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EFFICACY OF AVERMECTINS, CHITIN SYNTHESIS INHIBITOR AND FUNGICIDES AGAINST SPODOPTERA LITURA AND ASPERGILLUS FLAVUS

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ABSTRACT

The LC_{50} of the insecticides *viz.,* abamectin, emamectin benzoate, novaluron and lufenuron against *S.litura* was determined as 210.23, 102.12, 350.45 and 453.78 ppm, respectively, whereas the fungicides *viz.,* mancozeb, chlorthalonil, and carbendazim recorded LC_{50} values of 97.0, 1.16 and 40.94 ppm respectively. The test insecticides were non toxic to *A.flavus* at LC_{50}, field recommended and other concentrations, whereas fungicides were also non toxic to *S.litura.*

Key words: *Aspergillus flavus*, *Spodoptera litura*, Insecticides and Fungicides.

INTRODUCTION

During the past two decades, plant protection assumed greater importance as a consequence of tremendous increase in pest problems due to adoption of intensive agricultural practices. Frequently insect pest and diseases are occurring simultaneously causing enormous crop losses warranting the farmers to take up effective control measures. At present, highly effective fungicides and insecticides with novel modes of action are available and these are becoming increasingly important in modern agriculture as a component of integrated pest management and resistance management strategies. Avermectins are macrocyclic insecticides with low toxicity to non-target organisms and the environment. Chitin synthesis inhibitors are known to influence the insects by inhibiting or interfering with chitin deposition during and after moult and are reffered as insect specific insecticides. They are recommended for use against wide spectrum of lepidopteran pests attacking the foliage and fruits.

MATERIALS AND METHODS

Experiments were conducted in the Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad during 2009-2010. For assessing the biological effectiveness of the insecticides, tobacco caterpillar *Spodoptera litura* was used as the test insect. Similarly, for evaluating biological effectiveness of fungicides. *Aspergillus flavus* was selected as test fungus.

Egg clusters of *S.litura* were collected from castor crop from the fields of Student farm, Rajendranagar, Hyderabad and multiplied from a single egg mass of a single adult. The first instar larvae soon after hatching from the eggs, were transferred to glass trough containing fresh castor leaves. The larvae were provided with fresh diet by transferring them daily into fresh rearing trays till pupation and adequate care was taken to prevent the infection from fungal and other contaminants. The pupae were collected and transferred to a cage and the cotton swab soaked in 5 per cent sucrose solution was

provided as food to the emerging adults in the cage. After a week's time, the adults started emerging. The eggs laid on cotton / cloth were removed daily and kept in glass trough containing moistened filter paper to facilitate hatching. Ten days old larvae of *S.litura* of same size and wheighing 30-35 mg were used for bio-assay experiments.

Four insecticides, abamectin (Dynamite, 1.9% EC) at 0.019%, emamectin benzoate (Proclaim5% SG) at 0.002%, novaluron (Rimon 10% EC) at 0.01% and lufenuron (Signa 5.4% EC) at 0.01% and Fungicides, mancozeb (Dithane M- 45) at 0.25%, carbendazim (Bavistin 50% WP) at 0.10% and chlorothalonil (Kavach 75%WP) at 0.20% were selected for the study.

The pure culture of *A. flavus* was maintained on Potato Dextrose Agar (PDA) medium by periodic subculture at regular intervals so as to obtain a regular supply of uniformly grown culture for compatibility studies. Conidial suspension of *A. flavus* was prepared from 10 days old culture. The conidia were scrapped from surface of the colonies and transferred to sterile distilled water, which was filtered through a double layered muslin cloth. The conidial suspension was diluted in such a way so as to contain 25-30 conidia per microscopic field when seen under the low power of a compound microscope and were utilized for carrying out the investigation.

Conidial suspension of *A. flavus* was prepared from 10 days old culture. The conidia were scrapped from surface of the colonies and transferred into distilled water and filtered through a double layered muslin cloth. The conidial suspension was diluted in such a way so as to contain 25-30 conidia per microscopic field when seen under the low power of a compound microscope and was utilized for carrying out the investigation.

Abamectin, novaluron and lufenuron of 52.6 ml, 10 ml and 18.5 ml respectively were measured and transferred to 100 ml volumetric flasks separately. Acetone was added to each of the flasks and made upto 100 ml with constant shaking of the flask care that the insecticides were thoroughly mixed with acetone. Emamectin benzoate (10 g) was weighed and transferred to 100 ml volumetric flask and 10-15 ml of distilled water was added to it, shaken thoroughly to form paste. Volume was made upto 100 ml by adding distilled water to obtain one per cent suspension. The flasks having one per cent of the active ingredient of insecticide was taken as the stock solution. These stock solutions were kept in refrigerator and were removed an hour before use so as to bring the solutions to room temperature. Further dilutions to desired concentrations were prepared following the serial dilution technique using distilled water as diluent. These working concentrations were prepared fresh just before their use every time.

Spodoptera litura was exposed initially to concentrations of wider range and on the basis of mortality recorded, a series of concentrations of narrow range were selected to which the test insect was again exposed. The same procedure was repeated till mortality data in a range of 20.0 to 80.0 per cent were recorded. The narrow range concentrations of selected insecticides which gave mortality in the above range are furnished below:

Abamectin:	350, 300, 250, 200, 150, 100 and 50 ppm
Emamectin benzoate:	180, 160, 140, 120, 100, 80 and 60 ppm
Novaluron:	600, 500, 400, 300, 200 and100 ppm
Lufenuron:	900, 700, 500, 400, 300 and 200 ppm

Mancozeb (133.33 mg), carbendazim (200 mg) and chlorothalonil (133.33 mg) were weighed and transferred to 100 ml volumetric flasks separately. Little quantity of water was added to fungicides and the flasks were shaken thoroughly to form into a paste. The paste was made up to 100 ml by adding distilled water while shaking simultaneously. In this way suspension of mancozeb1000 ppm (0.1 %), chlorothalonil 1000 ppm (0.1 %) and carbendazim 1000 ppm (0.1 %) was prepared. From these fungicide suspensions, different

graded concentrations were prepared by serial dilution method every time just before their use.

The test fungus, *A. flavus* was initially exposed to concentrations of wider range and on the basis of percentage of non-germinated spores, a series of concentrations of required range were selected. The spores were again exposed to these concentrations and the germination of spores was recorded. The same procedure was repeated till the percentages of non-germinated spores in a range of 20-80 were recorded. The concentrations of fungicides which gave percentages of non-germinated spores in the range (20-80) are given below.

Mancozeb	: 500, 250, 100, 50,25 and 10 ppm
Chlorothalonil	: 3.0, 2.0, 1.5, 1.0, 0.75 and 0.5 ppm
Carbendazim	: 250, 200, 100, 50, 25, 10 and 5 ppm

Topical application method was followed to test the toxicity of insecticides against larvae of *S.litura*. Moderately tender castor leaves were cut into discs of 5 cm diameter and placed in petri dishes. Larvae are released in petri dish and each larva is treated with insecticide by applying on thorax region with the help of micropipette. Observations on mortality were recorded at 72 h intervals after the treatment.

The toxic effect of fungicides to *A. flavus* was tested by using the slide germination technique recommended by Montegomery and Moore (1938). Glass cavity slides (3x1") were used for this purpose.The fungicidal fluid of 0.06 ml was placed by means of a micropipette and spread uniformly in the cavities. The slides were kept under a fan for quick drying of fungicidal fluid. After complete drying, spore suspension of *A.flavus* was transferred at the rate of 0.03 ml in the cavity by means of micropipette, spread evenly and were then incubated in moist chambers.

Rectangular plastic boxes with moistened tissue paper served as moist chamber. Over the moist tissue paper, 'U' shaped bent glass tube was placed, which served as a support for the slides. The sealed slides placed over the 'U' shaped glass tube inside the moist chambers were incubated for 24 h at room temperature (26-30 ^{o}C). The incubation period of 24 h was pre-determined as maximum number of conidia germinated by this period. After the specified period of incubation, the number of non germinated spores was counted by means of compound microscope under low power (10X). Hundred spores were counted for each replication and each treatment was replicated thrice.

The fungicide concentration that inhibited 50 per cent of spores (LC_{50}) of the test fungus was mixed with different concentrations of insecticides. The spores of *A. flavus* were exposed to these mixtures and the spore germination was recorded at 24 h after incubation. The per cent non-germinated spores was calculated for each mixture for assessing the occurrence of synergism, antagonism and no effect in respect of toxicity of fungicide to test fungus when mixed with the insecticides.

Calculation of LC_{50} values

To determine LC_{50} values of each insecticide and fungicide, the concentrations that gave 20 to 80 per cent mortality of the *S.litura* and per cent inhibition of spore germination of *A.flavus* were subjected to probit analysis (Finney, 1952). The data on LC_{50} values are presented for each insecticide and fungicide separately and used for further studies on bio-efficacy. The data on per cent inhibition of spore germination was subjected to Abbott's correction (Abbott, 1925) wherever ungerminated spores were recorded in control.

RESULTS AND DISCUSSIONS

Effect of insecticides against *S .litura*

Mortality rates of *S. litura* were recorded at 72 hrs after the treatments.

Effect of abamectin against S. litura:

The effect of abamectin on mortality of *S. litura* is presented in Table 1. Mortality of 89.99, 82.60, 77.33, 67.22, 43.00, 35.20 and 30.66 per

Table 1: Effect of Abamectin on the Mortality Of *S. Litura*

S.No.	Concentrationof abamectin (ppm)	Mean mortality over control (%)	Heterogeneity	Regression equation	LC_{50} (Fiducial limits)
1	350	89.99	$\chi^2 = 0.773$	Y=1.8510+ 0.37X	210.23 (79.591-228.267)
2	300	82.60			
3	250	77.33			
4	200	67.22			
5	150	43.00			
6	100	35.20			
7	50	30.66			
8	Control	0.0			

Table 2: Effect of Emamectin Benzoate on the Mortality Of *S. Litura*

S.No.	Concentration of emamectin benzoate (ppm)	Mean mortality over control (%)	Heterogeneity	Regression equation	LC_{50} (Fiducial limits)
1	180	80.00	χ^2= 1.672	Y= -4.90+ 1.151X	102.12 (67.877-94.783)
2	160	73.33			
3	140	66.66			
4	120	60.00			
5	100	46.66			
6	80	36.66			
7	60	23.33			
8	Control	0.0			

Table 3: Effect of Novaluron on the Mortality Of *S. Litura*

S.No.	Concentration (ppm)	Mean mortality over control (%)	Heterogeneity	Regression equation	LC_{50} (Fiducial limits)
1	600	77.35	χ^2=1.220	Y=0.07702+ 0.42873X	350.45 (301.2573-491.2354)
2	500	67.45			
3	400	52.63			
4	300	47.44			
5	200	30.32			
6	100	24.12			
7	Control	0.0			

Table 4: Effect of Lufenuron on the Mortality Of *S. Litura*

S.No.	Concentration (ppm)	Mean mortality over control (%)	Heterogeneity	Regression equation	LC_{50} (Fiducial limits)
1	900	76.66			
2	700	63.33			
3	500	53.33			
4	400	46.66	χ^2=0.762	Y= -6.44+ 1.05X	453.78 (374.716-532.792)
5	300	36.66			
6	200	23.33			
7	Control	0.0			

Table 5 : Effect of Mancozeb on Spore Germination Of *Aspergillus Flavus*

S.No.	Concentration (ppm)	Mean of non-germinated spores over control (%)	Heterogeneity	Regression equation	LC_{50} (Fiducial limits)
1	500	84.34			
2	250	67.91			
3	100	56.62			
4	50	42.20	χ^2= 1.875	Y= -2.00 0.43 X	97.00 (19.96-187.052)
5	25	32.76			
6	10	25.35			
7	Control	0.0			

Table 6: Effect of Carbendazim on Spore Germination Of *A. Flavus*

S.No.	Concentration (ppm)	Mean of non-germinated spores over control (%)	Heterogeneity	Regression equation	LC_{50} (Fiducial limits)
1	250	82.50			
2	200	75.40			
3	100	69.00			
4	50	62.47		Y= -1.41 + 0.38 X	40.94 (13.13-74.17)
5	25	44.25	χ^2= 5.24		
6	10	31.45			
7	5	23.14			
8	Control	0.0			

Table 7: Effect of Chlorothalonil on Spore Germination Of *A. Flavus*

S.No.	Concentration (ppm)	Mean of non-germinated spores over control (%)	Heterogeneity	Regression equation	LC_{50} (Fiducial limits)
1	3.0	85.59			
2	2.0	79.20			
3	1.5	57.77		Y= -0.17	1.16
4	1.0	43.22	χ^2= 0.502	+	(0.9654-
5	0.75	34.16		1.15X	1.343)
6	0.5	18.92			
7	Control	0.0			

cent was recorded at concentrations of 350, 300, 250, 200, 150, 100 and 50 ppm, respectively as against nil mortality in control. It is evident from the data that the mortality decreased steadily with the decrease in concentration of the insecticide. By subjecting the corrected mortality data to probit analysis (Finney, 1952), the LC_{50} value of abamectin against the test insect was found to be 210.23 ppm. The population of the test insect was homogenous as evidenced by heterogeneity test.

Effect of emamectin benzoate against S. litura:
Table 2 indicates the effect of emamectin benzoate on the mortality of *S. litura*. The mean of corrected mortality percentages recorded were 80.00, 73.33, 66.66, 60.00, 46.66, 36.66 and 23.33 at 180, 160, 140, 120, 100, 80 and 60 ppm concentrations of emamectin benzoate, respectively. From the data it is clear that there was a steady decrease in mortality of the test insect with decrease in concentration of the insecticide. The LC_{50} of emamectin benzoate against *S. litura* was found to be 102.12 ppm .

Effect of novaluron against S. litura:
The mortality of *S.liura* larvae observed with the different concentrations of novaluron is presented in Table 3. At the concentrations of 600, 500, 400, 300, 200 and 100 ppm, the mortality of *S.litura* recorded was 77.35, 67.45, 52.63, 47.44, 30.32 and 24.12 per cent, respectively. The mortality decreased progressively with the decrease in concentrations of insecticide. The LC_{50} value of the novaluron against *S.litura* was established to be 350.45 ppm. The chi-square test indicated that the *S.litura* larval population used in this study was homogeneous (χ^2 = 1.220).

Effect of lufenuron against S. litura:
Data on the effect of lufenuron against the mortality of *S.litura* is presented in Table 4. The per cent mortality of insect larvae recorded was 76.66, 63.33, 53.33, 46.66, 36.66 and 23.33 at 900, 700, 500, 400, 300 and 200 ppm concentrations of lufenuron, respectively. The mortality of the test insect in control was nil. There was gradual decrease in mortality with the decrease in concentration of the insecticide. The lethal concentration for affecting 50.0 per cent kill of the insect larvae (LC_{50}) was found to be 453.78 ppm. The populations of the test was homogenous as considered by heterogeneity test (χ^2 =0.762).

Effect of fungicides against *A. flavus*

Effect of mancozeb on spore germination of A. flavus:
The toxic effect of mancozeb on spore germination of *A. flavus* is presented in Table 5. At 500, 250, 100, 50, 25 and 10 ppm concentrations of mancozeb, the percentages of non-germinated recorded were 84.34, 67.91, 56.62, 42.20, 32.76 and 25.35, respectively

(Table-5). It is clear from the data that there was progressive decrease in the non germinated spores with decrease in concentration of the fungicide, exhibiting as high as 84.34 per cent inhibition at 500 ppm concentration. The LC_{50} of mancozeb against *A. flavus* was found to be 97.00 ppm.

Effect of carbendazim on spore germination of A. flavus:

The data on toxic effect of carbendazim on spore germination of *A. flavus* is presented in Table 6. The percentages of non germinated spores recorded were 82.50, 75.40, 69.00, 62.47, 44.25, 31.45 and 23.14 per cent of non-germinated spores over control were recorded at 250, 200, 100, 50, 25, 10 and 5 ppm concentrations, respectively. It is evident that there was steady decrease in the non-germinated spores with decrease in concentrations of the fungicides exhibiting as high as 82.5 per cent inhibition at 250 ppm concentration. The LC_{50} of carbendazim, which inhibited 50 per cent of *A.flavus* spores was found to be 40.94 ppm.

Effect of chlorothalonil on spore germination of A. flavus:

The per cent germinated of spores of *A. flavus* with six test concentrations of fungicide chlorothalonil is presented in Table 7. At 3.0, 2.0, 1.5, 1.0, 0.75 and 0.5 ppm concentrations, the percentages of non germinated spores were 85.59, 79.20, 57.77, 43.22, 34.16 and 18.92, respectively. It is clear that there was a progressive decrease in the non-germinated spores with decrease in the concentrations of the fungicides. The lethal concentration of chlorothalonil, which inhibited 50 per cent of spore germination against *A. flavus* was found to be 1.16 ppm.

Effect of fungicides against *S. litura*

Effect of mancozeb, carbendazim and chlorothalonil aginst S. litura:

All the fungicides *viz.,* mancozeb, carbendazim and chlorothalonil were found to be non-toxic to *S. litura,* at their respective LC_{50} values.

CONCLUSIONS

LC_{50} value of abamectin, emamectin benzoate, novaluron, lufenuron against the test insect was found to be 210.23 ppm, 102.12 ppm, 350.45 ppm, 453.78 ppm. Among all emamectin benzoate was found to be effective on *Spodoptera litura* with LC_{50} 102. 12 ppm whereas the fungicides *viz.,* mancozeb, chlorthalonil, and carbendazim recorded LC_{50} values of 97.0, 1.16 and 40.94 ppm against *A. flavus.* Chlorothalonil was found to be effective on *A. flavus* with LC_{50} 1.16 ppm. All the fungicides *viz.,* mancozeb, carbendazim and chlorothalonil were found to be non-toxic to *S. litura,* at their respective LC_{50} values.

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