Bioefficacy of *Vinca rosea* leaf powder on *Aedes aegypti* L.

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ABSTRACT

While most of the people consider mosquitoes as an annoyance, these tiny assassins have the potential and lethal capacity more than any other group of arthropods. The increasing resistance of mosquitoes to current commercial insecticides has made the problem still worst. An alternative conventional method for this is the utilization of natural products from plants. In this regard shade dried leaf powder of *Vinca rosea* were used to assess the bioefficacy on *Aedes aegypti*. The different sized particle powder of 150µ, 250µ, 500µ and 600µ were tested for their bioefficacy. Emergence inhibition (EI_{50}) of 150µ particle sized powder was found to be most effective with the (EI_{50}) of 24.81mg/100ml of water. Growth inhibitory effect was observed during the development.

Key words: *Aedes aegypti*, Particle, Natural product, *Vinca rosea*

INTRODUCTION

Although many synthetic and botanical insecticides are available, the increasing resistance in mosquitoes proves the importance of the above words by Sir. Ronald Ross. Since *Aedes aegypti* is a fresh water breeding mosquito it is very difficult to control it during rainy season. This results in the different diseases like chikungunya, dengue and haemorrhagic fever. The eradication or control of *Aedes aegypti* is considered as the only option. One of the approaches for control of these mosquito borne diseases is the interruption of disease transmission, either killing, preventing mosquitoes to biting human beings or by causing larval mortality in a large scale at breeding centers of the vectors (Raj Mohan and Ramaswamy, 2007). Over two thousand species of plants are known to possess insecticidal properties. Very recently Gloria *et al.* (2009) natural compound isolated from soil actinomycete has been used for the management of this pest. A survey of literature on larvicidal effects of plant products on mosquitoes indicates that most of the studies included well known horticultural and commonly grown plants. Work on the shade dried leaf powder on *Vinca rosea* is lacking, more than that the work on particle size of the leaf powder is scarce. Therefore an attempt is made to take up such studies on commonly available plant *Vinca rosea*.

MATERIALS AND METHODS

Vinca rosea is a short plant with an average of two feet height and glossy dark leaves. It is grown mostly as an ornamental plant in perennial tropical regions. Alkaloids present in *Vinca rosea* are known to be cytospecific and phase specific blocking mitosis resulting in dysfunction of mitotic spindle apparatus, freeze the metaphase stage and prevent cell proliferation. Vincristin and vinblastin from the root barks and leaves have anti diabetic properties (Kirtikar and Basu, 1987). The fresh leaves of *Vinca rosea* were collected locally, washed with distilled water, pressed between the sheets of blotting paper to remove excess moisture and the leaves were shade dried for 4-6 days. The dried leaves were pulverized, sieved through different micron sieves of 150µ, 250µ, 500µ and 600µ. Experimental larvae were collected from cyclic colony maintained in the rearing house at the Entomology laboratory Karnatak University Dharwad, at a temperature 28± 2^0C, RH 65± 5, and the pH of rearing water 7.4 - 7.6. Twenty five freshly moulted third instar larvae were introduced in to wide mouth plastic cups (200 ml capacity) containing 100 ml water. The food was provided in the form of dog biscuit and yeast (2:1 respectively). The dry test powder in different quantities 0.1, 0.2, 0.5, 1, 1.5, 2, 3, 4, 8, 12, 24, and 48 mg/100 ml, was added to these plastic cups. The treatments were replicated thrice. These were kept for twenty four hours for stabilization. The just moulted third instar larvae were introduced into the treated glasses. Three replicates were maintained. Observations were recorded every 24 hours. The mortality rate was recorded at 24hours interval. The adults were observed for deformities. The LC_{50} and LC_{90} were calculated using probit analysis.

RESULTS AND DISCUSSION

The shade dried leaf powder of 150µ, 250µ, 500µ, 600µ particle size was tested against third instar larvae of *Aedes aegypti* at different concentrations. It is interesting to note that, the mortality increased as the particle size of

Table 1. Effect of particle size of the shade dried powder of *Vinca rosea* on its bioefficacy.

Wt.	150µ	250µ	500µ	600µ
0.1mg	60±2.30^{bc}	41.3±2.66^{f}	4 ±2.30^{h}	8±2.3^{g}
0.2 mg	57.3±1.33^{bc}	54.6±7.4^{e}	10.66 ±3.52^{gh}	6.66±2.66^{g}
0.5 mg	80±4^{a}	57.3±2.6^{e}	17.33 ±4.80^{g}	24±2.30^{f}
1 mg	77.3±4.8^{a}	73.3±1.3^{cd}	32±4.61^{f}	32±4^{e}
1.5 mg	80±2.3^{a}	84±4^{abc}	42.6 ±7.42^{ef}	49.3±1.33^{d}
2 mg	80± 4^{a}	86.6±2.66^{ab}	49.3 ±2.66^{de}	50.6±3.52^{d}
3 mg	84±4^{a}	82.6±3.52^{abc}	57.33 ±5.33^{cd}	61.3±1.33^{c}
4 mg	80±0^{a}	89.3±1.33^{a}	70.6 ±1.33^{ab}	70.6±1.33^{b}
8 mg	80±4^{a}	76±2.30 ^{bc}	78.6 ±1.33^{a}	73.3±1.33^{ab}
12 mg	64±4^{b}	77.3±3.52^{bc}	80 ±0^{a}	80±2.30^{a}
24 mg	58±2.6^{bc}	64±2.30^{abc}	78.6 ±3.52^{a}	81.3±.3.52^{a}
48 mg	52±4^{c}	60±6.11^{e}	64 ±2.30^{ab}	73.3±3.52^{ab}
Control	5.33±2.66^{d}	5.33±2.66^{g}	2 ± 2^{h}	10.6±1.33^{g}
F value	40.7	39.303	57.51	122.545
P value	<0.001	<0.001	<0.001	<0.001
EI_{50}	24.81	38.21	48.2	48.47
EI_{90}	64.59	95.87	179.2	325.89
X^2	11.51	12.18	43.76	59.6
DF	9	9	10	10

Results are Mean ± S.E, n = 75.
Means in the column followed by the same letter are not significantly different at 5 percent level by DMRT test.

the test powder was decreased. The 150ì particle sized powder showed the maximum per cent total mortality of 80 on addition of 0.5 mg /100 ml of water, 250ì powder needed 1.5mg to bring about 83% mortality and 500 and 600 needed 12mgs to bring about 80.5% mortality. Fine particle size increases the mortality rate hence found to be more effective. Quantity of the fine powder requirement is quite less than the coarser one (Table 1). This indicates that the fine powder is more effective than the coarser one. The fine particles of the powder might be releasing the toxic substance in the water in short period than the coarser one. This needs further conformation.

It was observed that the most of the pupae failed to emerge as adults and died in the pupal case itself. The sluggish movement and peculiar coiling of treated larvae may be due to some neuronal or muscular disturbance by some active principle or toxic substance released in the water by the powder, might cause the acute lethal effect. These results are more or less similar to those reported by Md. Ekramul Islam *et.al.*, (2003) in *Culex quinquefasciatus.* The delayed lethal effect of the compound is more likely to disturb the endocrine mechanisms that regulate moulting and metamorphosis. This mechanism of action has been already postulated previously for neem by (Zebitz, 1986).The larvae which survived in the larval and pupal stages and emerged as adults also faced some difficulty in the flight and died within an hour of emergence. Further studies pertaining to this are being carried out in our laboratory. Disruption of the process of digestion and absorption in the larvae exposed to test compounds was also observed. It has been documented that the root extract *Derris urucu* affected peritropic matrix structure of *Aedes aegypti* larvae causing damage to the midgut epithelium (Gusmao *et al*., 2002). In insects, the midgut is main sight of digestion and absorption (Chapman, 1998). The midgut lumen is lined by non-cellular membranous structure, “peritropic membrane”, which protects the mid gut, cells from toxic substances and pathogens that enter the midgut through food (Wigglesworth, 1965; Richards and Richards, 1971). The result suggest that the toxic component (s) released from the test powder had entered the midgut region and acted upon peritropic membrane (PM). The membrane might have separated and made free from the midgut cell lining and is thrown out along with the fecal matter mixed with food particles thereby exposing the midgut cells to the toxic components and disrupt the process of digestion and absorption (Fig 1). Similar results have been reported when the fourth instar larvae of *Aedes aegypti* were treated with 250-300mg/100 ml of water of shade dried leaf powder of *Clerodendron inerme* by Patil (2008).

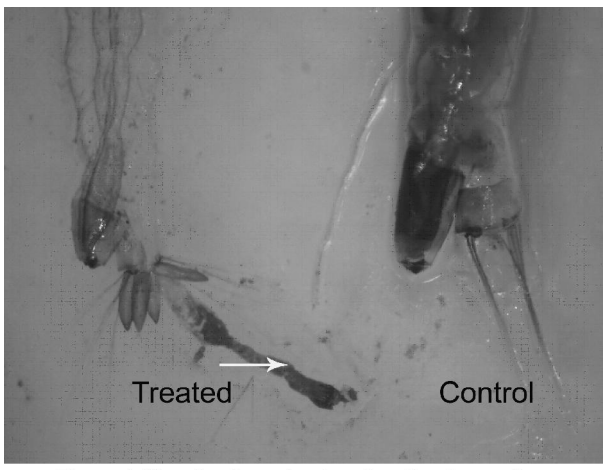
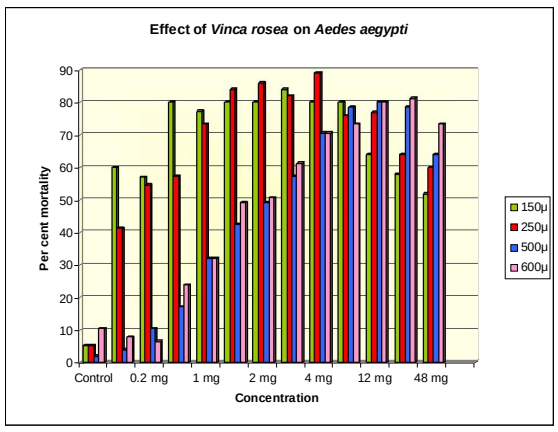


Figure 1. Showing the extrusion of peritropic membrane

CONCLUSIONS

It is suggested that the *Vinca rosea* leaf powder possesses good insecticidal effect when used in lower quantities with smaller particle size. Extrusion of peritropic membrane shows that the *Vinca rosea* leaf powder might be causing

a lot of tissue irritation. The death of adult mosquitoes might be due to the neurotoxic effect of the powder that influences the motor neuron function.



Graph showing the increase in the bioefficacy of the shade dried leaf powder of *Vinca rosea* as the fineness of the powder increases

Although the 250µ particle sized powder shows the highest peak in the graph but it require more quantity of powder than the 150µ particle sized powder. Hence 150µ may be considered as good for use.

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