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RESEARCH ARTICLE

LARVICIDAL EFFICACY OF *CATHARANTHUS ROSEUS* LINN LEAVES AND FLOWERS AGAINST THE MALARIA VECTOR *ANOPHELES STEPHENSI* LISTON (INSECTA: DIPTERA: CULICIDAE)

[1]Prasad, A., [2]Mathur, P., [3]Shrivastava, M., [4]Kumar, D and [5]Sharma E

Insect Microbial & Herbal Control Laboratory, Department of Zoology, Mohan lal Sukhadia University, Udaipur-313001

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ABSTRACT

Leaves and flowers of *Catharanthus roseus* L. are evaluated for larvicidal activity against *Anopheles stephensi* L. The larval mortality was observed at 24 and 48 hrs of time exposure. Highest larval percent was observed to be found in the flower extract of *Catharanthus roseus* L. plant with 100% mortality at 100mg/l concentration. LC_{50} value was calculated against different concentrations. It is recorded that lowest LC_{50} value was observed in the *Catharanthus roseus* L. flowers followed by leaves i.e. 37.15mg/l and 67.61mg/l respectively after 24 h of exposure time and 26.92mg/l, and 35.48mg/l. respectively at 48 h of exposure time. The present study indicates that phytochemical derived from *Catharanthus roseus* L. flower extracts are effective mosquito vector control agent and the plant extracts may be useful for further integrated pest management programme.

INTRODUCTION

Insect-transmitted diseases remain a major cause of illness and death around the world (Pavela, 2009). Vector and vector-borne diseases have become a challenging problem to public health in recent days as it has social and economical impact especially in subtropical and tropical countries (Klempner *et al*, 2007; Rahuman *et al*, 2008). Mosquitoes are vectors of many diseases affecting humans and domestic animals worldwide. Mosquitoes are the major vectors for the transmission of malaria, dengue, yellow fever, filariasis, Japanese encephalitis, *etc*., causing millions of deaths every year (James, 1992). Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions (Peng *et al*, 1999)

Anopheles stephensi is the primary vector of malaria in India and other west Asian countries and malaria remains one of the most prevalent diseases in the tropics. With 200 to 450 million infections annually, it causes up to 2.7 million deaths. The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved health worldwide. On a global scale, malaria causes 300–500 million cases and results in 1.5–3 million deaths annually. In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. India contributes 77% of the total malaria cases in Southeast Asia (WHO 2010).

Catharanthus roseus L (Apocyanaceae) also known as *Vinca Rosea*, is native to the Caribbean Basin and has historically been used to treat a wide assortment of diseases. European herbalists used the plant for conditions as varied as headache to a folk remedy for diabetes. *Catharanthus roseus* L., commonly known as periwinkle, is a perennial, evergreen plant that occurs in most warm regions of the world, including India. The plant has been historically used as a traditional remedy, as well as an insecticide (Singh *et al*, 2003). Hot water decoction of the leaves or the whole plant is used for treatment of diabetes in many countries (Novello and Sprague, 1957). *Catharanthus roseus* L. reported as antineoplastic agents to cure leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilms' tumor, and other types of cancers (Fischhof *et al*, 1996). Nayak (2006) reported that the dried or wet leaf extracts of *Catharanthus roseus* L. were applied as a paste on wounds in some communities. The fresh juice from the leaves of *Catharanthus roseus* L. made into a tea has been utilized by Ayurvedic physicians in India and other countries for external use to treat skin problems, dermatitis, eczema, and acne (El-Sayed and Cordell 1981). The leaves of *Catheranthus roseus* contain amyrin acetate and oleanolic acid, which have insect growth regulator (IGR) properties against the tobacco caterpillar Spodoptera litura F., green pod borer (*Helicoverpa armigera* Hub.), and various other pests (Patel *et al*, 1990; *Singh et al*, 2003).

The present study evaluated the larvicidal activity from leaves and flowers of *Catharanthus roseus* L. against the important malaria vector *Anopheles stephensi* L.

MATERIALS AND METHOD

Collection of plants

Fully developed leaves, flowers and seeds of the *Catharanthus roseus* L. were collected from in and around the Mohan lal Sukahadia University campus, Udaipur, Rajasthan, India. The dried plant materials were powdered by an electrical blender.

Extraction

The leaves were washed with tap water, shade-dried, and finely ground. The finely ground plant leaf powder (10g/ solvent) was loaded in Soxhlet apparatus (Vogel 1978). The solvents from the extracts were removed using a rotary

* Corresponding author: **Arti Prasad**
Insect Microbial & Herbal Control Laboratory, Department of Zoology, Mohan lal Sukhadia University, Udaipur-313001,

vacuum evaporator to collect the crude extract. Standard stock solutions were prepared at 1% by dissolving the residues in methanol. From this stock solution, different concentrations were prepared and these solutions were used for larvicidal bioassays.

Test organisms

The mosquitoes *Anopheles stephensi* L., were reared in the Insect Microbial and Herbal Control laboratory, Department of Zoology, Mohan Lal Sukhadia University. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and rabbit for blood meal. Mosquitoes were held at 28±2°C, 70–85% relative humidity, with a photo period of 14-h light and 10-h dark.

Larvicidal bioassay

The larvicidal activity of the plants crude extracts was evaluated as per the method recommended by World Health Organization (2005). Batches of 25 third instar larvae were transferred to small disposable test beakers, each containing 100 ml of water. The appropriate volume of dilution was added to 100 ml water in the beakers to obtain the desired target dosage, starting with the lowest concentration. Five replicates were set up for each concentration, and an equal number of controls were set up simultaneously using tap water. To this, 1 ml of appropriate solvent was added. The control mortalities were corrected by using Abbott's formula (Abbott, 1925). The LC_{50} and LC_{90} were calculated after 24 and 48 h by Probit analysis (Finney, 1979).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality } in\ control}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvaes}}{\text{Number of larvaes introduced}} \times 100$$

Statistical analysis

The mortality observed (mg/l)was corrected using Abbott's formula during the observation of the larvicidal potentiality of the plant extracts. Statistical analysis of the experimental data was performed with MS EXCEL 2003 to find the LC_{50}, regression equations (Y = mortality; X = concentrations) and regression coefficient values.

Table 1 Efficacy of leaves extract of *Catharanthus roseus* L. against *Anopheles stephensi* L. at 24 and 48 hrs

Concentration (mg/l)	Time (hrs)	% of mortality ± SD
20	24h	26.67± 0.58
	48h	40.00± 1.71
40	24h	36.67± 1.71
	48h	60.00± 0.96
60	24h	53.33± 0.58
	48h	73.33± 0.58
80	24h	63.33± 0.96
	48h	80.00± 0.00
100	24h	70.00± 1.00
	48h	86.67± 0.58

RESULTS

The extracts of different plant materials of *Catharanthus roseus* L. has been studied for use as ecofriendly insecticide instead of eco enemy synthetic insecticide. The effect of larvicidal activity was demonstrated in the present study confirm their potential for control of larval population. Effect of *Catharanthus roseus* L. on the third instar larvae increases with the increase in the concentration. Larval mortality, LC_{50} and LC_{90} value was observed. Regression analysis showed a concentration dependant significant correlation of the plant extract with larval mortality. The five different concentrations 20, 40, 60, 80, 100 mg/l of leaves and flowers were tested against malarial vector *Anopheles stephensi* L.

Table 2 Efficacy of flower extract of *Catharanthus roseus* L. against *Anopheles stephensi* L. at 24 and 48 hrs

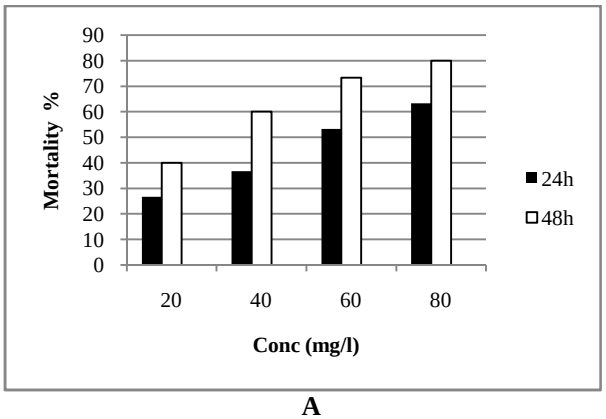
Concentration (ppm)	Time (hrs)	% of mortality ± SD
20	24h	43.33± 0.96
	48h	50.00±0.96
40	24h	50.00± 0.96
	48h	63.33±0.58
60	24h	70.00± 0.96
	48h	70.00±0.96
80	24h	73.33± 1.00
	48h	80.00±0.82
100	24h	83.33± 0.58
	48h	86.67±0.58

Table 3 Toxicity of leaves, flowers and seeds extract of *Catharanthus roseus* L. against *Anopheles stephensi* L. under 24h and 48 h exposure time.

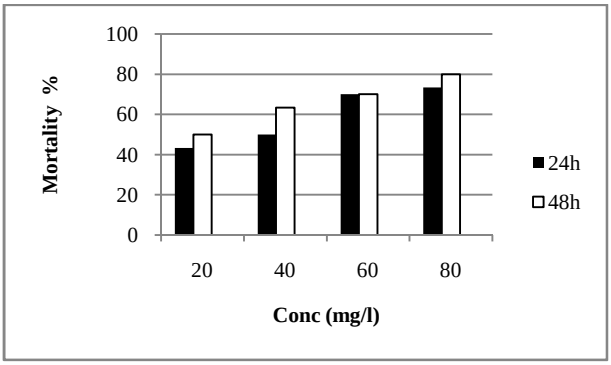
Plant material	Exposure time (h)	LC_{50} (mg/l)	Regression equation
Catharanthus roseus L. leaves	After 24 h	67.61	Y=1.78+1.75 X
	After 48 h	35.48	Y=1.63+2.47 X
Catharanthus roseus L. flowers	After 24 h	37.15	Y=1.63+2.44 X

Table 1 show the efficacy of leaf extracts of *Catharanthus roseus* L. against *Anopheles stephensi* L. at 24 and 48 hrs and generally found that highest larval mortality was observed in highest dose (100mg/l) concentration i.e. 86.67±0.58% at 48 h of exposure time. Larval mortality was highest in 48 h (80.00±0.00%) of exposure time as compared to the 24 h (63.33±0.96%) in 80ml/l concentration and this trend is generally observed in all the concentrations whereas it was lowest in the lower concentrations (20 mg/l) i.e. 26.67±0.58% and 40.00±1.71% at 24 and 48 hrs of time exposure. (Figure 1.A)

Table 2 shows the toxicity of flower extract at the different concentrations. Highest mortality (50.00±0.96%) was observed at the 100 mg/l concentration at 48 hrs and 43.33±0.96% at 24 hrs exposure whereas low larval mortality at low concentrations of 20 mg/l i.e. 43.33±0.96% and 50.00±0.96% at 24 and 48 hrs of exposure As compared to the leaf extract high larval mortality was observed at less time exposure. (Figure 1.B)



A



B

Figure 1 Dose response relationship for extracts of *Catheranthus roseus* L for 24h and 48h against *Anopheles stephensi* L A) Leaf extract B) Flower extract

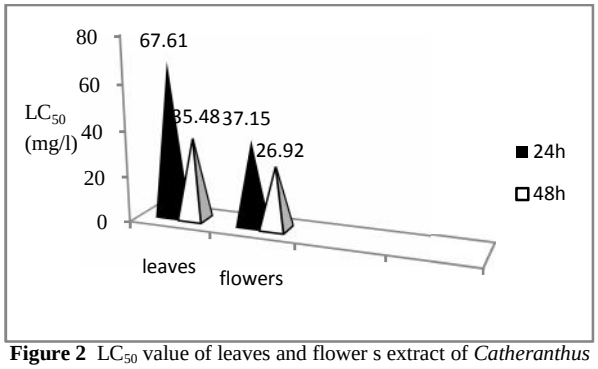


Figure 2 LC_{50} value of leaves and flower s extract of *Catheranthus roseus* against *Anopheles stephensi*

Table 3 shows the LC_{50} value and the regression equation which was calculated using the Probit analysis. LC_{50} value was calculated against different concentrations. It is recorded that lowest LC_{50} value was observed in the *Catharanthus roseus* L. flowers followed by leaves i.e. 37.15 mg/l for flowers and 67.61 mg/l for leaves after 24 h of exposure time and 26.92mg/l for flowers and 35.48 mg/l for leaves after at 48 h of exposure time. (Figure 2)

DISCUSSION

The 24 hrs and 48 hrs bioassay is the major tool for evaluating the toxicity of phytotoxins and a number of researchers have been applying this method to evaluate the toxic effect of different plant extraction against mosquitoes. The mosquito larvae exposed to plant extracts showed significant behavioural changes. The changes were observed within 30 min of exposure. The most prominent sign of *Anopheles stephensi* L. was inability to come to the surface. The larvaes also show loss of equilibrium, restlessness and finally led to dealth. The behavioural effect is more pronounced in case of flower extracts of *Catheranthus roseus* L. as compared to its leaves after exposure. These effects may be due to presence of neurotoxin compounds in *Catheranthus roseus* L. No such behavioural changes were obtained in the control sets.

Plants produce a wide spectrum of allelochemicals, however, many of such chemicals have not been explored for their physiological significance (Norduland and Sauls, 1981). These phytochemicals specifically inhibit growth, morphogenesis, metamorphosis and reproduction (Ahmad, 2007). Currently there is resurgence of interest in plant derived compounds for developing them commercially as ecofriendly insecticides. Tropical plants are more promising for the development of new insecticides (Jacobson and Crosby, 1971). Despite, the fact that hundreds of tropical plants are reported to possess insecticidal property, only few compounds (Azadirachtin) have been commercialized (Chopra *et al*, 1994).

Catheranthus roseus L. has more than 400 known alkaloids, some of which are approved as antineoplastic agents to treat leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilms' tumor, and other cancers. Its vasodilating and memory-enhancing properties have been shown to eliminate vascular dementia and Alzheimer's disease (Fischhof, 1996, Hindmarch *et al*, 1991). The two classes of active compounds in Vinca are alkaloids and tannins.

The major alkaloid is vincamine and its significantly related semi-synthetic derivative widely used as a medicinal agent, known as ethyl-apovincaminate or vinpocetine, has vasodilating, blood thinning, hypoglycemic and memory-enhancing actions (Chattopadhyay, 1991, Chattopadhyay *et al*, 1991). The extracts of Vinca have shown significant anticancer activity against numerous cell types (El-Sayed and Cordell, 1981)

CONCLUSION

The bioactivity of tested phytochemical extracts varied significantly with solvents used for the extraction and instar stage of the larvae. Reviewing the prospects of insecticidal for the management of pests and vectors. Jermy, 1990 and Ahmad, 2007 reported that plant extracts/compounds "with combined behavioral and toxic effect are more likely to have successful practical application than the compounds/extracts. Briefly, considering the information available in literature on insecticidal of plant extracts, the present study has shown that there is a wide scope for application of *C. roseus* as larvicidal agent in integrated pest management programs.

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