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# EVALUATION OF NIFA BIO-LARVICIDE IN NATURAL HABITATS OF *Culex quinquefasciatus* UNDER FIELD CONDITIONS AT DISTRICT MARDAN

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***Abstract***

*Mosquitoes transmit severe diseases in human beings, causing millions of deaths every year due to dengue fever. Indiscriminate use of synthetic chemicals in indoor and outdoor of mosquitoes breeding sites has caused physiological resistance and adverse environmental effects in addition to high operational cost. But due to environmental constraints, health hazards and resistance development in mosquitoes, other environment friendly vector control strategies are important. Previous researchers have been reported the bio pesticides as useful for the control of mosquitoes larvae. NIFA bio-larvicide was applied in selected breeding habitats of Aedes aegypti and Culex quinquefasciatus under the field conditions and reductions in the densities of 3rd and 4th instars larvae were compared with that of untreated matched as controls. Three different concentrations were used as 25, 50 and 100ppm. The present work discusses the potential of this product as potential larvicides for the control of mosquitoes. The results showed that an application of the formulation at the rate of 125ppm provided 19.67% reduction followed by 50 and 100 ppm as 94 and 98 % of larvae in different selected breeding sites on day 1; thereafter 100% reduction was recorded at after 4 and 5 days in all the tested concentrations. This product was found effective in suppressing mosquito larval population in different breeding sites under natural field conditions.*

**Key Words: NIFA Bio-larvicide, Natural habitats, Mosquitoes and Mardan**

## INTRODUCTION

Mosquitoes are one of the most important insect pests that affect human health and domestic animals worldwide. Under favorable environmental conditions, a vast population can occur in short period of time. Female mosquitoes require a blood meal for egg production, therefore, they visit human/animals and produce painful bite and transfer disease causing agents (Avery et al., 2001). Mosquitoes belong to the order diptera (Culicidae) including 39 genera with more than 3500 species worldwide. There are three main genus as Anopheles, Aedes and Culex are very important thus responsible for the spread of various diseases in humans and animals like Malaria, Dengue fever, Hemorrhagic fever, dengue shock syndrome, filarises and encephalitis (Reinert et al., 2004). *Aedes aegypti* and *Aedes Albopictus* are vectors of dengue, usually distributed in the tropical and subtropical zones. About two-thirds of the world population lives in areas infested with dengue vectors, mainly *A. aegypti*. In Pakistan, DF/DHF first outbreak reported with more than 5,522 cases including 2,000 positive cases and 50 deaths during 2006, while in 2008, more than 900 cases were documented in Karachi and Lahore. Recently in 2010 out of 36 districts in Punjab, 34 were reported with dengue (Khan & Khan, 2015). Therefore, dengue situation in Pakistan is alarming with tremendous risk of an epidemic. Dengue viruses, causative agents of dengue fever and more severe dengue hemorrhagic fever (DHF) /dengue shock syndrome infect over 100 million people every year (Hahn et al., 2001). More than 700 million people are affected by mosquitoes each year (Aregawi et al., 2008). According to world health organization (WHO. 2014) **t**here were about 198 million cases of malaria in 2013 and 584,000 deaths while according to an updated research in 2015, about 50 - 200 million dengue cases occur worldwide with more than 20,000 deaths annually (Khan & Khan, 2015). All mosquito species have four life stages: an egg; larval including four instars; a non-feeding pupal stage; and an adult. The mosquito biology mainly depends upon Laboratory conditions, large population can occur under the favorable environmental conditions like Temperature and Relative humidity. During in summer season, Eggs hatching may require at least 1-2 days, larvae may complete ( 1$^{st}$ to 4$^{th}$ instars) 7-10 days, Pupae take place about 2-3 days then adults eggs are laid either singly or together in a raft like structure that floats on water. The complete life cycle from the eggs hatching to new adult emergence, it may require 12-14 days.

There is no vaccine available or specific way for dengue control. Unfortunately the people using insecticides for the larvae control are harmful for human beings, Animals, environment as well as causing the resistance in Mosquitoes larvae (Hombach, 2007). Hence, more attention has been focused on botanicals that are likely not to induce adverse environmental and health effects. A large number of plant-based products have been reported to have larvicide or repellent activity towards mosquitoes (Phasomkusolsil & Soonwera, 2010; Greive et al., 2010). Many studies have been conducted on plant extracts as larvicidal properties, in the results found effective, eco-friendly, easily biodegradable, cheap and seem to be one of the possible alternatives to synthetic insecticides (Pitasawat et al., 2007). The researchers have been proved that the plant extracts has the good potential as larvicide against Dengue vectors *A.*

*aegypti* and *Albopictus* larvae in different countries of the world (Leuzzi et al., 2000; Robert, 2001).

## MATERIAL AND METHODS

**Research work:**The present work was carried out to evaluate the efficacy of NIFA Bio-larvicide in natural habitats of mosquitoes *Culex quinquefasciatus* under field conditions at Distract Mardan during July 2016.

**NIFA, Bio-larvicide Formulation:** NIFA Bio-Larvicide is an extract of Piper Nigrum (3%) and some other organic compounds as inert. This bio-larvicide has been added with some organic synergists to enhance its efficacy. Different concentrations were prepared from the stock solutions as 25, 50 and 100 ppm.

**Field Evaluation of larvicidal activity:** Before start of the study, a preliminary survey of various breeding sites of mosquitoes including *Aedes Aegypti* and *Culex quinquefasciatus* was carried out. Larval density was determined using standard dipper (300 ml capacity with 9 cm diameter) method. At the first day, randomly taken the mosquitoes larval densities with three pre-treatment depths of each concentrations, using a standard larval dipper of 300ml capacity considered as control or Pre-treatment. After the larvicide application, the same procedure was follow as post-treatment. The data was recorded after 1,2,3,4 and 5 days exposure regularly. The product was tested against existing larvae of different genera of mosquitoes in selection habitat. The larvicide was applied with the help of knapsack sprayers with flat-fan nozzle for uniform distribution after diluting the required quantity and applied as recommended by the manufacturers. The date were analyzed to check percent reduction in the average densities of III and IV instars in comparison with untreated controls using the following formula by (Haq et al., 2004): Percent reduction = 100 – [(C1 /T1) x (T2 /C2)] x100 Where, C1 and C2 are densities of III and IV instars in untreated control on Day 0 and on subsequent days of monitoring; and T1 and T2 in treated habitats before and after treatment respectively.

**Data Analysis:** Mean (±SE) mortality of *Culex quinquefasciatus* were calculated through SPSS 22.0 **(**IBM Statistics). The statistical differences among data related to the mean mortality were analyzed by one-way ANOVA followed by the Tukey post hoc test ($P < 0.05$) (IBM, SPSS Statistics). Graphs with error bars were generated using Sigma Plot 12.0 (Systat Software Inc., San Jose, CA).

## RESULTS AND DISCUSSION

NIFA, Bio-larvicide was sprayed on larvae of mosquito *C. quinquefasciatus* under a variety of habitats at District Mardan. It was applied at three different Concentrations i.e., 25, 50 and 100 ppm to check their larvicidal property. Among these Concentrations, 100 ppm has shown maximum reduction of mosquitoes larvae after four and five days of exposure time followed by 50 and 25ppm respectively. The pre-treatment and post treatment data were recorded as 318.89 and 16.000 at after 1 day exposure. At after 4 and 5 days, the pre-treatment and post treatment values was compared as 307.78, 305.56, 0.8889 0.0000 respectively (Table 1, 2 and Fig. 1, 2). Percent reduction of larvae was 91.67, 94.67, 97.67 after 1, 2, 3 days exposure time respectively of post application, while more than 97% reduction was observed up to 4 and 5 days. The same trend was observed, when the dose rate and exposure time of plant larvicide increased, the % reduction also increased. When the dose rate increased from 25 to 50, the percent reduction also increased with 94.0, 97.33 and 99.00 after the same days exposure while the complete % reduction was found at after 4 and 5 days exposure period at the same concentration followed by 100ppm. In the overall experiment at 100ppm dose rate found significant after 4 and 5 days of exposure time. Larvicidal property of the formulation against different genera of mosquitoes larvae in selected habitat is given in (Table 2, Fig. 2.).

Our results showed that maximum dose rate is most effective among the tested concentrations which is in accordance with the findings of (Dua et al., 2009) neem oil formulation was found effective to control mosquito larvae in different breeding habitats under natural field conditions and more than 80% reduction of *Anopheles*, *Culex* and *Aedes* larvae was observed up to three weeks of post application. The results of the present investigations are in agreement with the results of (Gianotti et al., 2008) who also reported the larvicidal effect of powdered seeds of neem trees and applied twice a week to known breeding sites for *An. gambiae* at the rate of 10 gm/m2 of pool surface area for effective larval control. Our results are further supported with (Gunasekaran et al., 2004) who found Bacillus thuringiensis israelensis (Bti) is effective against *C. quinqefasciatus* for up to 3 days only in drains. The result of the present work is strongly recommended with the finding of (Haq et al., 2004) who evaluated two bacterial larvicide as Bacticide and VectoBac. He found their mosquito larvicidal efficacy under the operational conditions of urban malaria control programme with the finding of VectoBac produced reduction in the density of III and IV instar larvae of *An. stephensi* (98–100%) and *Ae. aegypti* (100%) in the first week of application whereas Bacticide produced 71–100% reduction in *An. stephensi* and 100% in *Ae aegypti*.

**Table 1:** Mean number of early 3rd and 4th Instar larvae per dip in comparison to untreated control in larval habitat. Within the same column, different letters indicate significant differences at $P < 0.05$ level (one-way ANOVA followed by Tukey HSD tests).

| Days | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| **Pre-treatment** | 318.89 ± 1.231a | 305.56 ± 1.863a | 305.56 ± 1.654a | 307.78 ± 1.781a | 305.56 ± 1.246a |
| **Post-treatment** | 16.00 ± 1.00a | 8.666 ± 1.821b | 3.111 ± 1.862c | 0.8889 ± 0.821d | 0.000 ± 0.000d |

**Table 2:** Effect of three different concentrations of NIFA Bio-larvicide formulation against different genera of mosquitoes in natural habitats under field condition (% Reduction). Within the same column, different letters indicate significant differences at $P < 0.05$ level (one-way ANOVA followed by Tukey HSD tests).

| Dose/ Days | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 |
|---|---|---|---|---|---|
| **25 ppm** | 91.67 ± 1.706e | 94.67 ± 1.732d | 97.67 ± 1.606c | 99.00±2.234ab | 100.00 ± 1.876a |
| **50 ppm** | 94.00 ± 1.530d | 97.33 ± 1.633c | 99.00 ± 1.769ab | 100.00 ± 2.587a | 100.00 ± 1.543a |
| **100 ppm** | 98.00 ± 1.485bc | 99.00 ± 1.693ab | 99.67 ± 1.792a | 100.00 ± 1.770a | 100.00 ± 1.721a |

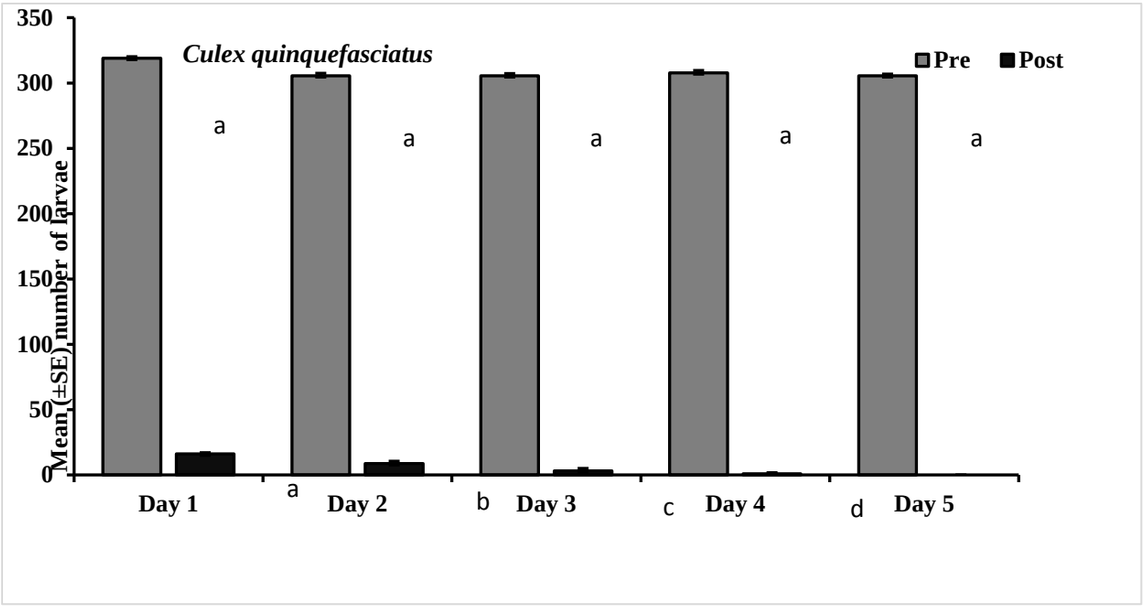


***Fig. 1*** *Mean (± SE) number of C. quinquefasciatus* ***at*** *pre and post-treatment. Columns bearing different letters are significantly different at P < 0.05 (one-way ANOVA followed by Tukey HSD tests).*

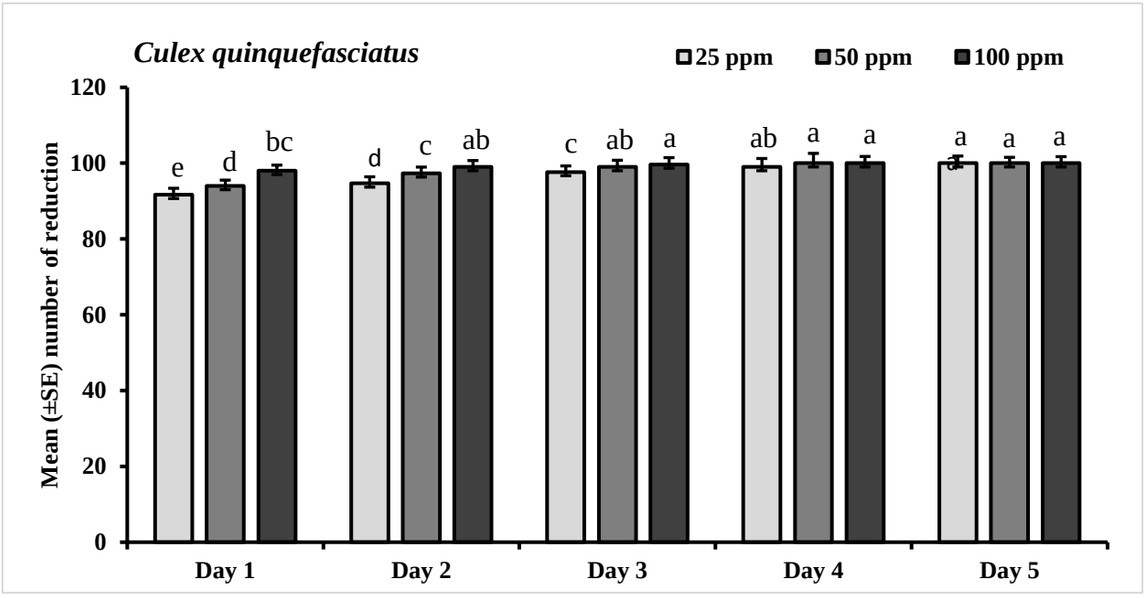


*Fig. 2. Mean (±SE) reduction of C. quinquefasciatus to different concentrations of NIFA Bio-larvicide. Columns bearing different letters are significantly different at P < 0.05 (one-way ANOVA followed by Tukey HSD tests).*

## CONCLUSION

It was concluded that up to 100 % reduction was induced in three different concentrations 25, 50 and 100 ppm after four and five days of exposure period. This research revealed that this product has high potential to replace the chemical pesticides for controlling *Culex quinquefasciatus* mosquitoes. Further research work is needed to explore the potential of NIFA Bio-larvicide which can prove as useful alternatives to chemical insecticides. It is also imperative to check the detrimental effects of this product on useful insects or animals and environment if any.

## REFERENCES


Aregawi, M., R. Cibulskis, M. Otten, R. Williams and C. Dye. 2008. World Malaria Report.p.190.

Avery DH, Eder DN, Bolte MA, Hellekson CJ, Dunner DL, Vitiello MV & Prinz PN. 2001. Dawn simulation and bright light in the treatment of SAD: a controlled study. Biological psychiatry 50: 205-216.

Dua VK, Pandey AC, Raghavendra K, Gupta A, Sharma T & Dash AP. 2009. Larvicidal activity of neem oil (Azadirachta indica) formulation against mosquitoes. Malaria journal 8: 124.

Gianotti RL, Bomblies A, Dafalla M, Issa-Arzika I, Duchemin J-B & Eltahir EA. 2008. Efficacy of local neem extracts for sustainable malaria vector control in an African village. Malaria journal 7: 138.

Greive KA, Staton JA, Miller PF, Peters BA & Oppenheim VJ. 2010. Development of Melaleuca oils as effective natural-based personal insect repellents. Australian journal of entomology 49: 40-48.

Gunasekaran K, Doss PB & Vaidyanathan K. 2004. Laboratory and field evaluation of Teknar HP-D, a biolarvicidal formulation of Bacillus thuringiensis ssp. israelensis, against mosquito vectors. Acta tropica 92: 109-118.

Hahn CS, French OG, Foley P, Martin EN & Taylor RP. 2001. Bispecific monoclonal antibodies mediate binding of dengue virus to erythrocytes in a monkey model of passive viremia. The Journal of Immunology 166: 1057-1065.

Haq S, Bhatt R, Vaishnav K & Yadav R. 2004. Field evaluation of biolarvicides in Surat city, India. Journal of vector borne diseases 41: 61.

Hombach J. 2007. Vaccines against dengue: a review of current candidate vaccines at advanced development stages. Revista Panamericana de Salud Pública 21: 254-260.

Khan J & Khan A. 2015. Incidence of dengue in 2013: dengue outbreak in District Swat, Khyber Pakhtunkhwa, Pakistan. Inter J of Fauna and Biolo Stud 2: 1-7.

Leuzzi U, Caristi C, Panzera V & Licandro G. 2000. Flavonoids in pigmented orange juice and second-pressure extracts. Journal of agricultural and food chemistry 48: 5501-5506.

Phasomkusolsil S & Soonwera M. 2010. Insect repellent activity of medicinal plant oils against Aedes aegypti (Linn.), Anopheles minimus (Theobald) and Culex quinquefasciatus Say based on protection time and biting rate. Southeast Asian Journal of Tropical Medicine and Public Health 41: 831.

Pitasawat B, Champakaew D, Choochote W, Jitpakdi A, Chaithong U, Kanjanapothi D, Rattanachanpichai E, Tippawangkosol P, Riyong D & Tuetun B. 2007. Aromatic plant-derived essential oil: an alternative larvicide for mosquito control. Fitoterapia 78: 205-210.

Reinert JF, Harbach RE & Kitching IJ. 2004. Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. Zoological Journal of the Linnean Society 142: 289-368.

Robert S. 2001. Discover a native plant extract that repels and kills termites. J. econ. Ent 94: 1200-1208.