Toxicity of Ivermectin in Inducing Larval Mortality in Bamboo Leaf Roller, *Crypsiptya coclesalis* Walker

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Abstract

Insecticidal activity of ivermectin (Ivecop- 12), a derivative of ivermectin B, produced by a soil actinomycete, *Streptomyces avermitilis*, was evaluated in laboratory against the larvae of bamboo leaf roller, *Crypsiptya coclesalis* Walker (Lepidoptera: Pyralidae), a major defoliator of bamboos in nurseries, plantations and natural forests. The study was conducted with eight treatment concentrations of lethal and sub-lethal doses. Results revealed that ivermectin is highly toxic and induce larval mortality when applied separately on host plant leaves and larvae, on leaves and larvae of the insect pest together and leaf rolls containing larvae. The nature of treatment affects the larval mortality. On the basis of two trials, results revealed that treatment of leaves and larvae together showed cent percent larval mortality by the 0.15% concentration, whereas treatment of leaves alone and leaf roll exhibited cent percent mortality by the concentration of 0.3%. Larval treatment showed hundred percent mortality by 1.2% concentration. The LC_{50} value of the tested product was worked out to be 0.012239, 0.076789, 0.010833 and 0.051116% for leaf and larval treatments separately, leaf and larval treatments together and leaf roll treatment respectively against the insect pest. This clearly shows that ivermectin is more effective in larval killing when ingested through food.

সার সংক্ষেপ

এই প্রবন্ধে মাটির একটিনোমাইসিটিস ব্যাকটেরিয়া (*Streptomyces avermitilis*) থেকে উৎপাদিত ivermectin নামক কীটনাশকের কার্যকারিতা নার্সারি, প্লানটেশন এবং প্রাকৃতিক বনের বাঁশের ডিফলিয়েটর লিফ রোলার *Crypsiptya coclesalis* Walker (Lepidoptera: Pyralidae)-এর লার্ভার উপর পরীক্ষা করা হয়। ivermectin এর আটটি ঘনত্বের মাত্রায় লিথাল এবং সাব লিথাল ডোজের মাধ্যমে কীটনাশকের কার্যকারিতা পরীক্ষা করা হয়। তীব্র মাত্রায় Ivermectin প্রয়োগের ফলে বাঁশের পাতাসহ লার্ভা, পাতা, লার্ভা এবং লার্ভাসহ রোল করা পাতার লার্ভা দমনের ক্ষেত্রে কার্যকরী ফলাফল পরিলক্ষিত হয়। লার্ভাসহ পাতার উপর ০.১৫% ঘনত্বে প্রয়োগ করলে অধিকাংশ লার্ভা মারা যায় এবং অপরদিকে শুধু পাতা ও মোড়ানো পাতার উপর ০.৩% ঘনত্বে অধিকাংশ লার্ভা মারা যায়। ১.২% ঘনত্বে লার্ভার উপর কীটনাশক প্রয়োগে সর্বাধিক লার্ভা মারা যায়। Ivermectin দ্বারা প্রস্তুতকৃত LC_{50} নামক কীটনাশক ০.০১২২৩৯, ০.০৭৬৭৮৯ ও ০.০১০৮৩৩ এবং ০.০৫১১১৬% ঘনত্বের মাত্রায় প্রয়োগ করে পাতা ও লার্ভার উপর পৃথকভাবে, পাতা ও লার্ভা একত্রে এবং মোড়ানো পাতার লার্ভা দমনের ক্ষেত্রে সুস্পষ্ট ফলাফল পরিলক্ষিত হয়।

Key words : *Crypsiptya coclesalis*, ivermectin, larval mortality, LC_{50}, toxicity

Introduction

Bamboo, the "poor man's timber" or the "green gold" is well known throughout the world. India is endowed with rich diversity of bamboo distributed in different agro-climatic zones of the country and is considered as one of the largest reserves of bamboo in the world. There are 136 indigenous and exotic species of bamboo belonging to 23 genera in an area of 10.03 million hectares (Mohanan 1990).

Bamboo has a rich complex of insect fauna and suffers assiduously from insect damage in varying degree, right from the seed to the finished products (Tewari 1992). Literature on bamboo entomology reveals about 200 odd species are associated with various species of bamboo in India and its adjoining countries. These belonging primarily to Coleoptera, Homoptera, Isoptera, Lepidoptera, and Thysanoptera (Beeson 1941, Bhasin *et al.* 1958, Mathur and Singh 1959, Singh and Bhandari 1988, Thakur 1988, Mathew and Varma 1990 and Singh 1990). Based on their food habits and nature of damage, the insect fauna of bamboo is grouped into seed pests (2 species), nursery pests (5 species), defoliators (48 species), sap-suckers (90 species), culm and shoot borers (12 species), borers (44 species) and termites (13 species) of felled and dried bamboos (Tewari 1992).

Defoliation is a serious problem in nurseries, plantations and natural forests of bamboo and defoliators are the main enemies. Among all, leaf rollers cause severe epidemic defoliation in bamboo (Mathur 1943). The most devastating bamboo leaf rollers that occur in Indian sub-continent are: *Pyrausta bambucivora* Moore and *Crypsiptya coclesalis* Walker (syn. *Pyrausta coclesalis, Algedonia coclesalis*) (Lepidoptera: Pyralidae: Pyraustinae) (Tewari 1992, Pal *et al.* 1996a). Intensive survey for seven years (1994-2001) in central India comprising of Madhya Pradesh, Maharashtra, Chhattisgarh and Orissa revealed that bamboo leaf roller, *C. coclesalis*, is a major pest, found abundantly in this region and causes severe damage of bamboo in nurseries and plantations (Roychoudhury and Joshi 2005). Recently, an exploratory research programme has been carried out to assess the damage caused by bamboo leaf roller and develop its bio-control techniques (Joshi *et al.* 2001) through integrated way by means of host resistance (Pal *et al.* 1996b) and use of microbial (Kalia and Joshi 1995), allelochemical (Kulkarni and Joshi 1998, Kulkarni *et al.* 1999, 2003), and potential parasitoids and predators (Joshi *et al.* 2003). Apart from these, information on the effective control measures of this defoliator is very scanty indeed. Therefore, the present study has been undertaken to examine the sensitivity and response of *C. coclesalis* to ivermectin, developed from avermectins that represent a novel class of natural compounds with potent pesticidal activities, produced by a soil actinomycete, *Streptomyces avermitilis* MA-4680 (NRRL 8165) (Campbell 1989). The subject is also little touched in context to leaf feeding insects.

Materials and Methods

Leaf rolls containing larvae of *Crysiptya coclesalis* were collected from the heavily infested plants of bamboos planted in the campus of Tropical Forest Research Institute, Jabalpur, (M.P.) and was reared in the insectary. Fresh leaves of host plants were provided daily to insects as food. One day old last instar larvae of weight ranged from 0.045-0.055 g (mean 0.051± 0.003 g) were separated out and preconditioned by starvation for about one hour.

The desired concentrations of ivermectin (Ivecop-12, manufactured by Oshin Laboratories Pvt. Ltd., Kundli, Haryana and marketed by Shalaks Pharmaceuticals Pvt. Ltd., New Delhi) were prepared in laboratory by dissolving in distilled water. Water dilution or solution was then uniformly sprayed by hand atomizer separately on host plant leaves (experiment I) and larvae (experiment II) as well as leaves together with larvae (experiment III) and leaf rolls alone (experiment IV). The sprayed leaves with unsprayed larvae (experiment I) and sprayed larvae with unsprayed leaves (experiment II) as well as sprayed leaves together with larvae (experiment III) and leaf rolls containing larvae (experiment IV) were then transferred to sterilized marked beaker of one litre capacity lined at the bottom with a piece of filter paper and covered with muslin cloth. Similarly, the untreated host plant leaves and larvae served as control. In all, three replications of each concentration were made with 10 larvae/rolls in each replication. The observations on the number of dead and moribund larvae after 24, 48 and 72 hours of treatment were recorded. Each experiment was conducted twice with nine treatments including untreated control. The data on larval mortality recorded in experiments I, II, III and IV after 72 hours of treatment were subjected to ANOVA (CRD) separately after angular transformation to conform to normal distribution (Snedecor 1950) for each trial. The pooled data of two trials were also evaluated and corrected by using Abbottís formula (Abbott 1925) and subjected to probit analysis (Finney 1952) with some modifications (Busvine 1957) for calculation of LC_{50} value of the product tested. The experiments were conducted in laboratory under the prevailing environmental conditions during the month of July-August, when the room temperature and the relative humidity varied from 30-37°C and 57-66% respectively.

Results and Discussion

C. coclesalis is an oligophagous insect, the larvae of which feed on different genus and species of bamboos (Roychoudhury and Joshi 2008). Generally, larval population of leaf rollers appeared with the onset of rain in the month of July, during the season of leaf flush in bamboo, when larvae had sufficient amount of leaves to feed. The population build-up reached their peak in August and September, during the monsoon rains and cause severe damage of bamboo in nurseries, plantations and natural forests (Pal *et al.* 1996a).

The percent larval mortality as recorded in the two trials of experiment I, II, III and IV due to treatment of different concentrations of ivermectin against *C. coclesalis* are summarized in Table 1, 2, 3 and 4 respectively. Results revealed that ivermectin induced larval mortality when applied separately on host plant leaves and larvae, on leaves and larvae together and leaf rolls containing larvae of the insect pest. Results exhibited that ivermectin induced larval mortality was significantly ($P<0.05$-$P<0.01$) different among their mean values in both the trials of experiments I, II, III and IV.

Among the treatment concentrations of ivermectin in experiment I and IV, hundred percent larval mortality was recorded by the minimum concentration of 0.3% and significantly different ($P<0.05$-$P<0.01$) from other lowest three concentrations, including control, in both the trials (Table 1 and 4). In experiment II, cent percent larval mortality was noticed by the highest concentration of 1.2% in both the trials and the treatment showed significant ($P<0.05$-$P<0.01$)

Table 1 . Larval mortality percentage in *C. coclesalis* due to leaf treatment with ivermectin in laboratory.

Treatment	Indices concentration (%)	Larval mortality (%)		
		Trial I (29.07.2007)	Trial II (02.08.2007)	Pooled mean
T_1	1.2	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_2	0.6	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_3	0.3	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_4	0.15	100.00 (90.00)	96.67 (83.86)	98.34 (86.93)
T_5	0.075	93.33 (77.71)	83.33 (66.14)	88.33 (71.93)
T_6	0.0375	90.00 (75.00)	76.67 (61.72)	83.34 (68.36)
T_7	0.01875	73.33 (59.21)	66.67 (54.78)	70.00 (57.00)
T_8	0.009375	43.33 (41.15)	50.00 (45.00)	46.67 (43.08)
T_9	Control*	3.33 (6.14)	0.00 (0.00)	1.67 (3.07)
	SEm	7.275	3.064	5.170
	C.D. at 1%	29.610	12.471	21. 041
	C.D. at 5%	21.615	9.104	15.360

* Without any treatment. Angular transformed values are inside parentheses.

Table 2 . Larval mortality percentage in *C. coclesalis* due to larval treatment with ivermectin in laboratory.

Treatment	Indices concentration (%)	Larval mortality (%)		
		Trial I (06.08.2007)	Trial II (10.08.2007)	Pooled mean
T_1	1.2	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_2	0.6	83.33 (66.64)	86.67 (72.79)	85.00 (69.72)
T_3	0.3	73.33 (59.00)	70.00 (56.79)	71.67 (57.90)
T_4	0.15	66.67 (54.78)	63.33 (52.86)	65.00 (53.82)
T_5	0.075	56.67 (48.93)	53.33 (45.00)	55.00 (46.97)
T_6	0.0375	40.00 (39.15)	36.67 (36.93)	38.34 (38.04)
T_7	0.01875	26.67 (31.00)	23.33 (28.28)	25.00 (29.64)
T_8	0.009375	16.67 (23.86)	10.00 (18.43)	13.34 (21.15)
T_9	Control*	3.33 (6.14)	3.33 (6.14)	3.33 (6.14)
	SEm	3.672	4.706	4.189
	C.D. at 1%	14.941	19.154	17.048
	C.D. at 5%	10.908	13.982	12.445

*Without any treatment. Angular transformed values are inside parentheses.

Table 3. Larval mortality percentage in *C. coclesalis* due to leaf and larval treatment together with ivermectin in laboratory.

Treatment	Indices concentration (%)	Larval mortality (%)		
		Trial I (11.08.2007)	Trial II (16.08.2007)	Pooled mean
T_1	1.2	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_2	0.6	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_3	0.3	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_4	0.15	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_5	0.075	96.67 (83.86)	90.00 (75.00)	93.34 (79.43)
T_6	0.0375	90.00 (71.57)	73.33 (59.00)	81.67 (65.29)
T_7	0.01875	70.00 (57.00)	60.00 (50.85)	65.00 (53.93)
T_8	0.009375	40.00 (39.06)	50.00 (45.00)	45.00 (42.03)
T_9	Control*	0.00 (0.00)	6.67 (12.29)	3.34 (6.15)
	SEm	3.080	3.758	3.419
	C.D. at 1%	12.536	15.295	13.916
	C.D. at 5%	9.152	11.167	10.160

*Without any treatment. Angular transformed values are inside parentheses.

Table 4. Larval mortality percentage in *C. coclesalis* due to leaf roll treatment with ivermectin in laboratory.

Treatment	Indices concentration (%)	Larval mortality (%)		
		Trial I (20.08.2007)	Trial II (24.08.2007)	Pooled mean
T_1	1.2	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_2	0.6	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_3	0.3	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_4	0.15	70.00 (57.00)	76.67 (61.22)	73.34 (53.93)
T_5	0.075	50.00 (45.00)	60.00 (50.85)	55.00 (47.93)
T_6	0.0375	40.00 (39.15)	36.67 (37.22)	38.34 (38.19)
T_7	0.01875	33.33 (35.22)	23.33 (28.78)	28.33 (32.00)
T_8	0.009375	16.67 (23.86)	13.33 (21.14)	15.00 (22.50)
T_9	Control*	3.33 (6.14)	0.00 (0.00)	1.67 (3.07)
	SEm	3.076	1.908	2.492
	C.D. at 1%	12.520	7.766	10.143
	C.D. at 5%	9.139	5.668	7.404

*Without any treatment. Angular transformed values are inside parentheses.

difference from others, including control (Table 2). In experiment III, percent larval mortality was registered by the minimum concentration of 0.15% in both the trials and found to be significantly ($P<0.05$-$P<0.01$) different from other treatment concentrations, including control (Table 3).

The dosage effect was found to be conclusive ($P>0.05$-$P>0.01$) only for experiment IV, whereas no significant ($P>0.05$) difference was noticed for experiment I, II and III. But, there was a gradual decline of larval mortality in respect of lowering concentrations of the ivermectin as evident from the data of the two trials of all the four experiments (Table 1, 2, 3 and 4) and also from pooled data based on percentage corrected larval mortality (Fig. 1).

The LC_{50} value of the tested product was worked out to be 0.012239, 0.076789 and 0.010833 and 0.051116% for leaf and larval treatments separately, leaf and larval treatments together and leaf roll treatment respectively against the early last instar larvae of *C. coclesalis* (Table 5). Earlier, Joshi *et al.* (2001) have carried out efficacy of two insecticides, such as endosulfan (endocel 35 EC) and monocrotophos (nuvacron 36 EC) against the early last instar larvae of *C. coclesalis* in laboratory and recorded highest larval mortality of 35 and 81% by the treatment concentrations of 0.04 and 0.08% respectively.

The present findings clearly suggest the toxic properties of the tested ivermectin against the larvae of *C. coclesalis* and possess potent insecticidal activities at very low concentrations when compared with chemical pesticides. Further, the results of the experiments indicate that ivermectin is more effective in larval killing when ingested through food. It has been reported

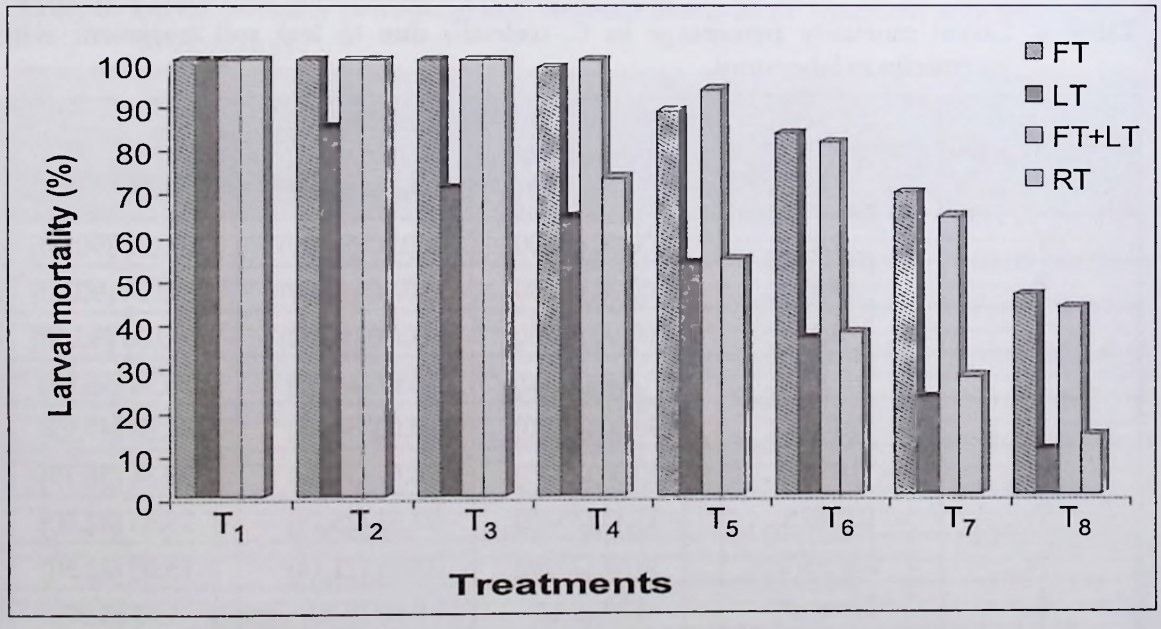

Figure 1 : Pooled data of percentage corrected larval mortality in *S. retorta* due to leaf treatment (FT), larval treatment (LT), leaf and larval treatment together (FT+LT) and leaf roll treatment (RT) with ivermectin.

Table 5 . Toxicity of ivermectin against the larvae *C. coclesalis* in laboratory.

Nature of treatment	Regression equation	Heterogeneity	Degrees of freedom	LC_{50}	Fiducial limits
Leaf treatment	Y=2.843+1.983x	$\chi^2 = 0.570$	3	0.012239	0.008515 0.017591
Larval treatment	Y=2.551+1.299x	$\chi^2 = 2.765$	6	0.076789	0.055949 0.105390
Leaf and larval treatment	Y=3.214+1.726x	$\chi^2 = 0.877$	4	0.010833	0.006863 0.017102
Leaf roll treatment	Y=2.186+1.647x	$\chi^2 = 3.696$	4	0.051116	0.03867 0.067559

Y = Probit kill, x = Log concentration, LC_{50} = Concentration calculated to give 50% mortality

that synthetic chemical studies following the discovery of the avermectins produced by a soil actinomycete, *S. avermitilis* during the process of fermentation, led to the synthesis of ivermectin, a semisynthetic avermectin (Lasota and Dybas 1991). Ivermectin is the common name for 22, 23-dihydroavermectin, produced commercially for veterinary and medical purposes (Campbell 1981). Ivermectin has demonstrated broad spectrum activity against a variety of helminthic species (Campbell *et al.* 1983) at a fraction of the concentrations of other antihelminthic compounds and currently used as a human drug for control of microfilaria that is transmitted by *Simulium* black flies (Green *et al.* 1989). The present study reports the insecticidal properties of ivermectin against phytophagous insect.

There is no published information regarding the toxicity of tested biological product against the larvae of *C. coclesalis*, with which the present findings could be compared. Therefore, the data on LC_{50} of the product recorded in the present work can be employed profitably for effective and economic use of ivermectin as a biological insecticide against *C. coclesalis* and as guidelines for further investigations.

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