem compared to cow dung alone in the laboratory. Standard error bars of the mean number of dead individuals are shown.

4.0 DISCUSSION

Currently, malaria vector control is thought to be one of the best approaches in controlling the high malaria incidences. Different studies have clearly demonstrated that it is far less costly and more effective to control mosquito populations as larvae, before they mature and disperse into the environment. Numerous studies have suggested that larval control is a more effective way of reducing the malaria vector [10]. In this study the role played by cow dung and neem *A. indica* in the control of malaria vector was investigated since these materials are less costly, easily applicable and biodegradable.

In this study, experiments on the effects of cow dung on immature stages of *Anopheles* mosquitoes have shown that, deliberate contamination of water with cow dung results in mortality of the immature stages of *Anopheles* spp. This was supported by the higher mortality of the larvae reared in cow dung than in the control treatment (rice canal water). Previous studies by [11] suggested that larvae imbibe micronutrients in the larval habitat in the process of filter feeding thereby ingesting harmful products that then results to their death. The lowest mortality was observed in the pupal stage indicating that pupae are less susceptible to contamination with organic materials such as cow dung. These could be attributed to the non feeding nature of pupae therefore avoiding ingesting of lethal materials.

In semi-field conditions, higher mortality in the immature stages in cow dung than those in rice canal water was recorded showing the potential of cow dung as a contaminant that can be used to reduce immature stages of mosquitoes. Early instar larvae (L1 and L2) were affected more than the late instar larvae (L3 and L4). These could be attributed to the fact that early instars larvae are more susceptible to mortality than the late instars larvae. Early instar larvae feed on the available organic material indiscriminately unlike the late instar larvae which specialize on feeding on particular organic matter[2]. This feeding habit could have caused the higher mortality of early instars than the late instar. The result of mortality induced by cow dung was in agreement with the findings of a study reported by Lee [12] who reported that the dung of herbivores exhibited larvicidal activity against immature stages of *Anopheles* mosquitoes. Further studies have also shown that the normal larval breeding habitats become unfavorable if they are accidentally or artificially polluted with animal wastes or organic matter [4]. On decomposition, cow dung reduces the amount of oxygen content available in water. Low oxygen tension then reduces the survival of mosquito immature stages therefore rendering the habitats unfavorable.

High larval mortality was observed in larval stages that were placed in bowls and sub-plots containing neem than in the rice canal water. Early instar larvae had a significantly higher mortality compared to the late instar in both the laboratory and semi-field experiments. This finding suggests the high toxicity of neem to the different developmental stages of *Anopheles* mosquitoes. Studies conducted in Nigeria using *Anopheles* larvae found a similar trend whereby all larvae died within 12 hours after neem extracts were applied in water [13]. The neem cake powder had greatly reduced-sized particles and was evenly mixed within the water with a few suspended particles on the water surface. The spread of these fine particles probably increased the efficacy of the

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neem since *An. gambiae* s.s. are small particle surface feeders [14]. When ingested, the neem product particles induce anti-feedancy in larvae either by altering the insect's chemoreception or by reducing the food intake due to its toxicity [15].

Other studies reported that azadirachtin, a botanical pesticide derived from the neem tree is generally considered less harmful to the environment than other commonly used pesticides [16] . Cow dung and neem (*A. indica*) are biodegradable and therefore after decomposition they not only kill mosquito larvae but also act as fertilizers. For instance, neem has been shown to have anti-larval properties and also act as a fertilizer [17]. Therefore neem has a dual purpose in that once applied in rice fields it could reduce mosquito immature stages and also act as a fertilizer [18]. When cow dung was mixed with neem a higher mortality of all the immature stages occurred in the water contaminated with cow dung and neem than in the control. Furthermore, this study revealed that the highest mortality was recorded in early larval instars (L1 and L2) than the late larval instars (L3 and L4). These observations revealed that cow dung and neem are efficacious against mosquito larvae and supports the findings by [19-20] who reported that larval control is a more effective strategy for reducing mosquitoes. In a combination of neem and cow dung, mortality was lowest at pupal stage. These findings can be attributed to the fact that pupa is a non-feeding stage thus they do not ingest the contaminants resulting from neem and cow dung that were used in this experiment. Other studies have also confirmed that pupal stage has a casing that prevents their contact with most of the contaminants thus conferring them protection against possible control strategies[21]. This implies that any control measure which targets the developmental stages of mosquitoes should target the larval stage and especially the early instars since they were more susceptible than late instars and pupae. When the combination of cow dung and neem was tested in semi-field conditions, a significantly higher mortality compared to rice canal water was noted.

In this study, the high larval mortality indicates that the combination of cow dung and neem is toxic. Early larvae instars also had higher mortality than the late larval instars. At this stage of growth, the larvae are still adapting to the environment and therefore contact with contaminants is likely to make the habitat unfavorable[22]. These could also be attributed to the indiscriminate ingestion of any suitably sized particles especially by the early instars larvae[22]. These findings of decreased mortality with increase in age support the reports by [23] who found out that the sensitivity of larvae to neem seed kernel decreased with increase in larval age and thus the late instar larvae were less susceptible to the larvicidal activity of neem. From this study, a combination of cow dung and neem caused mortality of the different stages of the mosquito larvae. Thus in integrated vector management combining both the reported organic materials would yield better results

5.0 CONCLUSION

This study demonstrated that cow dung, neem alone and a combination of neem and cow dung caused mortality of the immature stages and can thus be used in management of larval stages of Anopheles spp in the mosquito breeding sites.

6.0 CONFLICT OF INTEREST

The author declare that there is no conflict of interest with the funding organization

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