Int. J. Indust. Entomol. Vol. 4, No. 1, 2002, pp. 5~11

International Journal of *Industrial Entomology*

Screening of Fungicides and Natural Plant Products and Their Efficacy on Control of Aspergillosis in Silkworm, *Bombyx mori* L.

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(Received 25 August 2001; Accepted 26 December 2001)

Seven fungicides viz., salcylic acid, bavistin (Carbandazim 50% WP), bayleton (Triadimefon 25% WP), Dithane M-45 (Mancozeb 75% WP), captan (Captaf 50% WP) formaldehyde and benzoic acid at three concentrations (0.50, 0.75 and 1.0%) and ten plant products viz., Hena leaf, garlic bulb, tomato leaf, mango bark, cotton leaf, turmeric powder, onion, tulsi leaf, neem leaf and ginger at 1.0, 2.0 and 3.0% concentrations were screened against *Aspergillus flavus* and *A. tamarii* in vitro. Among fungicides, salcylic acid and bavistin and among plants Hena and Mango bark powder were found to be very effective at all concentrations tested. Based on *in vitro* screening, only selected six fungicides at 1.0, 1.5 and 2.0% and six plants at 2.0, 4.0 and 6.0% concentrations were tested *in vivo* for controlling Aspergillosis in silkworm. Salcylic acid and bavistin fungicides and Hena leaf powder and Mango bark powder have shown considerable effect in controlling *Aspergillus* infection in silkworm at all concentrations tested.

Key words : *Bombyx mori,* Aspergillosis, Fungicides, Natural plant products.

Introduction

Aspergillosis is a fungal disease caused by *Aspergillus* has been considered as an important silkworm disease, popularly called as Koji-Kabi disease in Japan (Ayuzawa *et al.*, 1972). More than ten species of *Aspergillus* have been reported to be pathogenic to silkworm in Japan (Kawakami and Mikuni, 1969; Ayuzawa *et al.*, 1972; Kawakami, 1975a). Incidentally, the climatic conditions (high temperature and high relative humidity) required for young age (chawki) silkworm rearing are quite favourable for the multiplication and growth of *Aspergillus* fungi (Kawakami, 1982b). As the *Aspergillus* disease occur particularly in the young stage of silkworm, the control of this disease is extremely important in chawki rearing centres where a large number of larvae are reared and distributed to the farmers for further rearing.

Kawakami (1973b) reported that dithiocarbamate fungicides were effective in controling Aspergillosis during silkworm rearing. Ludeman *et al.* (1979) has tested 32 fungicides in artificial diet of tobacco budworm and found 7 of them effective to control the growth of *Aspergillus niger* in diet. Chinnaswamy and Devaiah (1986), Anitha Peter (1988), and Manjunathan Gowda (1994) tested some fungicides and reported that bavistin and formalin were effective to inhibit the radial mycellial growth of *Aspergillus tamari.*

So far only chemical fungicides have been tested against Aspergillosis in silkworm, but no published information is available on the effect of natural plant products on Aspergillosis, though they are most safe for silkworm, human being and environment. Hence, in present study in addition to chemical fungicides, some plant products having fungicidal properties (Sanyal, 1924; Dey, 1980; Agarwal and Barin Agosh, 1989; Kaushik and Dhiman, 2000) have been evaluated and determined their efficacy on control of Aspergillosis disease in silkworm.

Materials and Methods

Seven fungicides and ten plant products were tested against *Aspergillus tamarii* and *A. flavus*. The fungicides used were salcylic acid, bavistin (Carbendazim 50% WP), bayleton (Triadimefon 25% WP), dithane M-45(Manco-

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zeb 75% WP), Captan (Captaf 50% WP), formaldehyde and benzoic acid. The plant products included, Tomato (*Lycopersicon esculentum*) leaf, Hena (*Myrtus communis*) leaf, Garlic (*Allium sativum*) bulb, onion , Tulsi (*Ocimum sanctum*) leaf, Neem (*Azadirachta indica*) leaf, Ginger (*Zingiber officianalis*), Turmeric (*Curcuma longa*) powder, Mango (*Mangifera indica*) bark, Cotton (*Gossipium*) leaf.

The fungicides were tested in *in vitro* at 0.5, 0.75 and 1.0 percent concentration and plant products at 1.0, 2.0 and 3.0 percent concentration. The fungicides were weighed out in required quantity and added to the 10 ml. sterilized potato dextrose liquid media separately in conical flasks to prepare individual concentrations. Similarly, the crued plant extracts were pipetted out in the required quantity and added to the culture medium. The conidial suspension of *Aspergillus flavus* and *A. tamarii* (1×10^7 conidia/ml.) was prepared separately in sterilized distilled water. Conidia per ml. were calculated by using haemocytometer. One ml. of *Aspergillus* suspension was added to the 10 ml. of treated culture media and incubated at the temperature of $28 \pm 1°C$ and $85 \pm 5\%$ relative humidity for 7 days. Each treatment was replicated three times for each species of *Aspergillus* separately. The growth of *Aspergillus* was observed regularly for 7 days and recorded as per the treatments and replications for both *Aspergillus* species separately.

Based on *in vitro* screening of fungicides/plant products, the selected ones were tried for their efficacy in controlling of Aspergillosis in silkworm. A popular bivoltine silkworm breed NB4D2 was used for *in vivo* efficacy of the fungicides and plant products. For *in vivo* screening the fungicides were tried at larger concentrations i.e. 1.0, 1.5 and 2.0% and plant products at 2.0, 4.0 and 6.0%. The leaves, bulbs and bark of respective plants were dried separately in shade for two days and then at 60°C temperature in oven for 24 hours and powdered with the help of mortar and pestle. Turmeric powder was used as available commercially in market. All the fungicides and powder of plant products were weighed out as per the required quantity and mixed properly with inert kaolin powder.

The newly hatched silkworms and newly moulted second and third instar silkworms were inoculated topically by spraying the suspension (1×10^7 conidia /ml) of different *Aspergillus* species separately on to the integument of the silkworm with the help of an automizer. Two ml of conidial suspension prepared from 7 days old culture was used to infect 100 silkworms. One hour after inoculation, larvae were dusted with the different concentrations of fungicides and plant products. Silkworms were fed with mulberry leaf after 30 minutes of dusting and reared under optimum temperature ($28 \pm 1°C$) and relative humidity (85 - 90%) as advocated by Krishanaswami (1979). Each treatment was replicated three times for both *Aspergillus* species separately. Mortality due to *Aspergillus* infection was recorded for 7 days for each treatment and replication separately. Data were statistically analysed by Completely Randomised Design to determine the efficacy of the treatments.

Results

In vitro efficacy of fungicides/plant products against *Aspergillus flavus* and *A. tamarii*

Observations on growth of *Aspergillus flavus* and *A.tamarii* in culture media after treatment with different concentrations of different fungicides /plant products were recorded and presented in Table 1a and b. Among seven fungicides tested, salcylic acid and bavistin were found effective in controlling the growth of both *Aspergillus* species in culture medium. In any replication of all tested concentrations of these fungicides, *Aspergillus* growth was not observed up to 7 days. Captan and dithane M-45 at 0.5 and 0.75% concentrations resulted in growth of *Aspergillus* in some of the replications, but at 1.0%, there

Table 1. In vitro screening of fungicides against *Aspergillus flavus* and *A. tamarii*

Name of Fungicides	Conc. (%)	*A. flavus* 1	2	3	*A. tamarii* 1	2	3
Salcylic acid	0.50	-	-	-	-	-	-
	0.75	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
Bavistin (Carbendazin 50 % WP)	0.50	-	-	-	-	-	-
	0.75	-	-	-	-	-	-
	1.00	-	-	-	-	-	-
Bayleton (Triadimefon 25% WP)	0.50	-	+	+	-	-	+
	0.75	+	-	-	-	+	-
	1.00	-	-	+	-	-	-
Dithane M-45 (Mancozeb 75% WP)	0.50	-	+	+	+	-	-
	0.75	-	+	-	-	-	+
	1.00	-	-	-		-	-
Captan (Captaf 50% WP)	0.50	+	-	-	-	-	+
	0.75	-	-	+	-	-	-
	1.00	-	-	-	-	-	-
Formaldehyde	0.50	+	+	+	+	+	+
	0.75	+	+	+	+	+	+
	1.00	+	+	+	+	+	+
Benzoic acid	0.50	+	+	+	+	+	+
	0.75	-	-	+	-	+	-
	1.00	-	+	+	-	-	+

+, Growth of pathogen observed.
-, No growth of pathogen observed.

Table 2. In vitro screening of Plants against *Aspergillus flavus* and *A. tamarii*

Name of plant	Conc. (%)	*A. flavus*			*A. tamarii*		
		1	2	3	1	2	3
Tomato	1.00	-	+	-	-	-	+
	2.00	-	-	+	-	-	-
	3.00	-	-	-	-	-	-
Hena	1.00	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	3.00	-	-	-	-	-	-
Garlic	1.00	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	3.00	-	-	+	-	-	-
Onion	1.00	+	+	+	+	+	+
	2.00	+	+	+	+	+	+
	3.00	+	+	+	+	+	+
Tulsi	1.00	+	+	+	+	+	+
	2.00	+	+	+	+	+	+
	3.00	+	+	+	+	+	+
Neem	1.00	+	+	+	+	+	+
	2.00	+	+	+	+	+	+
	3.00	+	+	+	+	+	+
Ginger	1.00	+	+	+	+	+	+
	2.00	+	+	+	+	+	+
	3.00	+	+	+	+	+	+
Turmeric	1.00	+	+	+	+	+	+
	2.00	-	-	+	-	-	+
	3.00	-	-	+	-	+	-
Mango bark	1.00	-	-	-	-	-	-
	2.00	-	-	-	-	-	-
	3.00	-	-	-	-	-	-
Cotton	1.00	+	+	+	+	+	+
	2.00	+	-	+	+	-	-
	3.00	-	+	+	+	-	-

+, Growth of pathogen observed.
-, No growth of pathogen observed.

was no growth of *Aspergillus*. Bayletone and benzoic acid were least effective to control the growth of both *Aspergillus* species in culture medium. Formaldehyde was not effective to control the growth of *Aspergillus* at all concentrations tested (Table 1).

Among the plant products Hena, Garlic and Mango bark inhibited the growth of both *Aspergillus* species at all concentrations tested in culture medium. Onion, tulsi, neem and ginger were not effective as the growth of *Aspergillus* was noted in all the replications at all concentrations tested. Omato resulted in partial inhibition of growth of Aspergillus 1.0 and 2.0 percent but complete inhibition of growth at 3 percent.Turmeric and cotton could not control the growth of *Aspergillus* at 1.0%, but partially inhibited the growth at 2.0 and 3.0% concentration in culture medium (Table 2).

Efficacy of fungicides/plant products in controlling *Aspergillus* infection in silkworm :

Only six fungicides (Salcylic acid, bavistin, bayleton, dithane M-45, captan and benzoic acid denoted by F1, F2, F3, F4, F5, and F6 respectively) based on in vitro study were tried for controlling Aspergillosis in silkworm. The larval mortality with *Aspergillus* infection in different treatments is presented in Tables 3 and 4.

The lowest mortality (5.33 ± 0.33 to 8.00 ± 0.57, 3.66 ± 0.33 to 6.66 ± 0.66, 1.33 ± 0.88 to 4.33 ± 0.66% in I, II and III instars respectively) with infection of *Aspergillus flavus* and (0.0 to 1.00 ± 0.57 % in I to III instar) with infection of *Aspergillus tamarii* was recorded when the larvae were dusted with 1.0, 1.5 and 2.0% of salcylic acid in inert kaolin powder followed by bavistin (14.33 ± 1.20 to 20.00 ± 1.15, 4.66 ± 1.20 to 10.66 ± 0.66, 3.00 ± 0.57 to 7.33 ± 1.45% in I, II and III instars respectively with *Aspergillus flavus* and (7.33 ± 0.66 to 11.00 ± 0.57, 4.00 ± 0.57 to 7.33 ± 0.66 and 2.00 ± 0.57 to 5.66 ± 0.33 % in I, II and III instars respectively) with *A. tamarii*. The highest mortality (48.00 ± 1.15 to 62.00 ± 1.52, 32.30 ± 1.45 to 35.33 ± 1.76, 14.30 ± 0.88 to 20.33 ± 1.76%) with *Aspergillus flavus* infection and (37.66 ± 1.45 to 43.00 ± 2.51, 27.33 ± 1.76 to 33.33 ± 1.76, 12.66 ± 1.20 to 18.33 ± 1.45% with *A.tamarii* infection was recorded in the silkworms dusted with 1.0, 1.5 and 2.0% Benzoic acid. Mortality range in the silkworms treated with captan, dithane M-45, and bayletone was between the mortality of bavestin and benzoic acid treated larvae in both the cases of *Aspergillus flavus* and *A. tamarii* (Table 3 and 4).

Out of ten, only six plants (Hena, garlic, mango bark, tomato, cotton and turmeric denoted by P1, P2, P3, P4, P5 and P6 respectively were selected based on *in vitro* screening for in vivo efficacy in silkworm. The percentage of mortality in silkworm with *Aspergillus* infection and plant products treatment are presented in Table 5 and 6. Among 6 plants Hena at 2.0, 4.0 and 6.0% resulted in lowest mortality (9.00 ± 1.00 to 13.66 ± 0.88, 6.00 ± 0.57 to 10.33 ± 0.88 and 4.66 ± 0.33 to 7.66 ± 0.88% in I, II and III instars respectively) in silkworm with *Aspergillus flavus* and 6.33 ± 0.88 to 10.33 ± 0.88, 5.00 ± 0.57 to 9.00 ± 0.57 and 4.00 ± 0.57 to 7.00 ± 1.52% in I, II and III instars with *A. tamarii* infection followed by mango bark, garlic, tomato and cotton and highest mortality of 34.33 ± 1.20 to 35.66 ± 1.20, 27.00 ± 1.76 to 29.33 ± 1.76 and 16.33 ± 1.33 to 20.33 ± 2.90% with *Aspergillus flavus* infection and 25.00 ± 1.15 to 29.00 ± 1.45, 25.66 ± 2.33 to 29.33 ± 1.33 and 14.00 ± 1.00 to 18.66 ± 2.33% with *A. tamarii* infection in I, II and III instars silkworms respectively was recorded

Table 3. Mortality in silkworm, *Bombyx mori* L. after *Aspergillus* infection (1×10^7) and fungicides treatments

Factor	Mortality (%) in silkworm with infection of					
	Aspergillus flavus			*Aspergillus tamarii*		
	I instar	II instar	III instar	I instar	II instar	III instar
Treatment F1	6.55 ± 0.44	5.44 ± 0.53	2.88 ± 0.56	0.55 ± 0.24	0.00 ± 0.00	0.00 ± 0.00
F2	16.88 ± 0.99	7.33 ± 0.99	4.77 ± 0.81	9.00 ± 0.62	5.55 ± 0.60	3.66 ± 0.57
F3	21.55 ± 1.33	8.66 ± 1.06	7.00 ± 0.92	14.55 ± 0.81	7.66 ± 0.89	6.11 ± 0.96
F4	24.15 ± 0.97	8.77 ± 1.06	8.22 ± 0.79	10.22 ± 0.52	8.00 ± 0.76	7.33 ± 0.89
F5	54.22 ± 2.17	34.33 ± 1.30	16.44 ± 1.14	40.22 ± 1.22	30.88 ± 1.26	16.00 ± 0.91
F6	35.55 ± 1.95	34.88 ± 0.93	21.00 ± 1.20	24.00 ± 1.06	30.33 ± 1.00	18.22 ± 1.34
Inoculated control	68.66 ± 0.98	52.00 ± 1.73	36.66 ± 2.40	58.66 ± 2.99	44.66 ± 2.60	30.00 ± 2.60
Conc.C1 (1.00%)	30.77 ± 4.22	19.50 ± 3.21	11.94 ± 1.76	18.44 ± 3.30	15.83 ± 3.09	10.27 ± 1.80
C2 (1.5%)	25.83 ± 3.57	17.00 ± 3.42	9.77 ± 1.54	16.27 ± 3.07	13.66 ± 3.10	8.22 ± 1.62
C3 (2.00%)	23.05 ± 3.27	17.83 ± 4.37	8.44 ± 1.67	14.55 ± 2.95	11.72 ± 2.76	7.16 ± 1.53
F1 X C1	8.00 ± 0.57	6.66 ± 0.66	4.33 ± 0.66	1.00 ± 0.57	0.00 ± 0.00	0.00 ± 0.00
F1 X C2	6.33 ± 0.33	5.33 ± 0.57	3.00 ± 0.57	0.66 ± 0.33	0.00 ± 0.00	0.00 ± 0.00
F1 X C3	5.33 ± 0.33	3.66 ± 0.33	1.33 ± 0.88	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
F2 X C1	20.00 ± 1.15	10.66 ± 0.66	7.33 ± 1.45	11.00 ± 0.57	7.33 ± 0.66	5.66 ± 0.33
F2 X C2	16.33 ± 0.88	6.66 ± 0.66	4.00 ± 0.57	8.66 ± 0.66	5.33 ± 0.88	3.33 ± 0.33
F2 X C3	14.33 ± 1.20	4.66 ± 1.20	3.00 ± 0.57	7.33 ± 0.66	4.00 ± 0.57	2.00 ± 0.57
F3 X C1	25.00 ± 2.88	12.00 ± 2.00	10.33 ± 2.00	16.66 ± 0.88	11.00 ± 1.00	8.66 ± 2.02
F3 X C2	18.66 ± 1.52	8.33 ± 0.33	8.33 ± 2.00	15.00 ± 0.57	6.33 ± 0.33	5.33 ± 0.66
F3 X C3	15.66 ± 0.66	6.33 ± 0.88	5.60 ± 0.66	12.00 ± 1.15	5.66 ± 0.33	4.00 ± 1.00
F4 X C1	27.33 ± 1.45	12.00 ± 1.57	10.00 ± 1.00	11.66 ± 0.88	10.66 ± 0.66	9.00 ± 1.52
F4 X C2	24.33 ± 0.88	8.00 ± 0.57	8.33 ± 1.20	10.00 ± 0.57	7.66 ± 0.33	7.00 ± 2.00
F4 X C3	22.00 ± 1.15	7.30 ± 0.33	6.33 ± 1.33	8.66 ± 0.57	5.66 ± 0.33	6.00 ± 1.00
F5 X C1	62.00 ± 1.52	35.33 ± 1.76	20.33 ± 1.76	43.00 ± 2.51	33.33 ± 1.76	18.33 ± 1.45
F5 X C2	52.66 ± 1.45	35.30 ± 1.33	16.66 ± 0.66	40.00 ± 1.52	32.00 ± 1.73	15.00 ± 1.50
F5 X C3	48.00 ± 1.15	32.30 ± 1.45	14.30 ± 0.88	37.66 ± 1.45	27.33 ± 1.76	12.66 ± 1.20
F6 X C1	42.33 ± 1.45	37.00 ± 1.52	22.66 ± 1.20	27.33 ± 1.00	32.66 ± 1.45	20.66 ± 3.46
F6 X C2	34.33 ± 1.76	35.00 ± 0.57	20.33 ± 2.33	23.33 ± 1.45	30.66 ± 1.45	18.33 ± 2.02
F6 X C3	30.00 ± 1.15	32.66 ± 1.76	20.00 ± 2.88	21.33 ± 0.88	27.66 ± 1.20	16.66 ± 1.45
Inoculated control	68.66 ± 0.88	52.00 ± 1.73	52.00 ± 1.73	58.66 ± 2.96	44.66 ± 2.60	30.00 ± 2.60
Fungicide CD 5%	2.20	2.15	2.36	1.77	1.67	2.39
Conc. CD 5%	1.55	1.35	1.67	1.25	1.18	1.69
Interaction CD 5%	3.81	3.79	4.09	3.07	2.89	4.14

Table 4. Anova - for mortality in silkworm, *Bombyx mori* L. after *Aspergillus* infection (1×10^7) and fungicides treatment

Source of variation	Mean sum of square					
	Aspergillus flavus			*Aspergillus tamarii*		
	I instar	II instar	III instar	I instar	II instar	III instar
Treatments	2464.00**	1787.28**	454.52**	1750.72**	1611.05**	456.17**
Conc.	275.38**	29.16 N.S.	56.16**	68.35**	76.12**	45.05**
Treatments x Conc	15.38**	79.85 N.S.	4.38 N.S.	2.88 N.S.	5.08 N.S.	2.30 N.S.
Treatments x Control	5040.03**	3264.03*	2012.64*	5071.11*	2718.22*	1347.92*

when the larvae dusted with 2.0, 4.0 and 6.0% turmeric powder (Table 5 and 6).

Inoculated and normal controls for both fungicides and plant products treatments were same. The mortality in inoculated control was 36.66 ± 2.40 to 68.66 ± 0.88% with the infection of *Aspergillus flavus* and 30.30 ± 2.60 to 58.66 ± 2.96% with the infection of *A. tamarii* in I, II and III instar larvae. In normal control no mortality was recorded.

Table 5. Mortality in silkworm, *Bombyx mori* L. after *Aspergillus* infection (1×10^7) and treatment with plant products

Factor	Mortality (%) in silkworm with infection of					
	Aspergillus flavus			*Aspergillus tamarii*		
	I instar	II instar	III instar	I instar	II instar	III instar
Treatment P1	11.66 ± 0.83	8.11 ± 0.71	6.22 ± 0.57	8.11 ± 0.73	6.88 ± 0.63	5.44 ± 0.74
P2	22.55 ± 0.95	14.11 ± 0.88	9.88 ± 0.80	13.55 ± 1.13	8.88 ± 0.84	7.33 ± 0.74
P3	18.00 ± 0.86	9.77 ± 0.82	6.22 ± 0.74	13.66 ± 1.17	5.00 ± 0.76	5.55 ± 0.06
P4	34.33 ± 1.55	26.77 ± 0.77	15.44 ± 1.40	24.55 ± 1.22	21.66 ± 1.01	11.33 ± 0.95
P5	36.44 ± 1.60	27.22 ± 0.64	17.55 ± 1.09	27.00 ± 1.38	24.00 ± 0.88	13.77 ± 1.07
P6	36.55 ± 1.02	29.44 ± 0.91	18.88 ± 1.33	28.55 ± 1.21	27.33 ± 0.98	16.00 ± 1.13
Inoculated control	68.66 ± 0.88	52.00 ± 1.73	36.66 ± 2.40	58.66 ± 2.96	44.66 ± 2.60	30.00 ± 2.60
Conc. C1 (2.00%)	29.11 ± 2.49	20.55 ± 1.98	14.33 ± 1.52	22.88 ± 2.09	17.66 ± 2.18	11.77 ± 1.21
C2 (3.00%)	26.77 ± 2.42	19.22 ± 2.07	12.27 ± 1.36	18.94 ± 1.82	15.27 ± 2.07	9.83 ± 1.05
C3 (4.00%)	23.88 ± 2.35	17.94 ± 2.46	10.50 ± 1.28	15.88 ± 1.84	13.94 ± 2.36	8.16 ± 1.06
P1 X C1	13.66 ± 0.88	10.33 ± 0.88	7.66 ± 0.88	10.33 ± 0.88	9.00 ± 0.57	7.00 ± 1.52
P1 X C2	12.30 ± 0.88	8.00 ± 0.57	6.33 ± 0.88	7.66 ± 0.88	6.66 ± 0.33	5.33 ± 1.33
P1 X C3	9.00 ± 1.00	6.00 ± 0.57	4.66 ± 0.33	6.33 ± 0.88	5.00 ± 0.57	4.00 ± 0.57
P2 X C1	25.00 ± 1.15	16.66 ± 0.88	11.00 ± 1.00	16.66 ± 0.66	11.33 ± 0.66	9.00 ± 1.00
P2 X C2	23.00 ± 1.52	14.66 ± 0.33	10.66 ± 1.76	14.66 ± 0.33	9.33 ± 0.66	8.00 ± 1.15
P2 X C3	19.66 ± 0.33	11.00 ± 0.57	8.66 ± 1.00	9.33 ± 0.66	6.00 ± 0.57	5.00 ± 0.57
P3 X C1	20.33 ± 1.45	11.33 ± 1.33	7.66 ± 0.66	17.33 ± 0.66	6.33 ± 0.66	7.33 ± 0.66
P3 X C2	17.66 ± 0.88	10.66 ± 0.66	6.33 ± 1.85	13.66 ± 1.20	5.33 ± 1.33	5.33 ± 1.20
P3 X C3	16.00 ± 1.15	7.33 ± 1.20	4.66 ± 0.66	10.00 ± 1.15	3.00 ± 1.15	4.00 ± 0.57
P4 X C1	38.00 ± 1.15	27.33 ± 0.66	19.33 ± 2.33	28.66 ± 1.76	25.00 ± 1.52	13.00 ± 2.08
P4 X C2	32.00 ± 1.15	26.33 ± 1.85	14.00 ± 1.00	23.33 ± 0.88	20.66 ± 0.66	11.33 ± 1.33
P4 X C3	30.00 ± 1.73	26.66 ± 1.76	12.66 ± 1.85	21.66 ± 0.88	19.33 ± 0.88	9.66 ± 1.45
P5 X C1	42.00 ± 1.15	28.33 ± 0.88	19.66 ± 2.33	32.00 ± 1.15	24.66 ± 0.33	15.33 ± 2.33
P5 X C2	35.00 ± 0.57	27.00 ± 1.52	16.33 ± 2.02	26.00 ± 0.57	22.66 ± 1.45	13.66 ± 1.45
P5 X C3	31.00 ± 0.88	26.33 ± 0.88	16.00 ± 1.20	23.00 ± 0.57	24.66 ± 2.40	12.33 ± 2.02
P6 X C1	35.66 ± 1.20	29.33 ± 1.76	20.33 ± 2.90	29.00 ± 1.45	29.33 ± 1.33	18.66 ± 2.33
P6 X C2	39.66 ± 1.45	28.66 ± 1.76	20.00 ± 2.51	28.33 ± 0.88	27.00 ± 1.00	15.33 ± 1.76
P6 X C3	34.66 ± 1.20	27.00 ± 1.76	16.33 ± 1.33	25.00 ± 1.15	25.66 ± 2.33	14.00 ± 1.00
Inoculated control	68.66 ± 0.88	52.00 ± 1.73	36.66 ± 2.40	58.66 ± 2.96	44.66 ± 2.60	30.00 ± 2.60
Plants CD 5%	1.89	2.01	2.71	1.63	1.96	2.43
Conc. CD 5%	1.34	1.42	1.92	1.15	1.38	1.17
Interaction 5%	3.28	3.48	4.71	2.82	3.40	4.20

Table 6. Anova for mortality in silkworm, *Bombyx mori* L. after *Aspergillus* infection (1×10^7) and treatment with Plant Products

Source of variation	Mean sum of square					
	Aspergillus flavus			*Aspergillus tamarii*		
	I instar	II instar	III instar	I instar	II instar	III instar
Treatments	1024.47**	835.84**	289.05**	652.46**	860.96**	179.30**
Conc	123.18**	30.68**	66.24**	221.68**	64.01**	56.96**
Treatments x Conc	14.03**	5.59 N.S.	4.35 N.S.	3.55 N.S.	4.17 N.S.	0.74 N.S.
Treatment x Control	5031.17*	3051.05*	1677.72*	4417.77*	2396.31*	1185.77*

Discussion

Among seven fungicides tested for their efficacy *in vitro* against *Aspergillus flavus* and *A. tamarii*, salcylic acid and bavistin were noted to be very effective as no growth of *Aspergillus* was observed at all the concentrations tested. These present observations are in agreement with Krishnaprasad *et al.* (1978), Chinnaswamy and Devaiah (1986),

Anitha Petter (1988) where they have stated that bavistin inhibited 100% growth of *Aspergillus*. Kawakami (1973b) reported 66.0% survival of silkworms after dusting of salicylic acid against *Aspergillus flavus*. Benjoic acid was least effective and formaldehyde was not effective in present study which is similar to the results of Wadee *et al.* (1972) who reported the resistance in *Aspergillus* species to formalin. Kawakami and Mikuni (1969, 1973) observed both resistance and susceptibility among different isolates of *Aspergillus* species to formalin. Similarly Manjunathan Gowda (1994) observed the resistance of *Aspergillus tamarii* to formalin and benzoic acid.

Other fungicides i.e. bayleton, dithane M-45 and captan were partially effective in *in vitro* as the growth of *Aspergillus* was observed in some of the replications. Similarly Chinnaswamy and Devaiah (1986) found bayleton and dithane M-45 less effective as he has noted less zone inhibition percentage of the *Aspergillus* fungus. Manjunathan Gowda (1994) reported that dithane M-45 and captan inhibited the growth of *Aspergillus tamarii*.

Among ten plants used in *in vitro* efficacy, Hena leaf, garlic and mango bark found to be very effective in controlling the growth of *Aspergillus*. Tomato and cotton leaf were partially effective. However, information on use of these plants against *Aspergillus* is not available. All the plants used in the present study are either antifungal or antibiotic (Sanyal, 1924; Dey, 1980; Agarwal and Barin Ghosh, 1989; Kaushik and Dhiman, 2000).

Though, all the treatments of fungicides tested in *in vivo* have shown significantly ($p < 0.01$) lower mortality due to Aspergillosis in silkworm than inoculated control. But the mortality in silkworm was lowest when the silkworms were treated with different concentrations of salicylic acid and followed by bavistin (Table 3 and 4). These results are more or less similar to the results of Kawakami (1973b) who found 92.0 to 100.0% survival of silkworms wih organosulfurous fungicides and 66.0% survival with salcylic acid. Chinnaswamy and Devaiah (1986) and Anitha Peter (1988) noted comparatively higher larval survival with bavistin treatment. Dithane M-45, benzoic acid and bayleton were not effective in present study as the larval mortality was higher. Similarly Chinnaswamy and Devaiah (1986) found dithan M-45 not to be effective in controlling *Aspergillus* in silkworm, but recorded good larval survival in bayleton treated silkworms. Anitha Peter (1988) tested five fungicides for their efficacy in controlling *Aspergillus* in silkworm and found benzoic acid and dithane M-45 less effective.

Six plants were tested for their efficacy against *Aspergillus* and *A. tamarii* in present investigation. All the treatments of plant products have shown significantly lowere mortality in silkworm due to Aspergillosis than inoculated control, but it was significantly ($P < 0.01$) lowest when the silkworm dusted with Hena leaf powder and followed by mango bark powder and garlic at all concentrations tested (Table 3 and 4). The plants used in present investigation have antifungal and antibiotic properties (Sanyal, 1924; Dey, 1980; Agarwal and Barin Ghosh, 1989; Kaushik and Dhiman, 2000). However, their antifungal action on *Aspergillus* disease in silkworm has not reported in the past.

The treatments of fungicides and plant products and different concentration of them were significantly different ($P < 0.01$) at I, II and III instar in case of both *Aspergillus flavus* an *A tamarii* infection in silkworm. However the interaction of treatment and concentration was significantly different at I instar than II and III instar in case of both *Aspergillus flavus* and *A. tamarii* infection in silkworm (Table 2 and 4).

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