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Influence of Rice-farming Herbicide (2, 4-Dichlorophenoxyl Acetic Acid) on the Development of Culex pipiens pipiens (Diptera: Culicidae), a Major Swamp-breeding Mosquito Vector of Filariasis

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A B S T R A C T

Wetland rice-fields have been adjudged one of the most productive mosquito larval habitats, partly due to the influence of rice-farming chemical inputs on rates of development and survival of immature stages of mosquitoes. This study was, therefore, carried out to situate, appropriately, potential contributions of application of 2,4-Dichlorophenoxyl Acetic Acid (2,4-DAA) herbicide to mosquito production in rice-farming. The bio-assay involved rearing of approximately one-day old first instar larvae of Culex pipiens pipiens mosquitoes in a series of increasing concentration of 2,4-DAA, ranging from 0.18 – 0.72g/litre. The larvae were monitored daily for survival and duration of development of immature life stages. The results showed that Total Larval Duration (TLD) was significantly ($P<0.05$) elongated from 8.39±0.79 days in the Control mosquitoes (i.e., larvae not exposed to herbicide) to about 15 days in those raised in the highest concentration (i.e., 0.72g/l) of herbicide tested. Though, duration of development was dependent on herbicide concentration, the pattern of influence differed considerably among the four larval instar stages (i.e., L1 – L4). On the other hand, however, duration of pupal development was not significantly affected (range = 1.84±0.04 days in 0.18g/l to 2.02±0.05 days in 0.72g/l). While, Total Immature Survival (TIS) was only significantly reduced in mosquitoes exposed to 0.72g/l herbicide (i.e., 51.16±2.13%), the larval stage survivorship was inversely related to herbicide concentration (range = 67.56±14.60 to 38.06±2.92%). The findings of this study revealed that application of 2,4-DAA herbicide in rice-farming can be modified and used as an integral tool for reducing mosquito production from wetland rice-fields.

Key words: *Developmental Rate, Herbicide, Immature Stage, Larva, Pupa and Survival Rate*

Introduction

Culex mosquito-borne diseases including filariasis, elephantiasis, encephalitis, etc, constitute serious health and socio-economic burdens in the Tropics thus, impeding the progress of the region towards achieving many of the Millenium Development Goals (Snow et al., 2005; Sachs and Malaney, 2002). Filariasis, for example, threatens the health of about 1.3 billion people in 83 countries while, about 15 million cases of elephantiasis and 40 million sufferers of encephalitis have been reported worldwide (WHO, 2004; WHO, 2011). The vectorial capacity of mosquitoes for the diseases transmitted is largely influenced by the intensity of larval production from breeding habitats (Depinay et al., 2004). To a great extent, Culex mosquitoes breed principally in large water bodies especially wetland rice-fields (WHO, 1989), where they constitute the dominant species. To underscore the dominance of Culex mosquito production in wetland rice-fields, Pierre-Armand (1989) re-stated observations made by earlier authors, that mosquito productivity of rice-fields range from about $2/m^2$/day for Anopheles, to about $20/m^2$/day for Culex.

The relatively high Culex mosquito production in wetland rice fields have been attributed to a number of interdependent factors including, physicochemical and quantity of rich larval diets. These factors are in turn influenced significantly by rice-farming chemical inputs such as fertilizer and pesticide applications. For example, pesticidal inputs are known to promote mosquito larval development by eliminating their competitors (**refs**). However, another school of thought opines that, being toxic, rice-farming pesticides including herbicides may actually act to suppress mosquito production in wetland rice fields. Thus, judging by the relatively high Culex mosquito productivity often associated with rice fields, which makes them important larval habitats to be targeted for effective vector control operations, there is an urgent need to put in a clearer perspective, the contributions of pesticides to mosquito production in the rice agro-ecosystem, in order to ensure sustainable rice farming. This study was, therefore carried out to elucidate the effects of a popular rice-farming herbicide (i.e., 2, 4-Dichlorophenoxyl Acetic Acid) on survival and developmental rates of Culex pipiens pipiens mosquitoes, an important rice swamp-breeding species.

Materials And Methods

Sources of 2,4-DAA Herbicide and Culex pipiens pipiens Mosquitoes.

The herbicide amide, 2,4-DAA used for the bio-assay in this study was obtained from an agro-chemical outlet in Minna (Long. 6° 33´ E and Lat. 9° 27´ N), the capital city of Niger state, Nigeria. The herbicide was stored at 4°C until needed for bio-assay. The Culex pipiens pipiens mosquitoes came from a colony maintained in the laboratory of the Department of Biological Sciences, Federal University of Technology, Minna. The mosquito colony was maintained at ambient laboratory conditions, following standard procedures (Olayemi & Ande, 2008-Colony maintainance).

Preparation of Graded Concentrations of 2,4-DAA and Experimental Set-up

A pre-study questioning of rice farmers and agrochemical dealers was carried out in Minna to establish the concentration range of 2,4-DAA application for /in rice-farming in the area. Guided by information obtained from the users of 2,4-DAA, four (4) graded concentrations of the herbicide namely 0.18, 0.36, 0.54, and 0.72g/l were separately prepared for bio-assay. Then 1000ml each of the test herbicide concentrations was put in plastic bowls (25×5cm) in four replicates. A control experiment was similarly set-up as the tests, except the control bowls contained only 1000ml of distilled water, i.e. no addition of 2,4-DAA whatsoever.

Experimental Bio-assay of 2,4-DAA against Culex pipiens pipiens Mosquitoes

A batch of 100 apparently healthy 1-day old 1st instar larvae of Cx. Pipiens pipens were introduced into each of the replicated bowls of herbicide concentration treatment as well as the control. The larvae were fed with fish feed (Coppens ®), and maintained as described by Olayemi et al. (2012). The bio-assay was carried out at 30.00±2.00°C, 64.00±4.00% relative humidity and 12 hours day : 12 hours darkness photoperiod.

The water of the culture media was replaced daily with same original herbicide concentration for each treatment. This was done to prevent scum formation and, also, to ensure that the larvae of each treatment remained continuously exposed to constant herbicide concentration desired. The experiments were monitored at 0600 and 1900 hours, for mortality and ecdysis or metamorphosis, till all the immature mosquitoes have either died or emerged as adults. Mortality and developmental rates of immature life stages of the mosquitoes were determined as described earlier (Olayemi et al., 2012). The whole experiment was repeated within one week of the termination of the first exercise, thus, resulting in the exposure and monitoring of 800 larvae per herbicide treatment concentrations as well as the control.

Data Analysis

Data for mortality and duration of development of the various immature life stages of the mosquitoes were processed as mean±SD using SPSS (version 16.0) software. Statistical significance of differences in mean values of both mortality and developmental rates among the herbicide- concentration treatments were determined using the Chi-square test at P = 0.05.

Results

The effects of 2,4-Dichlorophenoxyl Acetic Acid on duration of developmental life stages of Cx. p. pipiens mosquitoes are shown in Table 1. Total larval Duration (TLD) significantly (P<0.05) increased from 8.39±0.79 days in the Control mosquitoes, to about 15 days in the group exposed to the highest concentration of herbicide investigated (i.e., 0.72 g/l). However, there was no significant difference (P>0.05) in the extension of larval duration of mosquitoes raised in 0.54 and 0.72 g/L. Though, developmental duration got longer with increasing herbicide concentration, the pattern of influence varied considerably among the four larval instar stages (i.e., L1 - L4). While, duration of L1 and L2 instars of the mosquitoes exposed to increasing herbicide concentrations were more-or-less not significantly different from those of the Control mosquitoes, such insignificant differences in instar duration during L3 and L4 stages were limited to those between the Control and 0.18g/l herbicide treatment. However, even when herbicide concentration treatments above 0.18g/l significantly extended the duration of L3 and L4 larval instars, such extension were more-or-less not significantly (P>0.05) different with increasing herbicide concentration.

Though, the Control group of mosquitoes completed pupal development in the shortest time (1.73±0.03 days), pupal duration of mosquitoes exposed to herbicide treatment was not significantly extended even with increasing concentration (range = 1.84±0.04 days in 0.18 g/l to 2.02±0.05 days in 0.72 g/l). Total immature Duration (TID) was 10.12±0.82 days in the Control mosquitoes, but significantly (p<0.05) extended with increasing herbicide concentration, by as much as one week (17.39±1.79 days) in the highest herbicide concentration treatment (i.e., 0.72 g/l).

Survivorship of immature life stages of the mosquitoes, under the influence of exposure to increasing concentrations of 2,4-Dichlorophenoxyl Acetic Acid is presented in Table 2. Total immature survival (TIS) of the exposed mosquitoes was only significantly (P<0.05) reduced in the group raised in 0.72 g/l treatment concentration (51.16±2.13%), from the >90% achieved by the Control mosquitoes and those exposed to 0.18 g/l herbicide concentration treatment. A similar trend of survival distribution was recorded in the pupal stage. However, the Control mosquitoes and those reared in 0.18 - 0.54 g/l treatments achieved more than 90% pupal survival.

However, mean larval survival (MLS) was significantly influenced by increasing herbicide concentration, mean larval survival (MLS) reduced from 67.56±14.60% in the Control mosquitoes to 38.06±2.92% in the group exposed to the highest concentration of herbicide. In a pattern that also characterized the distribution of larval instars, increasing herbicide concentration had no significant (P>0.05) effect on larval survivorship during the L1 and L2 instars except those of 0.72 g/l herbicide concentration treatment, in the case of survivorship distribution. Survival of the L1 larvae was generally >90%, except those of 0.72 g/l treatment which recorded 76.25±3.02% survival. On the other hand, survival during the L2 instar stages of the exposed mosquitoes was consistently <90%, and dropping as low as about 55% in the group of larvae maintained in 0.72 g/L herbicide concentration treatment. Larval survival of the Control mosquitoes during the L3 and L4 instars, were 83.50±1.13% and 79.00±1.60% respectively; and decreased significantly with increasing herbicide concentration to abysmal low levels of 17.50±1.30% and 3.25±12.20%, respectively, in the mosquitoes maintained in 0.72 g/l herbicide concentration treatment.

Discussion

The results of this study indicated significant extension of duration of total larval stages of mosquitoes exposed to increasing concentrations of 2,4-Dichlorophenoxyl Acetic Acid (2,4-DAA), especially, in concentrations above 0.72 g/l. This finding suggests toxicity of the herbicide to the larvae although; the mosquito species demonstrated tolerance level up to 0.72 g/l. This finding contradicts literature reports from some quarters, that aquatic invertebrates are generally not negatively affected by 2,4-DAA (Pesticide News, 1997; USEPA, 2003). By significantly extending the duration of the larval stages of Cx. p. pipiens, 2,4-DAA appears to possess an Insect Growth Regulatory (IGR) effect on the mosquitoes. Such IGR effects result from disruption of endocrinological activities that regulate ecdysis and metamorphosis in insects (Zebitz, 1986). Mosquitoes undergo four ecdysial instar stages to complete larval development (Olayemi et al., 2012), and any disruption in hormonal secretions will equally modify rates and pattern of immature development.

The suppressive influence of 2,4-DAA on duration of larval development was only significant in the latter larval instar stages (i.e., L3 and L4). Thus, it seems that, in addition to disruption of hormonal secretions, 2,4-DAA also impacts negatively on other activities and/or biology of L3 and L4 larval instar stages. According to Briegel (2003), a major activity during latter instar stages of mosquito larvae, is the accumulation of teneral reserve from ingested food. Therefore, if 2,4-DAA interferes with feeding during the late larval stages, for example, the mosquitoes are likely to remain as larva for a longer time, in order to slowly accumulate the threshold teneral reserve for oogenesis during the adult stage. This physiological inadequacy, perhaps, further explains the significant extension of larval duration by 2,4-DAA observed in this study. Exposure to 2,4-DAA did not significantly affect duration of pupal stage in the mosquitoes. This observation is not surprising, as the pupal stage is non-feeding, thus reducing the intake of toxic 2,4-DAA by the mosquitoes. Also, it's possible that the pupae were able to excrete or detoxify the 2,4-DAA ingested during the larval stage. Muturi et al. (2007), observed that the immature stages of Cx. quinquefasciatus were able to repair developmental damages elicited by exposure to inorganic fertilizers.

Unlike the duration of development, immature survivorship was only significantly reduced among mosquitoes raised in the highest concentration of herbicide tested. This finding suggest that rate of immature development and survivorship, in the mosquito are differentially vulnerable to 2,4-DAA. The processes of development in insects generally are more complex and environmentally-dependent (Pfadt, 1985), than those of survivorship. This, perhaps, explains the considerable variation in vulnerability of duration of development and survivorship of the immature stages to 2,4-DAA.

The findings of this study also revealed significant mitigating effects of 2,4-DA on the development and survivorship of immature stages of Cx. p. pipiens. Therefore, contrary to literature reports (Heathcote, 1970; Lacey and Lacey, 1990; De Plean et al., 2003), such effects should suppress mosquito production from wetland rice-fields, where the herbicide 2,4-Dichlorophenoxy Acetic Acid is a popular farming input. However, viewed from an informed-perspective, the explanation for the seeming controversy may lie in the difference in settings (i.e. field and laboratory conditions) of the probable influence of 2,4-DAA on mosquito production in rice-fields and as observed in this laboratory study. The negative effects of 2,4-DAA on Cx. p. pipiens immature stages recorded in this study may not be obtainable under field conditions, for a number of reasons.

Firstly, excess 2,4-DAA (i.e., not absorbed by the targeted weeds), usually degrade quickly within few days, thus, reducing the amounts imbibed by the mosquito larvae as well as duration of exposure. This is contrary to the situation in this study whereby the mosquito larvae were maintained in more-or-less constant concentrations of bio-active 2,4-DAA; as the water-media of the cultured larvae were changed every alternate day to ensure freshness. Secondly, the herbicide 2,4-DAA is recommended to be applied once in the life time of the crop, usually between 8 to 9 weeks old. This, therefore, means about two months of uninterrupted intense mosquito production from wetland rice-fields, before the once in a life-time and short-lived application of 2,4-DAA for weed control. Finally, in the field, 2,4-DAA promotes the growth of certain phyto-planktons serving as a viable source of organic carbon. Such phyto-plankton, in turn, enhance mosquito population development by providing rich larval diet, in addition to enriching dissolved oxygen (DO) of the water, for rapid developmental metabolic activities (Sunnish and Pocuben, 2001).

Table 1. Mean duration (days) of immature life stages of Culex pipiens pipiens mosquitoes exposed to increasing concentrations of the rice-farming herbicide, 2.4-Dichlorophenoxyacetic acid

Herbicide Treatment (g/litre)	Larval Instar L1	L2	L3	L4	Total Laval Development	Pupal Stage	Total Immature Development
0.00 (Control)	1.74±0.07[a]	1.85±0.08[a]	2.28±0.02[a]	2.52±0.04[a]	8.39±0.79[a]	1.73±0.03[a]	10.12±0.82[a]
0.18	2.05±0.47[a]	2.08±0.05[a]	3.06±0.10[a]	3.20±0.86[a]	10.39±1.48[b]	1.84±0.04[a]	12.23±1.52[ab]
0.36	2.15±0.03[a]	2.42±0.03[a]	3.68±0.05[b]	3.85±0.08[b]	12.19±0.19[c]	1.89±0.02[a]	13.99±0.21[b]
0.54	2.19±0.02[a]	3.08±0.06[b]	3.73±0.04[b]	4.49±1.30[bc]	13.94±1.42[d]	1.90±0.02[a]	15.84±1.44[bc]
0.72	2.27±0.26[a]	3.42±0.22[b]	4.12±0.07[b]	5.56±0.01[c]	15.37±16.74[d]	2.02±0.05[a]	17.39±16.79[c]

[*]Values followed by similar alphabets, in a column, are not significantly different at P = 0.05.

Table 2. Mean survival rate (%) of immature life stages of Culex pipiens pipiens mosquitoes exposed to increasing concentrations of the rice-farming herbicide, 2.4-Dichlorophenoxy Acetic Acid

Herbicide Treatment (g/litre)	Larval Instar L1	L2	L3	L4	Larval Stage	Pupal Stage	Total Immature Stage
0.00 (Control)	97.00±21.60[b*]	90.75±34.40[b]	83.50±1.13[c]	79.00±1.60[c]	87.56±14.60[c]	98.00±0.62[b]	92.78±7.61[b]
0.18	95.00±0.73[b]	89.50±0.16[b]	83.25±0.03[c]	77.25±0.15[c]	86.25±0.27[b]	96.25±0.30[b]	91.25±0.28[b]
0.36	94.00±0.46[b]	86.75±1.48[b]	79.00±0.13[bc]	71.00±1.66[b]	82.75±3.52[b]	95.00±0.43[b]	88.87±1.97[b]
0.54	93.75±0.44[b]	85.00±1.69[b]	77.50±2.75[b]	70.50±1.43[b]	81.68±1.57[b]	94.25±0.07[b]	87.96±0.82[b]
0.72	76.25±3.02[a]	55.25±5.30[a]	17.50±1.30[a]	3.25±1.22[a]	38.06±2.92[a]	64.25±1.34[a]	51.16±2.13[a]

[*]Values followed by similar alphabets, in a column, are not significantly different at P = 0.05.

Conclusion

The findings of this study revealed that the herbicide 2,4-Dichlorophenoxyl Acetic Acid is significantly toxic to immature stages of the filarial vector mosquito, Cx. p. pipiens, by suppressing rates of development and survival. However, while developmental responses are herbicide concentration-dependent, those of survival are not. Though, 2,4-DAA was directly toxic to the larvae under laboratory conditions, as observed in this study, field application of the herbicide may not suppress mosquito production from wetland rice-fields, as a result of certain environmental influences that reduce its toxicity to aquatic invertebrates. Therefore, there is need to integrate adequate mosquito larviciding measures into rice-farming cultural practices, to reduce mosquito production from such sites, thus, guaranteeing sustainable rice production.

References

Br iegel H. 1990b. Metabolic relationship between female body size, reserves and fecundity of Aedes aegypti. J. Insect Physiol. 36: 165-172.

Briegel H. 1990a. Fecundity, Meabolism and body size in Anopheles (Diptera: Culicidae), vectors of malaria. J. Med. Entomol. 27: 839-850.

Briegel H.2003. Physiological bases of mosquito ecology. J. Vector Ecol. 28: 1-11.

De Plean R, Geneau R, Teuscher T, Koutoua A, Scka ML. 2003. Living in the paddies: A social science perspective on how inland valley irrigated rice cultivation affects malaria in Northern Cote d'Ivoire. Trop. Med. Int. Health 8: 459-470.

Depinay JO, Mbogo CM, Killeen G, Knols BJ, Carlson J, Dushoff J, Billingsley P, Mwambi H, Githure J, Toure AM, Mckenzie FE.2004. A simulation model of African Anopheles ecology and population dynamics for the analysis of malaria transmission. Malaria Journal 3:29 doi: 10.1186/1475-2875-3-29.

Lacey LA, Lacey CM. 1990. The medical importance of Riceland mosquitoes and their control using alternatives to chemical insecticides. J. Am. Mosq. Control Assoc. 2: 1-93.

Muturi EJ, Jacob BG, Shililu J, Novak R.2007. Laboratory studies on the effect of inorganic fertilizers on survival and development of immature Culex quinquefasciatus (Diptera: Culicidae). J. Vector Borne Dis. 44: 259-265.

Olayemi IK, Maduegbuna EN, Ukubuiwe AC, Chukwuemeka VI.2012. Laboratory studies on developmental responses of the filarial vector mosquito, Culex pipiens pipiens (Diptera: Culicidae), to Urea fertilizer. J. Med. Sci. 2012: DOI: 10.3923/jms.2012.

Pfadt RE.1985. Fundamentals of Applied Entomology. 4th ed. Macmillian Publishing Company, New York, pp. 128-161.

Sachs J, Malaney P.2002. The economic and social burden of malaria. Nature 415: 680-685.

Snow RW, Guerra CA, Abdisalan M, Myint HY, Hay SI. 2005. The global distribution of clinical episodes of Plasmodium falciparum malaria. Nature 343: 214-217.

Sunish I, Reuben R.2001. Factors influencing the abundance of Japanese encephalitis vectors in ricefields in India-I. Abiotic. Med. Vet. Entomol. 15: 381-392.

Zebitz CPW.1986. Effects of three neem seed kernel extracts and azadirachtin on larvae of different mosquito species. Journal of Applied Entomology 102: 455-463.