ORIGINAL ARTICLE

EFFECT OF N-P FERTILIZERS AND COW DUNG ON THE LARVAL DEVELOPMENT OF MOSQUITOES IN SIMULATED FIELD IN ALAGAE, CENTRAL ETHIOPIA

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ABSTRACT

Objective: *This study was conducted to assess the effect of N-P fertilizers and cow dung on mosquito larva development at Alagae, Central Ethiopia, from January 2007 to February 2007.*
Methods: *Cow dung, Urea and DAP were applied in thirty-three experimental ponds of 50 cm radius wide and 19 cm deep.*
Results: *Application of these fertilizers resulted in varied larval count of culicines and anophelines mosquito species. Culicine mosquito larvae count (83.1% and 97.2%) was higher than that of anophelines (16.9% and 2.8%) in manure and N-P treated ponds respectively. Higher larval number was collected in ponds treated with both cow dung and N-P fertilizers (87.7%) than the control (12.3%), (P<0.001). Moreover, application of these fertilizers in a pond resulted in three-fold increase in larval population when compared with the number in control ponds. Dose variation in urea and cow dung also resulted in larva count variation. Accordingly, more larvae were collected from 10 gm urea-treated ponds than 5gm and 15gm treated ones. On the other hand, ponds treated with 300gm cow dung carried higher number of larvae compared to 600gm and 900gm treated ponds.*
Conclusion: *The current flourishing irrigated agricultural system coupled with the extensive usage of fertilizers in all agro-ecological zones of Ethiopia may create suitable environmental conditions for the breeding of different mosquito species.*
Key words: *Fertilizers, mosquito, larvae population, Ethiopia.*

INTRODUCTION

The distribution of mosquitoes is associated with various environmental factors including type of habitat (1), feeding behaviour of mosquito species (2, 3, 4), availability of oviposition sites (5) and natural resting sites (4, 6). Mosquitoes breed in water which must be more or less quiescent, since the wave action in large bodies of water is a deterrent unless the larvae are able to obtain shelter among vegetation (7).

Successful larval surveillance requires the ability to identify larval habitats and distinguish between sites with high and low vector populations in a timely manner (8). Natural and artificial habitats are equally productive (9). The biological and physico-chemical attributes of the aquatic environment may alter larval population and adult vector competence (10).

The occurrence and abundance of immature mosquitoes are significantly correlated with the water pH, dissolved oxygen concentration, absence/presence of fauna particularly predators, electro-conductivity and temperature (11).

Man-made larval habitats in close vicinity to human habitations are known to play an important role in mosquito proliferation, especially in the dry season when supported by human activity (12, 13).

Accordingly, understanding where mosquitoes breed and why they prefer certain water bodies to others is vital for the exploration of sound mosquito control strategy and is particularly important in areas where large scale irrigation is being practiced (14).

For example, in the case of *Anopheles arabiensis*, availability of numerous small pools created by the footprints of rice workers were found preferable habitats by the mosquito (3). In connection with this, previous studies proved that the application of fertilizers was found more effective to increase larval

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population of different species of mosquitoes (3, 15, 16, 17, 18, 19, 20).

In Ethiopia, even though farmers have been practicing manure (organic fertilizers) application in their crop field for more than a century, inorganic fertilizer application began in the early 1960s.Currently all farmers of the country use both organic and inorganic fertilizers for better crop production. In Ethiopia, farmers adapt 100kg/ha blanket rate of Urea and DAP application (21).

Previously studies were not carried out to verify effect of existing Ethiopian fertilizers application practice on mosquito larval development and population. This study is the first in the country to investigate the subject matter that information obtained would have been used as an input while mosquito control strategy is designed.

Therefore, the objective of this investigation is to reveal the effects of application of inorganic and organic fertilizers on larval development of mosquitoes.

METHODS

Study area

This study was conducted at Alagae Agricultural TVET College 217 km south of Addis Ababa with an elevation of 1,600 meters above sea level on a longitude of about 38^0 30' East and a latitude of $7^0$30' North. The area is characterised by semiarid climate of 10^0c - 25^0c environmental temperature and relative humidity of 65-70%. The area experiences a bimodal rainfall distribution with an average annual rainfall of 700-900 mm. The dominant soil types of the area are clay soil (Vertisol) and sand silt clay with a pH of 7.9. The Gido River is the only surface water source for both homestead consumption and irrigation agriculture in the area.

General experiment design

A complete randomized blocking experimental design was used to conduct the study for two months (January 2007 to February 2007). Thirty-three holes (here- after called ponds) were prepared in which plastic washbasins of 100 cm diameter wide and 19 cm deep were half-buried in the ground. The ponds were arranged 5m apart to each other in parallel rows.

In each pond a nutritionally unanalysed two kilograms of dry soil was added. And it was homogenously dissolved in field water fetched from the common canal of the area. The original water level (12 cm high from the bottom of the ponds) and volume (~ $0.01m^3$) was maintained uniformly by topping-up all the ponds with canal water whenever necessary. These ponds (both experimental and control types) were left open throughout the study period.

Mosquito larvae were then collected from each pond twice a week for six consecutive weeks after the first fertilizer application using 230 ml holding capacity scoop (6). Ten scoops in not more than 20 minutes time gap were taken at each pond in the morning (8-12 am) per a single round of sampling. The number of collected larvae of mosquitoes was recorded per 10 scoops and the average recorded number in the six weeks of data collection was taken to be the result for this study.

Mosquito larval instars (1^{st}L, 2^{nd}L, 3^{rd}L and 4^{th}L) and mosquito species identification was done according to Walker identification criteria (22). Instar larvae identification was done mainly based on larval size and external morphological features. Similarly, culicine and anopheline larvae were identified based on their orientation on water surface and presence or absence of siphon at the end of the abdominal region of the larvae. Hand lens was used when necessary to magnify the first larval instars and morphological structure of all larvae. Once samples were taken, larvae were counted, recorded and returned to the breeding ponds they were taken from.

N-P fertilizers and cow dung application

A mixture of Urea and DAP (1:1 ratio) and urine-wetted stale cow dung alone were used for this experiment trial. The cow dung was then dried and milled to fine powder and then weighed (dry weight) to the designed weight before being applied into the ponds. A corresponding amount of currently used amount (100kg/ha each) of N-P fertilizers in Ethiopia (21) was the initial amount being added for this trial. However, the amount of cow dung was calculated from the rate used by Victor and Reuben (23).

Treatment types were:

A. Inorganic fertilizers: Urea 5g and DAP 5g (mixture)
B. Milled cow dung 300g
C. Control (without fertilizer)

The treatments were done in three replicates

Treatment type	Urea and DAP Mixture	Cow dung	Control
Replicate (No. of containers)	○ ○ ○	○ ○ ○	○ ○ ○

The prepared fertilizers were divided and applied in intervals at a rate of 50%, 25% and 25% of their total weight into the 3 containers. The 50% weight was applied 2 days after the ponds were filled with water. And the remaining two quarters of the total weight of fertilizers were added at 15 days interval after the first application. This is done by adopting the existing fertilizer application trend in Ethiopia.

Different dose application

The pattern and dosing reference of fertilizer application was similar with section 2.2.1. Urea and DAP mixture (5gm, 10gm and 15gm) and cow dung (300gm, 600gm and 900gm) treatment categories were considered in replicates with respective dose applications. Control breeding ponds were prepared for each category of treatment group.

Urea and DAP mixture

Treatment type	5gm	10gm	15gm	Control (without fertilizer)
Replicate (No of Ponds)	○○○	○○○	○○○	○○○

Cow dung

Treatment type	300gm	600gm	900gm	Control (Without fertilizer)
Replicate (No of ponds)	○○○	○○○	○○○	○○○

Water turbidity

Water turbidity following fertilizer application was measured using Turbidity meter (LAMOOTTE, Model 2020 TURBID meter, 9v, 500mA, USA, 1997) for each pond and the number of larvae collected in the respective ponds was recorded. The reading of the turbidity meter was reported as Nephelometric Turbidity Unit (NTU). The lower reading was an indication of less turbidity of the sampled water.

Data analysis

The results of the study were analysed using SPSS version 12. Univariate analysis and ANOVA were used. The weekly and total frequencies of larvae collected per treatment and pond type were computed to measure the effect of fertilizers on larval growth. Results were considered statistically significant when $P<0.05$ at 95% CI.

RESULTS

N-P Fertilizers and cow dung vs. mosquito larval population

This study was done to identify the effect of fertilizers on mosquito larvae development in Alagae TVET College, Central Ethiopia. In this study, the number of larvae collected was higher (87.7%, 907) in ponds to which fertilizers were added than the controls (12.3%, 127) and this difference was statistically highly significant ($P<0.001$)(Table 1).

Table 1: *Mosquito larvae collected from ponds treated with N-P fertilizers and cow dung per 10 scoops*

Treatment	Cumulative number of larvae	Mean	SD	Relative frequency	
				Culicine	Anopheline
Inorganic	587	12.8	12.6	83.1	16.9
Manure	320	7.8	9.1	97.2	2.8
Control	127	3.5	4.1	71.7	28.3

$P<0.001$

The number of culicine larvae was higher than anopheline larvae in both inorganic (83.1 % vs 16.9 %) and cow dung (97.2%vs 2.8%) treated ponds than the control (71.7%vs 28.3%) (Table 2 and 3).

Table 2: *Relative frequency of larvae population per 10 scoops in relation to doses of urea*

Treatment	Weeks 1	2	3	4	5	6	7	Total
5g	0.6	15.6	20.0	20.0	27.2	10.0	6.7	180
10g	0.8	20.6	20.6	15.3	26.6	10.1	6.0	248
15g	1.1	16.4	26.8	27.9	9.8	9.3	8.7	183
Control	1.6	14.5	15.6	17.7	19.4	16.1	14.5	86

Table 3: *Association of pond water turbidity with larval count per treatment groups*

	Treatment Group								
	N-P Fertilizer			Cow dung			Control		
		Larval count(%)			Larval count(%)			Larval count(%)	
Week	Turbidity in NTU	Anopheline	Culicine	Turbidity in NTU	Anopheline	Cu-licine	Turbidity in NTU	Anopheline	Cu-licine
0	17.5	0	0	17.8	0	0	17.6	0	0
1	11.5	26.3	20.2	28.8	30.8	22.6	21	18.8	20.0
2	9	19.3	17.4	28.4	38.5	11.4	22.2	18.8	17.1
3	13.8	16.7	13.8	38.8	30.8	22.3	29.8	6.3	7.6
4	56.1	14.0	12.1	61.8	0.0	11.7	85	15.6	8.6
5	57.5	13.2	16.0	75.7	0.0	14.8	85.7	15.6	16.2
6	95.1	10.5	15.0	99.8	0.0	7.3	113.7	21.9	8.6
7	97.6	0.0	15.6	100	0.0	9.9	116.8	3.1	21.9
Total count	-	99	488	-	9	311	-	36	105

Population compared with that of cow dung treated ponds. In addition, the number of anopheline larvae in cow dung treated ponds was very low (Table1) and disappeared from the ponds in later periods of the study (Figure 1).

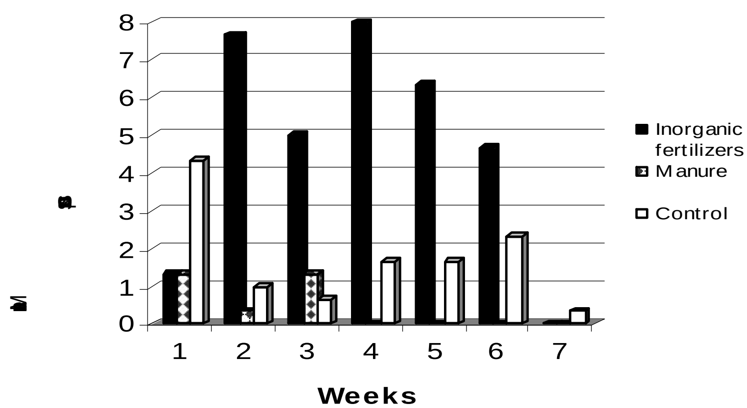

Figure 1: *Effect of N-P fertilizers and cow dung on anopheline larval population*

The population of the first instar larvae collected from cow dung and inorganic (N-P) fertilizer-treated ponds was high when compared with controls (Figure 3) .

However, N-P fertilizer-treated ponds had more number of first instar larvae(L1) of both genera than cow dung-treated ponds and this difference was highly significant (P<0.001).

Similarly, the L1 larvae density was higher than the other larval stages (L2, L3 and L4) throughout the study period, although this number proportionally declined in the later study period. Generally, addition of N-P fertilizers led to a 3-fold increment in larval count compared with the control.

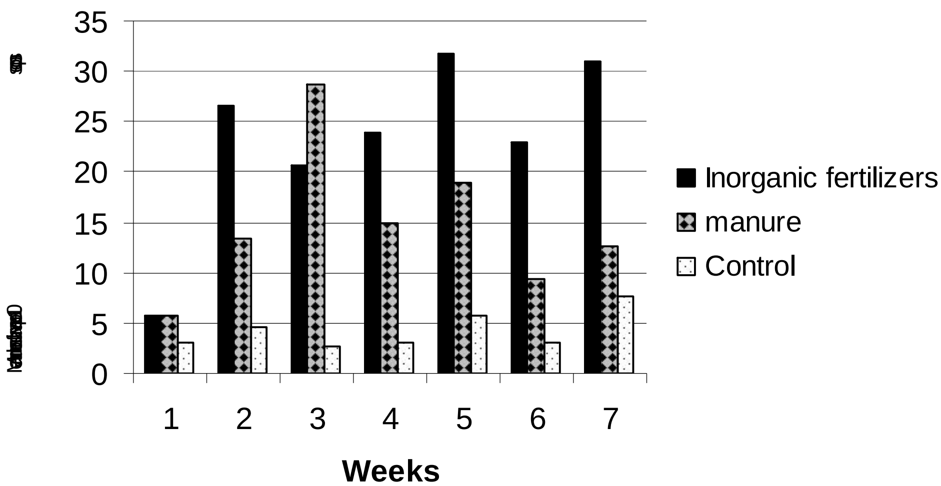

Figure 2: *Effect of N-P fertilizers and cow dung on larval population of culicine mosquitoes*

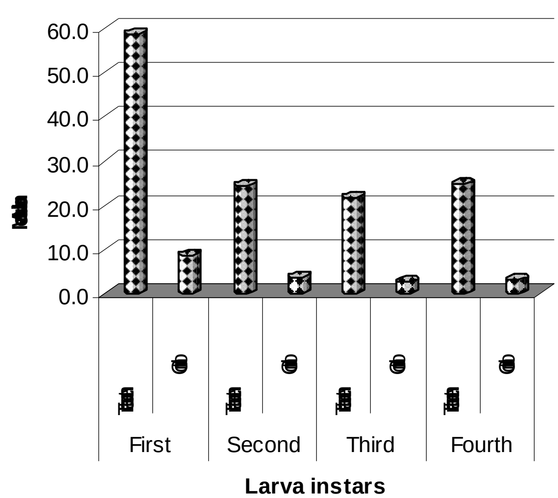

Figure 3: *N-P fertilizer and cow dung effect on population of mosquito larval instars*

Effect of organic and inorganic (N-P) fertilizer dose difference on larva count

All ponds treated with different rates of urea had higher population of larvae count than the controls (P<0.001) (Table 2) .

Similarly, ponds treated with 10 gm inorganic fertilizer carried a higher number of both anopheline and culicine larvae (dominated by L1) and the difference was a highly significant (P<0.001).

The population density of culicine larvae was proportionally higher in 10 gm followed by 5 gm N-P fertilizer-treated ponds and the difference was statistically significant (P<0.005).

In contrast, anopheline larvae were abundant in ponds treated with 5 gm than other ponds. Application of the fertilizers with time interval did affect larval population. The first instar larvae population was higher in the first week of larva collection after application of N-P fertilizers.

The application of cow dung with different doses (Figure 4) indicated the presence of great variability of larval count (P<0.001).

Accordingly, higher numbers of larvae were collected from 300gm and 600gm cow dung treated ponds than in 900gm treated ponds.

The type of collected mosquitoes was mainly culicine larvae even though anopheline larvae were collected in the early periods that disappeared later from all cow dung treated ponds.

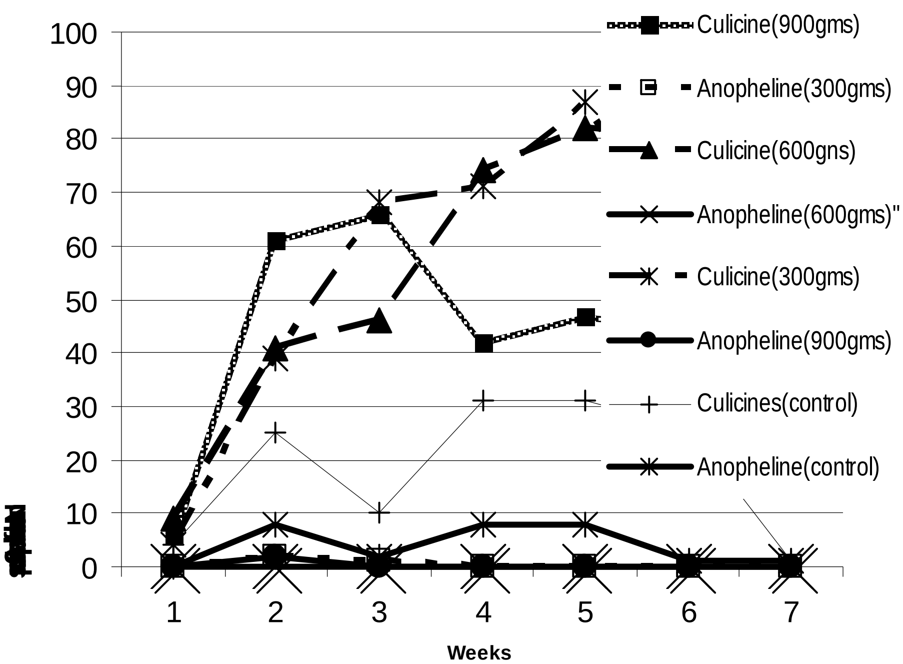

Figure 4: *Dose effects of cow dung on larval population of mosquitoes*

Turbidity

Application of N-P fertilizers decreased water turbidity of ponds (Table 3). However, as time went on, the water became turbid. The turbidity of manure treated breeding ponds was constantly high due to which it was not even possible to see the bottom stratum of the pond. Control ponds were more turbid compared with N-P fertiliser-treated ponds but less turbid than that of manure-treated ponds.

DISCUSSION

The study proved the intrinsic influence of both N-P fertilizers and cow dung on mosquito larval population in relation to their application in artificial ponds. Previous studies confirmed that the application of different types of fertilizer in natural conditions led to increased larval population of culicine and *An. arabiensis* (3, 19, 20).

Simpson and Roger (18) and Mwangangi *et al*., (16) also found out that there was a greater population of mosquito larvae in rice plots where nitrogenous fertilizer (urea) was applied than where no such fertilizer was used.
The higher population of the first instar larvae in fertilizer-treated ponds of the current study may be due to the oviposition of gravid mosquitoes attracted by the ammonia emitted from these ponds. This was in agreement with a study done in Kenya where nitrogenous fertilizer application during rice plantation proved to stimulate oviposition that enhanced mosquito larval densities (17).

Another study done in India also indicated that the application of nitrogenous fertilizers in the paddy field resulted in higher concentration of ammonia and nitrates in the water which was positively associated with the abundance of early instars (19). Similarly, Saxena *et al*. (24) found out that a higher amount of free ammonia available in the water was responsible for the higher population density of *Anopheles stephensi* first instar larvae.

The current study also found out that a high density of all stages of larvae, which may probably be due to the favourable condition created by the application of fertilizers for the multiplication of micro organisms that can act as feed sources of the larvae. This is supported by a study done by Kaufman and Walker (25) who reported that soluble nitrogen when mixed with particulate matters existing in mosquito breeding sites increases its rate of conversion to microbial biomass as feed for browsing larvae. Similarly, Sunish and Reuben (19) confirmed that application of nitrogenous fertilizers accelerated the multiplication of microorganisms which are the main diet of mosquito larvae.

N-P fertilizers were found to stimulate higher population of mosquito larvae than cow dung did. This agrees with the result of a study done by Victor and Reuben (20). This may be due to the rapid oxidation of ammonia observed because of the loose condition of cow dung and the gas which escapes easily into the environment before nitrification takes place (26).For this reason, the hatched larvae could not get enough feed to grow.

Anopheline larvae were very few in number in the cow dung-treated ponds. This might be due to specific breeding habitat preferences of the anopheline as they are usually found in clean and clear water (27, 30).

In this study, the application of different amounts of urea resulted in varied larval population count which is in agreement with a previous study done in India (20). Ten grams (10g) of N-P-treated ponds were found to carry a higher larval population which declined at a later period (5^{th} -7^{th} weeks) of the study. The probable reason might be lower nitrate concentration due to evaporation and transpiration from small habitats like ponds in this case (19, 23).

Despite the positive effect of cow dung for larval development, a larger dose of cow dung was found to carry lower numbers of larvae compared with lower doses. This could be due to the deterrent effect of higher feed availability since larvae that are overfeed by nutritionally valuable bacteria died of feeding effect (7).

The current study also confirmed that the larval density of anophelines was much lower compared with that of culicine. The dry season of the study area could have decreased the anopheline population density because the early breeding of culicine larvae might cause interspecies competition and become repellent for the few gravid anopheline mosquitoes to lay their eggs in the breeding habitats. This is in agreement with a study done in Eritrea and other countries (27, 28 and 29).

The water from the treated ponds in this study was less turbid upon N-P fertilizer application which is similar to the findings of previous studies (3). The high abundance of larvae in N-P fertilizer-treated ponds may be due to the less turbid ponds which are visually more attractive to egg laying gravid mosquitoes (3). In addition, mosquito larvae could easily fetch their feed if the breeding water is clear (27, 30).

In conclusion, it has been indicated that the application of fertilizers on experimental ponds (as simulation of the natural mosquito breeding sites) favours mosquito larvae breeding.

Therefore, the current flourishing irrigated agricultural system coupled with the extensive usage of fertilizers in all agro-ecological zones of Ethiopia may create suitable environmental conditions for the breeding of different mosquito species. This will indirectly affect both public and animal health status of the country. Since 85% of the Ethiopian people support themselves with agricultural practices that are mainly adapted to fertilizer utilization, it is strongly recommended that integrated vector management strategies be implemented in the country through the collaboration of Ministry of Agriculture and the Ministry of Health.

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