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BIOINSECTICIDAL POTENTIAL OF *VINCA ROSEA* AGAINST THE TOBACCO CATERPILLAR, *SPODOPTERA LITURA* FABRICIUS (LEPIDOPTERA: NOCTUIDAE)

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Abstract

The tobacco caterpillar *Spodoptera litura* Fabricius has emerged as a serious and dominant pest on many agricultural crops causing enormous losses. The use of plant extracts of various medicinal plants against the different developmental stages of *S. litura* to test their efficacy in organic farming is the need of the day. A crude aqueous extract of leaves of *Vinca rosea* at different concentrations from 2 per cent to 40 per cent at an increment of 5 per cent was administered to lab-reared; pre starved fourth instar larvae topically as well as through food. The fourth instar larvae treated topically with the 25 per cent extract exhibited a total larval mortality of 91.66±1.66 per cent and the maximum total per cent mortality was 93.33±1.66 during its development to the adult stage. The larvae that were administered the plant extract through their food showed an 85.0±2.88 per cent total larval mortality and 93.0±1.66 per cent total mortality at 25 percent concentration. Deformities were observed in the dead larvae, pupae and adults. The LC 50 and LC 90 for topical application was 18.87 and 42.98 .The same for leaf application was 21.07 and 45.38. The botanical showed its continued bioinsecticidal effect in the F1 generation. There was a reduced hatchability of eggs (47.66 p±0.66 per cent compared to the 65.33±0.88 per cent in the absolute control, for topical application.) and also a significant per cent mortality of first instar larvae within two hours of hatching (53.36±0.66 per cent for topical application and 21.36±0.33 per cent for leaf application) crude aqueous leaf extract of *Vinca rosea* was found to be effective in the management of the polyphagous pest *S litura* F.

Key Words: Biological insecticide; *Spodoptera litura* Fabricius; *Vinca rosea*.

Introduction

The lepidopteran pest *Spodoptera litura* (Fabricius) is a serious pest on tobacco. It also has been recorded on several other crops like cauliflower, castor, cotton, banana and mulberry. The fully grown caterpillars are the most voracious feeders and cause extensive damage by defoliation. Use of insecticides for controlling this pest is on the rise and has the ability to develop resistance to many insecticicides (Murugesan *et al* 1995) Chemicals of botanical origin used in pest control programmes may prevent several adverse effects caused due to synthetic insecticides (Gayatri *et al* 2003*)*

In the present study an attempt has been made to investigate insecticidal properties of crude aqueous extracts of fresh leaves of *Vinca rosea* on the fourth instar larvae of *Spodoptera litura* F.

Vinca rosea*: Lochnera rosea* (The Rose periwinkle) The Dogbane family:Apocynaceae.

Punjab: Rattanjot (Kirtikar *et al* 1987)

Small shrub found everywhere in Indian gardens, flowers throughout the year. Drought stops the growth of plants, but doesn't seem to affect *Vinca rosea.* It has a strong woody stem, covered with a very tough and leathery bark.The sap in the stem is slimy. Leaves: elliptic, opposite, shiny above and provided with a thick epidermis. A fine coat of down is present all round the epidermis. Flowers grow in pairs in the axil of leaves. Fruits two, erect, cylindrical. Follicles dehiscing each in its ventral suture. seeds numerous. (P.Fleiderer 1990)

The juice of the leaves is employed in Orissa as an application to wasp stings. The macerated root is given

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as a tonic and a stomachic in La Reunion. It is used as a diabetes remedy, particularly in Natal as well as in other parts of south Africa, and in Queensland. It is also used in treating menorrhagia. An infusion of the leaf by the mouth is the usual mode of administration (Kirtikar *et al* 1987)

The roots of this plant constitute the drug that has gained more importance than sarpagandha. This is because the roots contain more ajmalicine than those of sarpagandha. The drug is used as a sedative as well as in in the treatment of hypertension .Experiments on animals have shown that extracts of this plant containing vincrystine may prove to be a boon in the treatment of leukemia (Cowley *et al* 1928). In the present investigations, effects of a crude aqueous extract from *Vinca rosea* leaves in different concentrations of 2, 5,10, 15, 20,25, 30, 35 and 40 per cent on the survival rate at all the developmental stages, deformities in the adults, feeding inhibition and on hatchability of eggs,survival rate of the F1 larvae of first instar stage of *S. litura* have been studied.

Materials and Methods

Freshly laid batches of eggs of *S. litura* were collected from the soybean fields at the University of Agricultural Sciences, Dharwad, Karnataka, India. The eggs were allowed to hatch under laboratory conditions, and the larvae were reared in the laboratory in sterilized earthen pots of 2 l capacity with the mouths of the pots covered securely with a clean sterilized white muslin cloth. The larvae were fed on fresh castor (*Ricinus communis* L.) leaves, and maintained at a temperature of 26± 1 ^{0}C , a relative humidity of 65± 5 %, a photoperiod of L10:D14. The laboratory reared pre-starved fourth instar larvae were used as test insects.2, 5, 15, 20, 30, 35, and 40 per cent crude aqueous extracts of *Vinca rosea* leaves were used for the Topical and Leaf application tests.

Fresh leaves of *V. rosea* (500 gms) were cooked for 15 min under 15 lb pressure without adding any water, then allowed to cool. These cooked plant products were ground to a fine paste in an electric mixer-grinder using distilled water to obtain a total stock solution of 1 litre. This extract was first filtered through a clean, sterilized muslin cloth and then through ordinary filter paper. From this fresh stock, solutions of 2, 5, 10, 15, 20, 25 30, 35, and 40 per cent concentration were prepared using distilled water and were immediately used for trials.

As the fourth, fifth and sixth instar larvae cause the maximum damage to the foliage, fourth instar larvae were selected for topical as well as leaf application tests. Under each type of application, the first nine groups were treated with the extract of 2, 5, 10,15, 20,25, 30, 35 and 40 per cent concentrations. The tenth group was the carrier (distilled water control), the eleventh , the absolute control. Each group comprised 20 larvae, kept in 20 separate sterilized earthen pots of 1 liter capacity each and were fed on fresh castor leaves. Extract (2 ml) to be tested was sprayed on the larvae (topical application) and on similar sized castor leaves (leaf application) with a Chromatographic sprayer. An ordinary cold-air blow dryer was used to hasten the process of drying of the extract on the leaves. Three replications were maintained. Observations were made to assess the total per cent mortality, per cent mortality at the individual stages of development of the insect, feeding deterrence and deformities in the adults, per cent egg hatchability, survival of first instar larvae in F1 generation .

The per cent mortality and the per cent deformity were corrected using Tukey's honestly significant test. LC_{50} and LC_{90} were calculated using probit analysis (Finney, 1971).

Results

Feeding was totally stopped by the topically treated larvae for the initial 10-15 minutes of application, at 2, 5, 10 and 15 per cent concentrations. A total inhibition of feeding for the initial 20-25 minutes was exhibited by the larvae treated topically with 20, 25, 30, 35 and 40 per cent extract. Feeding depression may have been caused by behavioural effects. Pellets were normal for all the concentrations of the botanical tested.

In the leaf application group, this behavior persisted for the initial 25-30 minutes in the 2, 5, 10, 15 and 20 per cent concentration subgroups. Larvae subjected to 25, 30, 35 and 40 per cent leaf application test abstained from feeding for 60-70 minutes. Feeding when resumed was slow and intermittent in the next 24 hours. Pellets were normal for the 2-15 per cent treatment, but were extremely moist and mushy in larvae fed with leaves treated with 20-40 per cent concentration of the botanical.

Table 1. Bioefficacy of crude aqueous leaf extract of *Vinca rosea* on development profile of the tobacco cutworm, *Spodoptera litura* Fabricius following a topical treatment on the fourth instar larvae.

Percent concentration	Percent larval(IV,V&VI instar)mortality Mean±SE	Percent pre-pupal(shrunken stage) mortality Mean ±SE	Percent mid-pupal(larval-pupal intermediate)mortality Mean± SE	Percent pupal mortality Mean± SE	Percent deformed Adults Mean± SE	Percent Total mortality Mean± SE
2	63.33±1.66 ^{d}	0 ^{b}	0	5.00±2.88^{ab}	0 ^{b}	68.33±1.66b
5	78.33±1.66^{bc}	0^{b}	0	0^{b}	3.33±1.66^{ab}	78.33±1.66^{ab}
10	75.00±2.88^{cd}	0^{b}	0	13.33±4.41^{A}	0^{b}	88.33±1.66^{A}
15	88.33±1.66^{ab}	0^{b}	0	8.33±1.66^{ab}	0^{b}	96.66±1.66^{A}
20	80.00±2.88^{a-c}	0^{b}	0	11.66±3.3^{ab}	0^{b}	91.66±1.66^{A}
25	91.66±1.66^{A}	0^{b}	0	1.66±1.66^{ab}	0^{b}	93.33±1.66^{A}
30	88.33±3.33^{ab}	0b	0	6.66±3.33^{ab}	0^{b}	95.00±0.00^{A}
35	90.00±2.88^{ab}	3.33 ±1.66^{A}	0	0^{b}	6.66±1.66^{A}	93.33±4.41^{A}
40	86.66±3.33^{a-c}	0 ^{b}	0	10.0±2.88^{ab}	1.66±1.66^{ab}	96.66±1.66^{A}
Carrier(distilled water control)	35.33±1.66^{e}	0^{b}	0	1.33±1.66^{ab}	3.33±1.66^{ab}	36.66±1.66^{c}
Absolute control	30.00±2.88^{f}	0^{b}	0	1.66±1.66^{ab}	3.33±1.66^{ab}	31.66±1.66^{c}
SE	2.44	0.503	0	2.45	1.03	3.83
"F" test	66.30	4.00	0	3.54	0.90	38.82
CD (0. 05)	7.20	1.48	0	7.23	NS	11.29
CD (0. 01)	9.82	2.02	0	9.86	NS	15.41

Means followed by the same letters do not differ significantly from each other at P<0.05(Tukey's honestly significant test)

LC 50 = 18.87 LC 90 = 42.90

Table 2. Bioefficacy of crude aqueous leaf extract of *Vinca rosea* on development profile of the tobacco cutworm, *Spodoptera litura* Fabricius following a leaf application treatment on the fourth instar larvae.

Percent concentration	Percent larval(IV,V&VI instar)mortality Mean ±SE	Percent pre-pupal(shrunken stage) mortality Mean±SE	Percent mid-pupal(larval-pupal intermediate)mortality Mean±SE	Percent pupal mortality Mean±SE	Percent deformed Adults Mean±SE	Percent adult mortality Mean ±SE	Percent total mortality Mean ±SE
2	78.33 ±1.66^{ab}	0	0	2.33±1.66^{b-d}	3.33±1.66^{ab}	0 ^{A}	80.66±1.66^{ab}
5	82.00 ±1.66^{ab}	0	0	0^{d}	1.66±1.66^{ab}	0A	82.00±1.66^{ab}
10	81.66 ±1.66^{ab}	0	0	5.00±1.66^{b-d}	0^{b}	0^{A}	86.00±1.66^{ab}
15	70.00 ±7.63^{b}	0	0	19.00±4.41^{A}	1.66±1.66^{ab}	0^{A}	89.00±1.66^{A}
20	83.33 ±1.66^{ab}	0	0	8.33± 1.66^{b-d}	0^{b}	0^{A}	91.66±1.66^{A}
25	85.00 ±2.88^{ab}	0	0	8.00±1.66^{cd}	0^{b}	0^{A}	93.00±1.66^{A}
30	75.00 ±2.88^{ab}	0	0	18.33±1.66^{ab}	3.33±1.66^{ab}	0^{A}	93.33±1.66^{A}
35	88.33 ±4.41^{A}	0	0	6.66±1.66^{cd}	0^{b}	0^{A}	95.00±2.88^{A}
40	80.00 ±2.88^{ab}	1.66±1.66	0	14.00±4.41^{a-c}	8.33±1.66^{A}	0^{A}	95.66±3.33^{A}
Carrier(distilled water control)	38.33 ±3.33^{c}	6.66±1.66	0	0 ^{d}	1.66±1.66^{ab}	0^{A}	45.00±2.88^{c}
Absolute control	30.00 ±2.88^{c}	0	0	1.66±1.66^{cd}	3.33±1.66^{ab}	0^{A}	31.66±1.66^{c}
SE	3.19	0.72	0	2.16	1.38	0.67	1.84
"F" test	36.51	0.90	0	10.00	2.87	0.90	134.47
CD (0. 05)	9.40	NS	0	6.39	4.08	NS	5.42
CD (0. 01)	12.80	NS	0	8.72	NS	NS	7.40

Means followed by the same letters do not differ significantly from each other at P<0.05 (Tukey's honestly significant test)

LC 50 = 21.07 LC 90 = 45.38

Maximum total mean per cent mortality of 93.33 was observed at 25 per cent concentration in topical test. A large per cent mortality (91.66) was recorded at the larval stage at 25 per cent concentration in topical treatment and this indicated the direct insecticidal action of the plant extract (Table 1). Deformities in adults occurred in both the topical and leaf application groups: the maximum being 6.66 per cent at 35 per cent concentration for topical applicationThe adult deformities were higher (8.88 per cent) in the leaf application group. The severely deformed adults were flightless because of their curly or stubby wings. They fed normally on the 10 per cent honey provided and the females laid unfertilized eggs. Some of the adults showed incomplete emergence from their pupal case and died within 4-6 hours from the time of beginning of emergence. Results from table 2

(leaf application) show that more than 50 per cent total mortality was observed for all concentrations tested .The maximum total mortality was 93.00 at 25 per cent concentration. Maximum mortality occurred at the larval stage (88.33 per cent mortality at 35 % concentration) upon leaf application. The larvae, 20 minutes after feeding on the treated leaves oozed a green fluid through the body openings and death occurred within 2-3 hours

Table 2a. Effects on hatchability of eggs and survival rate of I instar larvae of *Spodoptera litura* F that had been treated topically with a crude aqueous extract of *Vinca rosea* at their fourth instar stage.

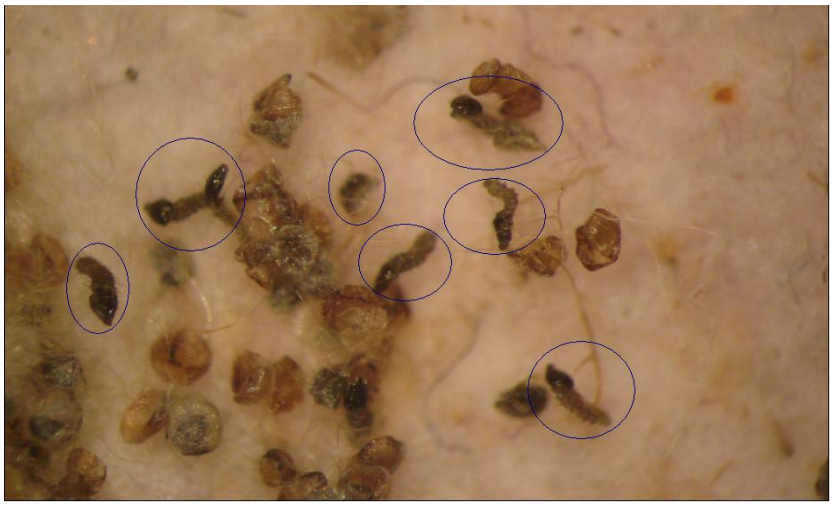
Per cent concentration	Per cent hatchability of eggs Mean ±SE	Per cent mortality of I instar larvae Mean ± SE
2	63.0±1.0	7.30±0.33
5	62.33±0.33	8.02±0.57
10	59.33±0.33	12.80±0.33
15	56.33±0.33	17.05±0.33
20	55.33±0.33	19.53±0.57
25	51.66±0.88	24.41±0.66
30	49.66±0.33	27.41±0.66
35	48.0±0.57	31.87±0.33
40	47.66±0.66	53.35±0.66
Absolute control	65.33±0.88	5.51±0.33

Table 2b. Effects on hatchability of eggs and survival rate of I instar larvae of *Spodoptera litura* F that had been fed castor leaves treated with a crude aqueous extract of *Vinca rosea* at their fourth instar stage.

Per cent concentration	Per cent hatchability of eggs Mean ±SE	Per cent mortality of I instar larvae Mean ± SE
2	64.66±0.33	7.12±0.33
5	63.66±0.33	8.80±0.33
10	63.0±0.57	8.80±0.66
15	60.33±0.66	9.95±0.57
20	60.0±1.52	11.0±0.33
25	59.33±0.33	13.49±0.57
30	57.66±0.66	15.62±0.33
35	57.66±0.88	17.36±0.57
40	57.66±0.33	21.35±0.33
Absolute control	65.33±0.88	5.50±0.33

Fig.1. Effect of crude aqueous extract of *Vinca rosea* leaves on *Spodoptera litura* F

Fig.2. Mortality of F 1 larvae within two hours of hatching. (Parental generation larvae treated with crude aq.extract of *Vinca rosea* leaves)



Per cent hatchability of eggs was affected, as was first instar larval survival rate. From Tables 2a and 2b, it is observed that the former was 47.66± 0.66 at 40 per cent concentration topical application compared to 65.33±0.88 of the absolute control group The latter was 53.36± 0.66 per cent for topical application and 21.36±0.33 per cent for leaf application.

Discussion

Malformed adult emergence might be due to inhibition of chitin synthesis as observed in *S .litura* treated with diflubenzuron, a chitin synthesis inhibitor (Nelson,Jeyarajan *et al* 2006). Some dead pupae showed incomplete chitinization in the thoracic and cephalic region. Death in the pupal stage is ascribed to the slow action of plant products on growth stages of insect or due to the enhanced activity of plant constituents when assimilated in insect tissues. Similar effects were documented by (Senthamizselvan.M *et al* 1992) in *S.exigua*. The incomplete chitinization in pupae suggests that the botanical used may have caused an inhibition in chitin synthesis.

It is suggested that imbalance in the enzyme activity would have caused collapse of the digestive system which would have resulted in the oozing of internal body contents (Nelson,Jeyarajan *et a* 2006*l*) Larval mortality could be attributed to direct insecticidal action (as a contact poison) or due to feeding inhibition or gustatory repellency or impairment in the food assimilation.

Death at the larval stage in some larvae occurred due to a strong moult inhibition; the larvae were unable to shed off their skin completely and died within 48 hours of topical treatment.Deformities and death in larval,larval and pupal stages(figure 1) may be due to change in the ecdysteroid titre as demonstrated by (Leuschner,K *et al* 1972) against coffee bug when it was treated with methanol extract of Neem leaves. Nelson et al. (1996) also observed similar morphogenetic effects of *Azadirachtin* rich fractions against *Spodoptera litura* (Nelson Jeyarajan *et a*l 2006)The reduced hatchability in eggs and lowered per cent survival in the newly emerged ,first instar larvae indicates the prolonged effect of the botanical.The just hatched larvae did not survive for more than two hours.

(Figure 2)The present study indicates that both topical and leaf applications of crude aqueous extract of *V.rosea* leaves are highly effective in controlling the lepidopteran pest *S. litura* by causing a heavy mortality (more than 50 per cent) at the larval stage under laboratory conditions. This plant product is also eco-friendly, easily available and economically viable. Biopesticides are considered to be safe to natural enemies and free from any residue problem on the crop and in the environment. (Mukherjee,U *et al* 2006) Considering the overall performance, the crude aqueous leaf extract of *V.rosea* may be utilized in the management *of S .litura* after evaluating its effects against *S. litura* under field conditions.

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