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ASSESSMENT OF larvicidal PROPERTIES OF AQUEOUS EXTRACTS OF FOUR PLANTS Against *Culex quinquefasciatus* LARVAE

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Abstract

Aqueous extracts of four plants were tested in a laboratory for larvicidal properties against the most serious vector of filariasis and avian malaria, *Culex quinquefasciatus* Say (Diptera: Culicidae). The endeavour is to further explore the use of natural insecticides in integrated vector management programmes to control larvae of *Culex*. Laboratory reared larvae were exposed to 1, 2, 3, 4 and 5 ppm concentrations of the extracts of *Azadirachta indica* Juss., *Gymnema sylvestre* R.Br., *Nerium indicum* Mill and *Datura metel* L. respectively in Zoology research laboratory of D.A-V Degree College, Kanpur, India. Larvicidal assays were conducted according to standard WHO procedure 1981. Result showed that the plant *A. indica* elicited 70-99% mortality, followed by *G. sylvestre* 44-89%, *N. indicum* 41-74% and *D. metel* elicited 19-54% mortality to larvae. The extracts of *A. indica* and *G. sylvestre* were found to be significantly effective in controlling *Culex* larvae. The results indicate that the natural insecticides could be taken in the place of synthetic insecticides and save our environment from chemical hazards.

الملخص

مستخلص 4 نباتات تم دراستها في المختبر لمعرفة تأثيرها على يرقات أخطر بعوض *Culex quinquefasciatus* الناقل الملاريا وداء الخيطيات ، وهذا لمعلاقة فعالية المبيدات الحشرية النباتية للإدماجها في برنامج المكافحة ضد يرقات *Culex*.
تم في المختبر معاملة الطور اليرقي بالتركيز التالية 1، 2 ، 3 ، 4،و 5 ppm بالنسبة لكل من *Azadirachta indica, Gymnema sylvestre*, *Nerium indicum Datura metel* عل التوالي في مختبر جامعة كانبور –الهند ، حيث تمت المعاملة حسب طريقة المنظمة العالمية للصحة (WHO)1981 أظهرت النتائج ان نسب الموت تراوحت بين (70-99 ٪) عند نبات *Azadirachta indica* ، تليها *Gymnema sylvestre* (44-89 ٪) , *Nerium indicum* بـ 41- 74٪، *Datura metel* (19-54 ٪) ، كذلك بينت النتائج ان *Azadirachta indica* أكثر فعالية على يرقات البعوض تليها *Gymnema sylvestre* ثم *Nerium indicum* وفي الأخير *Datura metel*
وتشير النتائج أن استخدام المبيدات الطبيعية فعال، ولهذا يمكن اتخاذها في مكان المبيدات الاصطناعية وإنقاذ بيئتنا من المخاطر الكيميائية.

Keywords: Gymnema sylvestre, Culex quinquefasciatus, malaria, larvicide, LC_{50}.

1. Introduction

Control of mosquito is essential as many species of mosquitoes are vectors of malaria, filariasis, dengue and many other viral diseases and they cause unbearable biting irritations (Curtis, 1994; Collins and Paskewitz, 1995; Gubler, 1998). *Culex quinquefasciatus* is a vector of lymphatic filariasis which is widely distributed tropical disease with around 130 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard et al., 2003)

Control of mosquito larvae frequently depends on continuous use of organophosphates and insect growth regulators (Yang et al., 2002). The organophosphate insecticides target and depress acetylcholinesterase activity in a dose-dependent manner, leading to an excessive acetylcholine output, nerve paralysis and finally death. The acetylcholinesterase inhibition is non-specific, affecting the whole body systems via the cholinergic, muscarinic and nicotinic receptor pathways. Since the body systems affected are the central nervous system, the autonomic nervous system, as well as the peripheral muscular pathways (Robbin, 1991; Hassal, 1982; Purdey, 1994). Therefore the problem can be resolved by developing some new strategies for mosquito control with some less harmful, biodegradable, non toxic larvicidal natural products. Plant extracts may be alternative source to control mosquito larvae, many researchers have reported on the effectiveness of plant extracts or plant oils against mosquito larvae. Secoy & Smith (1983) stated that the roots of Peganum harmala Linn. contain toxic alkaloids for lice and mosquitoes.

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The seed extracts of *Sterculia guttata* Roxb. (Katade et al., 2006b); fruit extract of Balanites aegyptiaca Del. (Wiesman et al., 2006); root extract of Solanum xanthocarpum Schrad and Wendl. (Mohan et al., 2007); leaves of *Artemisia annua* L. and *Azadirachta indica* Juss. (Tonk et al 2006); the acetone extract of *Nerium indicum* Mill. and *Thuja orientelis* L. (Sharma et al., 2005) been tested against the mosquitoes larvae. Oils from 41 plants tested against Aedes larvae, and out of these only 13 plants gave 100% mortality (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum and sandalwood) after 24 hrs. The best oils were tested against third instar larvae of Aedes, Anopheles and Culex in 1, 10, 50, 100 and 500 ppm concentration and found extremely prominent results (Amer and Mehlhorn, 2006).

The Meliaceae plant family is known to contain a variety of compounds that show insecticidal, antifeedant, growth regulating properties (D'Ambrosio and Guerriero, 2002). Azadirachta indica commonly known as Neem, is a deciduous tree native to northwestern India. Its dentate leaves have long been recognized for insecticidal properties. Gymnema sylvestre (Gurmar) belongs to family Asclepiadaceae is an herb native to the southern and central India. The major bioactive constituents of Gymnema sylvestre is a group of oleanane type triterpenoid saponins known as gymnemic acid. Leaves of this species yield acidic glycosides and anthroquinones. Datura metel (Angels trumpet) is perennial shrub belongs to family Solanacae. All parts of plants contain dangerous level of poison; the principal toxic elements are tropane alkaloids. Nerium indicum (Kaner) belongs to family Apocynaceae, it's a green shrub with milky juice. Root, bark and seeds contain cardioactive glycosides. The bark also contains scopoletin and scopoline and small quantity of tannin, found in gangitic plains and Madhya Pradesh, India. The present investigation is conducted to study the larvicidal effect of Azadirachta indica, Gymnema sylvestre, Nerium indicum and Datura metel against larvae of C. quinquefasciatus Say.

2. Materials and Methods

For preparing the plant extracts to be tested, seeds of *A. indica*, leaves of *G. sylvestre*, bark and leaves of *N. indium* and leaves of *D. metel* were collected and their identity were confirmed at Botany department of D.A-V Degree College, Kanpur, India. After that material is cleaned, chopped, dried in shade and ground to fine powder with the help of electric grinder. All plants used in present study were kept safe as voucher specimen in Museum of Botany department. Extraction of all plant parts was carried out in a simplest way, thinking that it could be easy for the local communities to adopt this method. Twenty gram of each powder was placed in separate glass 100 ml of tap water was added and mixed vigorously. The mixture was kept for 24 hours with occasional shaking, after that, mixture was filtered using a fine muslin cloth and the final volume adjusted to 100 ml. A series of concentrations 1,2,3,4 and 5 ppm were prepared by using the stock solution using the tap water.

Culex quinquefasciatus larvae were collected from stagnant surface water of pools with the help of jar and stored in enamel trays containing tap water. They were maintained at 27± 2^0C temperature, 70± 5^0C relative humidity under 12:12 light and dark photo period cycle. The larvae were fed with the fresh food containing finely grounded dog biscuits and yeast extract in a ratio of 3:2. Pupae emerged were transferred to new trays containing tap water and placed in screened cages (30x30x30 cm), where adult emerged. Adult mosquitoes were fed on a 10% sucrose solution and 10% multivitamin syrup and periodically blood fed from fresh blood of rabbit. The egg masses produced due to adults mating were kept to continue next generation.

Laboratory reared IV instar larvae of *Culex quinquefasciatus* were tested with different concentration (1-5 ppm) of selected plants extract in Zoology Research Laboratory, D.A-V Degree College, Kanpur, India during May to July 2009 according to the standard WHO procedure (1981). A total of 25 fourth larvae were introduced in 500 ml glass beaker containing various concentrations of different plant extracts. The treatments were replicated four times, and each replicate set contained one control. Mortalities were reported after 24 hours of the exposure period. Laboratory room temperature was maintained at 27±2^0C during the experiment period. The moribund and dead larvae in four replicates were combined and expressed as percentage mortality for each concentration. Dead larvae were acknowledged when they failed to move after probing with a needle. Moribund larvae were those unable of rising to the surface within reasonable period of time. The percentage mortality was calculated and analysis of data was carried out by employing probit analysis (Finney, 1971) and corrections for mortality if needed were done by using Abbott formula (Abbotts, 1925).

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

$$\text{Corrected percentage of mortality} = 1 - \frac{n \text{ in T after treatment}}{n \text{ in C after treatment}} \times 100 \quad (2)$$

Where n= number of larvae, T= treatment and C= control

3. Results

The larvicidal activities of aqueous extracts of four plants tested are summarized in Table 1, 2 and Figure 1. It was observed that larvae became slowly inactive within 10 hours and began to fall towards the bottom of the glass beaker. The treated larvae showed curling up, anxiety and vigorous body movements. The larvicidal activity of aqueous extract of *Azadirachta indica* seeds showed 70, 80, 83, 91 and 99% of death with the use of 1, 2, 3, 4 and 5 ppm concentrations, respectively. The 4 ppm concentration killed more than 90% of the larvae. However 99% mortality was observed only at 5 ppm concentration (Fig 1). Aqueous extract of leaves of *Gymnema sylvestre* causes 44, 58, 76, 83 and 89% mortality after 24 hours. The larval mortality was above 50% from 2 ppm concentration, the maximum 89% mortality shown at 5 ppm concentration as mentioned in table 1. Extract of bark and leaves of *Nerium indium* showed greater than 50% mortality when 3 ppm concentration was used, while 5 ppm concentration showed 74% larval mortality. Extract of *Datura metel*

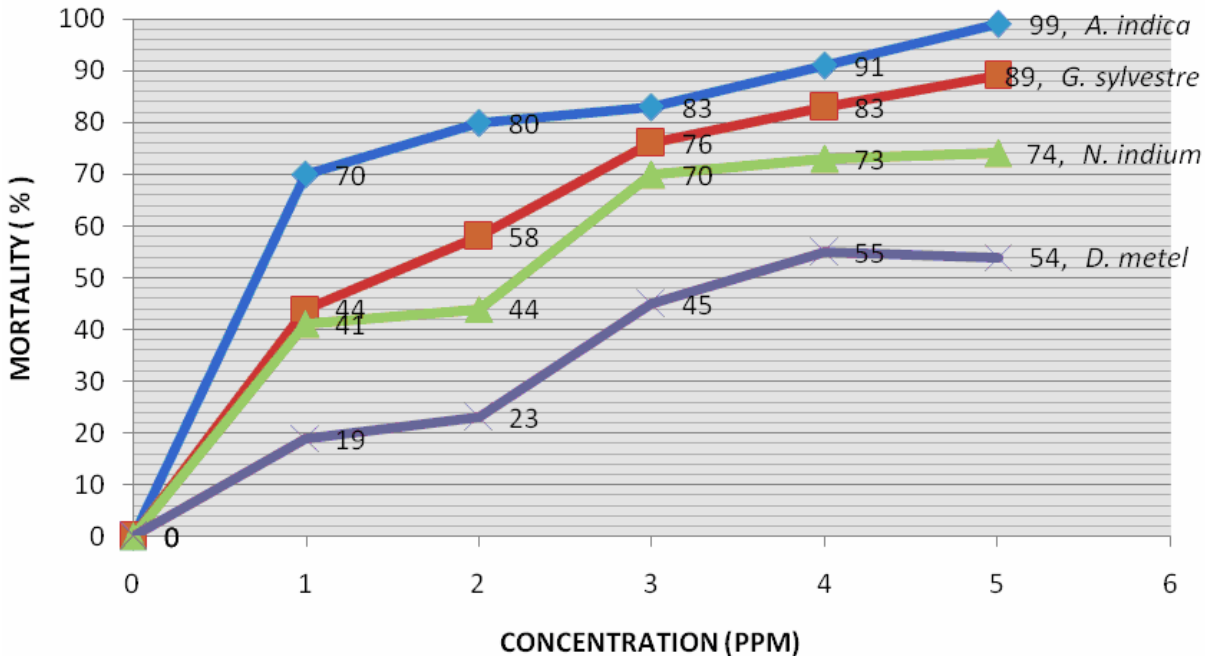

Figure 1. Dose response relationship for selected plant extracts, applied for 24 hours on c.quinquefasciatus Say.

Table 1. Larvicidal activity of various plant extracts to the fourth instar larvae of *C. quinquefasciatus* Say.

Plant Extracts	Observed mortality in percentage after 24 hrs				
	1 ppm	2 ppm	3 ppm	4 ppm	5 ppm
Azadirachta indica	70±2.57	80±1.74	83±0.69	91±1.88	99±2.54
Gymnema sylvestre	44±1.37	58±1.51	76±1.06	83±1.96	89±0.28
Nerium indicum	41±0.38	44±1.96	70±1.85	73±1.47	74±1.88
Datura metel	19±0.58	23±0.51	45±0.37	55±0.92	54±0.68

*Values are the means of 4 (n=4± SE)

Table 2. LC_{50} and LC_{90} with fiducial limits (95%) of tested plant extracts against larvae of *C. quinquefasciatus* Say.

Plant Extracts	Activity (ppm) (95% FL)	
	LC_{50} (LCL-UCL)	LC_{90} (LCL-UCL)
Azadirachta indica	0.53 (0.25-0.79)	3.423 (2.78-4.65)
Gymnema sylvestre	1.31 (1.02-1.57)	5.95 (4.76-8.41)
Nerium indicum	1.67 (1.25-2.03	12.90 (8.43-28.66)
Datura metel	3.97 (3.35-5.05)	23.85 (14.23-61.25)

FL= Fiducial limits, UCL= upper confidence limit, LCL= lower confidence limit

leaves showed the least effective results, it killed 54% larvae at the 5 ppm concentration.

Among these extracts, seed extract of *A. indica* and leaves extract of *G. Sylvestre* are the most promising ones. More accurate data on the toxicity of the plant extracts were obtained by calculating their LC_{50} & LC_{90} (Table 2). *A. indica* showed high toxicity with a LC_{50} of 0.53 ppm and LC_{90} of 3.42 ppm. *G. Sylvestre* and *N. Indicum* also show LC_{50} values less than 2.00 ppm, while *D. metel* showed 3.97 ppm value for LC_{50}. The LC_{90} value for all extracts ranges between 3.42 ppm to 23.85 ppm. *Datura metel* needed 23.85 ppm concentration to kill 90% larvae where as just 3.42 ppm of *Azadirachta indica* caused 90% mortality. It is clear from figure 1, that the aqueous seed extract of *A. indica* is highly lethal followed by *G. sylvestre*, *N. indium* and *D. metel* respectively.

4. Discussion

Biopesticides may serve as suitable alternative to chemical insecticides in future as they are relatively safe, inexpensive and available everywhere in the world. This work demonstrates the potency of Neem seed extract as an effective larvicide against *C. quinquefasciatus* larvae; it was highly toxic to mosquito larvae. The high rates of larval mortality observed at 3 to 5 ppm within 24 hrs with LC_{50} value 0.53ppm indicate the high toxicity of the extract. Previous studies have shown that *A. indica* extracts possessed significant larvicidal activity. According to Mustafa and Al Khazraji (2008) *Azadirachta excels* Jack showed excellent larvicidal properties at low concentrations against *Culex pipiens molestus*. Its LC_{50} value after 1 day was 62.5µg/mL. Dua et al. (2009) stated that, emulsified concentration of neem oil formulation showed 95.5% reduction in larval population of *C. quinquefasciatus* in one day under field trails and thereafter 80% reduction was achieved up to the third week.

The major bioactive constituents of *Gymnema sylvestre* is triterpenoid saponins (5.50%) and tannins (1.00%). Wiesman et al. (2006) reported that saponin extracted from the fruit of *Balanites aegyptiaca* showed 100% larvicidal activity against *Aedes aegypti* mosquito larvae. Aqueous extract of *Gymnema sylvestre* causes 31, 45, 45, 71 & 100 % mortality to *C. quinquefasciatus* at 1, 2, 3, 4 and 5% concentration respectively (Khanna and Kannabiran, 2007). Results of present study are in line with earlier work done. *Nerium indicum* showed moderate larvicidal properties when compare with other two plants extracts with 74% mortality 5 ppm. Sharma et al. (2005) tested alcoholic and acetone extracts of *N. indium* leaf against *Anopheles stephensi* and found results of LC_{50} at 185.99 ppm after 24 hours of exposure. Srivastava *et al*. (2003) examined the aqueous and methanolic extract of *N. indium* lattices against *Culex quinquefasciatus* and obtained that different dilutions of the lattices delay the post embryonic development of *Culex* larvae, methanolic extract is 1.8 times more toxic than aqueous extract. *Datura metel* showed less activity among the tested plant extracts with 54% mortality at 5 ppm. Mustafa and Al Khazraji (2008) tested the *Datura stramonium* seed extract against larvae of *Culex pipiens* at 20µg/mL, caused very low mortality up till seven days of exposure. These findings have re-emphasised the need to explore the possibility of using plant based larvicide and reduce the chemical hazards in the environment. The seed extract of *A. indica*, leaf extract of *G. sylvestre* and bark & leaf extract of *N. indium* were very promising. Furthermore, all these plant materials can be easily collected from the nature. Therefore, plant originated insecticides can be used as sustainable larvicide in a mosquito control programme.

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