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Effects of organic fertilizers on fitness cost of *Culex quinquefasciatus* Say

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Abstract

Culex quinquefasciatus Say is a cosmopolitan distributed pest. It is a major vector of diseases in Asia. An experiment was conducted in the laboratory to assess the effects of artificial nitrogenous fertilizers i.e., Urea, Nitrophos (NP) and Diammonium phosphate (DAP) on the various fitness parameters of *Cx. quinquefasciatus*. Fifty first instar larvae were reared in different media of fertilizers, four different strains i.e., urea strain, NP strain, DAP strain and field strain were named. Larvae were monitored throughout their immature stages. Larval duration, pupal duration, pupal weight, survival from first instar to pupae and emergence (%) were recorded. The survival of immature from first instar to pupae was lower in urea and NP strain then field and DAP strain. Developmental time was reduced in DAP strain. None of the fertilizers dose had significant impact on emergence of immature of *Cx. quinquefasciatus*. These findings demonstrate that urea and NP fertilizers are toxic for the immature of *Cx. quinquefasciatus.*

1. Introduction

About 3500 species of mosquito are reported in the tropical and subtropical region (Ghosh et al., 2012). There is a concept that mosquito is world most dangerous insect (Mendki et al., 2015) because the mosquito is a vector of many viral, bacterial and protozoans endemic diseases (Kalita et al., 2013). Major genus of mosquitoes that act as a vector for various diseases is Culex. Among species of this genus, *Culex quinquefasciatus* (Diptera: Culicidae) is most abundant all over the globe. *Cx. quinquefasciatus* has cosmopolitan distribution (Bhattacharya et al., 2016). The origin of *Cx. quinquefasciatus* is southeast Asia (Fonseca et al., 2006). *Cx. quinquefasciatus* is a vector of many pathogenic diseases like bancroftian filariasis and *Dirofilaria immitis* (Lai et al., 2000). It also transmits the virus of West Nile virus (Goddard et al., 2002), Rift Valley fever virus (Meegan, 1979), avian pox (Atkinson et al., 2000). It has been reported a vector of St. Louis encephalitis virus (Tsai & Mitchell, 1989) in USA and Japanese encephalitis virus in Japan (Reuben et al., 1994) and in India (Sunish & Reuben, 2001).

After the green revolution, use of inorganic fertilizers has increased. These fertilizers i.e., Urea, DAP, Nitrophos, Ammonium sulphate are the main sources of minerals which plant required for better growth. Nitrogen, phosphorus, and potassium have great effects on plant growth. These essential minerals are required in specific amount (Yagoub et al., 2012). Rice is an important crop of Pakistan. In rice the nitrogenous fertilizer are used to get maximum yield (Ehsanullah et al., 2012). *Cx. quinquefasciatus* is abundant in irrigated rice agro-ecosystems (Muturi et al., 2006). The dense tillers of rice do not allow the sunlight to reach the water surface, so it provides suitable atmosphere for the growth and development of mosquito larvae (Muturi et al., 2007).

Nitrogenous fertilizer is recommended in rice field to get its higher yield (Ehsanullah et al., 2012). It was observed in many studies that the field of rice treated with nitrogenous fertilizer has higher population densities of mosquito immature (Victor & Reuben, 2000). The fertilizers having nitrogen, phosphorous and potassium are attractant for gravid female mosquitoes to lay more eggs

(Darriet & Corbel, 2008). The NPK-fertilizers affect all stages of mosquito including eggs, larvae, pupae and adult (Darriet, 2016). The mosquito immature survival and developmental time is also affected by the fertilizer concentration (Olayemi et al., 2012). But there is no report on effect of fertilizers on complete life cycle and fitness cost of mosquito. Moreover, it is necessary to manage the *Cx. quinquefasciatus* efficiently by economic means. Therefore, the study is planned to determine the effect of the main inorganic fertilizer i.e., Urea, Diammonium phosphate and Nitrophos on the fitness cost of *Cx. quinquefasciatus* immature and emergence of adults.

2. Material and Methods

2.1 Insect Collection and Rearing

The experiment was conducted by collecting *Cx. quinquefasciatus* in larval stage from sewerage water from Bahauddin Zakariya University (30º 11'44" N, 71º 28'31" E) Multan, Pakistan. Larvae were brought and maintained in Insecticide Resistance Laboratory, Department of Entomology, Bahauddin Zakariya University Multan, Pakistan. The Larvae were kept in a container (15x15 cm) filled with one-litre water and fed on crushed fish food (CPPRIMA, Indonesia) mixed in 70-90mg / 1000 mL. When the pupae were developed, they transferred in plastic pupal cup (100ml capacity) with the help of dropper. The pupal cup is placed in an adult cage (30×30x×30 cm). A cotton wick soaked in 10% sugar solution put in a Petri dish and it was placed in adult cage as an adult diet. After 4-5 days of adult emergence, female fed on pigeon in darkness over night for blood meal. A plastic cup (120 ml) having 30 ml tap water was placed in the adult cage for oviposition (egg laying). The female lay egg within 3-4 days after blood meal. Using a small camel hairbrush, laid egg rafts were shifted to a new plastic container for hatching. Whole experiment was done at 27±2°C and 70% ±5 relative humidity.

2.2 Fertilizers Used

Fertilizers used in this experiment are urea, Nitrophos and Diammonium phosphate and their doses were 139.7 mg, 292.5 mg and 357.5 mg respectively. These doses dissolved in one litter of water. These doses were per unit area recommended doses applied in rice fields in Pakistan. Fertilizers were taken from Department of Soil Science Bahauddin Zakariya University Multan, Pakistan.

2.3 Fitness Cost

Three fertilizers (Urea, NP, DAP) concentrates and one simple water without fertilizer were total four treatments of this experiment. There were three replications of each treatment. Tested fertilizers were mixed in one litre of water in a larval container (15×15 cm) separately as per recommendation given above in each replication. Within 6–12 h of egg hatching, fifty first instar Cx. *quinquefasciatus* were transferred to larval container (15×15 cm) in each replication. The larvae were fed with fish food (CPPRIMA, Indonesia) mixed in 70-90mg / 1000 mL. The larval container was examined after 24 hr. The larval and pupal duration were noted. Pupae were weighed singly. Each pupa was transferred into a separate pupal cup having 30 ml of respective fertilizer concentrate. Pupae from each replicate of a treatment were placed and emerging adults were counted and recorded. The experiment was terminated when all pupae had emerged.

3. Statistical Analysis

All the fitness parameters were analyzed using the Complete randomize Design in General Linear Model by Statistix 8.1 (Analytical software;(Anonymous, 2005)) at the 5% level of significance to check the effects of fertilizers concentrate on all studied tested fitness parameters. Analysis of variance of each parameter was made. Least significant difference test at $P<0.05$ was further used to examine pair-wise differences of means among treatments in cases where a significant effect of treatment was observed.

4. Results

Means of fitness parameters of four different strains of *Cx. quinquefasciatus* were given in Table 1. The larval duration of field, urea and NP strains were similar, but DAP strain was significantly lower larval duration than other three tested strains ($F=1.91$, $df=3$, $P=0.21$). Moreover, Pupal duration of DAP strain was significantly lower ($F=36.4$, $df=3$, $P=0.0001$) than all other tested strains followed by NP strain while urea and field strain have similar and highest pupal duration. The pupal weight was non-significant in all tested strains ($F=0.95$, $df=3$, $P=0.46$). Furthermore, survival of mosquito larvae from first instar to pupa in urea strain was similar with NP strain but

significantly lower than Field and DAP strain (F=27.3, df=6, P=0.0001). The percentage of Adult emergence (F=1.54, df=3, P=0.27), male emergence (F=1.10, df=3, P=0.41) and female emergence (F=0.81, df=3, P=0.53) did not show statistical difference among all four tested strains. However, in DAP strain the adult emergence was 100% as compared to other tested strains.

Table 1: Means of fitness parameters of four different strains of *Cx. quinquefasciatus*

S. No	Fitness Cost Parameters	Field Strain	Urea strain	NP Strain	DAP Strain
1	Neonates	150	150	150	150
2	Larval duration	6.73±0.41	7.07±0.67	7.04±0.77	5.46±0.08
3	Pupal duration	2.00±0.01	2.00±0.01	1.79±0.10	1.27±0.04
4	Pupal weight	3.80±0.04	3.81±0.19	4.02±0.06	3.93±0.07
5	Survival from first instar to pupa (%)	89.33±1.33	68.00±6.11b	57.33±3.33	98.00±1.15
6	Adult emergence (%)	87.12±9.67	89.58±4.81	82.53±5.04	100.00±0.00
7	Male emergence (%)	60.97±9.95	42.93±10.25	51.37±7.48	61.10±4.01
8	Female emergence (%)	39.47±10.06	57.07±10.25	32.63±17.95	38.90±4.01

Mean values with no letters in respective rows show non-significant differences according to LSD at 5% level (±SE)

5. Discussion

According to an estimate about 180 million tons of fertilizers are being used globally every year (FAO, 2012). Fertilizers are the main source of inorganic nutrients that can be used for increasing the growth and yield of the crops to get more income by farmers (Kumar et al., 2015). Fertilizer affects the growth and development of the mosquito. In rice, the nitrogenous fertilizer is used to get maximum yield (Ehsanullah et al., 2012). *Cx. quinquefasciatus* is abundant in irrigated rice agro-ecosystems (Muturi et al., 2006). The dense tillers of rice do not allow the sunlight to reach the water surface, so it provides suitable atmosphere for the growth and development of mosquito larvae (Muturi et al., 2007).

The results of present study showed that the development time (including larval duration and pupal duration) of DAP and NP strains were significantly reduced compared to urea and field strains. As the presence of plant matter (consisted of commercial rodent food (grassland hay) and NPK fertilizers faster the growth of larvae and decrease the developmental time of immature of *An. gambiae* (Darriet, 2017). The reduction in the development time and faster growth is due to production of organo-mineral complex in water which is abundant in nutrients i.e., Organic nitrogen, carbon, nitric nitrogen, phosphorus, potassium, ammonia nitrogen which favors the growth of algae and fungus in water (Xiao-hui et al., 2010) which increase the food biomass in the breeding site and thus faster the growth of larvae and reduce the development time (Darriet et al., 2012; Darriet et al., 2010).

There is no significant difference were found in pupal weight between all tested strain similar result was found by Alam et al., (2017) in which pupae weighed for all tested strain i.e., UNSEL-strain, SUS-strain and Dimeth-SEL strain has no significant difference. to the reason behind non-significant difference could be non-feeding habit of the pupal stage (Olayemi et al., 2012). It is concluded that there is no significant effect of fertilizers and insecticides on the pupal weight of mosquitoes.

The results of present study indicated that urea and NP strain had negative effect on survival of larvae from first instar to pupa. Concentration of urea was toxic to *Cx. p. pipiens* larvae (Olayemi et al., 2012). Urea forms neutral solution while NP form highly acidic solution pH 3.5 in water which cause mortality and affect the survival of mosquito. DAP form acidic solution which form basic solution which favors the larval growth.

In present study there was no significant difference recorded in male and female emergence percentage. These observations support the previous finding that the application of the fertilizers does not affect the male and female emergence percentage of *Ae. aegypti* and *An. gambiae* (Darriet, 2017). Our result demonstrated

that there is no significant difference in adult emergence. In contrast to our results NPK has reduce (0-20%) of adult emergence in *Ae. aegypti* and *An. gambiae* (Darriet, 2017). Moreover, similar results were found in which nature of breeding site (presence of fertilizer) did not affect the male and female emergence (Darriet, 2017). Apart from this the sex determination is controlled by genes (Adelman & Tu, 2016). There is no significant effect of fertilizers on the sex-determination of mosquitoes.

6. Conclusion

Based on our findings, it can be concluded that urea can be used for good management of mosquito under field conditions. Because urea causes high mortality and reduce the survival rate of immature of the mosquito. In this study, the NP and DAP strain of mosquito was at a disadvantage when compared to the Field population cause reduce in development time of immature. It is hoped that the findings of our study will help to develop the appropriate use of fertilizers in the production technology ensuring the high production of rice and efficiently manage the mosquito population in the rice field of Pakistan.

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