Int. J. Indust. Entomol. Vol. 15, No. 2, 2007, pp. 101~106

International Journal of
Industrial Entomology

Efficacy of Pesticides and Growth Hormones against Root Disease Complex of Mulberry (*Morus alba* L.)

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(Received 30 October 2007; Accepted 22 November 2007)

During mulberry cultivation, root disease complex caused by the association of root knot nematode (*Meloidogyne incognita*) with root rot pathogens like *Fusarium solani* and *Botryodiplodia theobromae* poses serious loss in leaf production. Therefore, an attempt was made to assess the efficacy of eight pesticides (Metayalaxyl+Mancozeb, Thiophanate methyl, Mancozeb, Bitertanol, Phenomiphos, Phorate, Thionazin & Carbofuran) and two growth hormones (Salicylic acid and Indole 3 acetic acid) at 0.1 and 0.2% concentrations under *in vitro* conditions against nematode (hatching of eggs and mortality of larvae) and root rot pathogens (poisoned food technique) for short listing the treatments to develop an IDM strategy. Results revealed that among the pesticides and growth hormones, Carbofuran followed by Salicylic acid were found to be effective at 0.2% concentration against both nematode and pathogenic fungi. Both the chemicals inhibited the hatching of nematode eggs by 83.5-78.9% and 80-76% larval mortality over the control and reduced the mycelial growth of both the pathogenic fungi to an extent of 75.5-77.8%. Though Mancozeb inhibited both the pathogenic fungi strongly (77 -80%), it did not show any effectiveness against nematode. The rest of the chemicals were found either moderately or poorly effective in reducing the growth of pathogenic fungi, hatching of nematode eggs and enhancing the mortality of larvae. The two effective chemicals *viz.*, Carbofuran and Salicylic acid, which rated as strong inhibitors against both nematode and pathogenic fungi, can be exploited in developing an IDM package as one of the component for better management of root disease complex in mulberry.

Key words: Pesticides, Mulberry, Root disease complex, Root knot nematode, Pathogenic fungi.

Introduction

Mulberry (*Morus alba* L.) a sole food plant of silkworms (*Bombyx mori* L) is affected by two major soilborne diseases *viz*., root knot (*Meloidogyne incognita*) and root rot (*Fusarium solani* and *Botryodiplodia theobromae*), which affect the established plantations resulting in severe leaf yield loss up to 20% besides altering the nutritional status of the leaf (Sharma *et al*., 2003; Sharma and Gupta, 2005). Recently, root disease complex [association of root knot nematode (*M. incognita*) with root rot pathogens like *Fusarium solani* and *B. theobromae*] is a major production constraint during mulberry cultivation resulting in leaf yield loss to an extent of 15% (Naik and Sharma, 2004; Naik, 2006). Reports on the management of this disease are negligible (Sharma *et al*., 2003; Sharma and Gupta, 2005).

Furthermore, to obtain good quality mulberry leaf, it is essential to manage the array of pathogens that infest mulberry crop and the interactions among them. Till today, chemicals are being used as the most common component especially for soilborne diseases for developing an Integrated Disease Management package (Nehra, 2005). Various nematicides/fungicides have been tested against root disease complex in certain agricultural/horticultural crops and successfully reduced the severity of disease and improving the plant growth characters (Verma, 1993; Poornima and Vadivelu, 1993; Singh and Goswami, 2001). Besides, growth hormones are not only known to produce the pathogenesis related (PR) proteins such as chitinase, B-1 and 3-glucanase, which induce systemic resistance in plants against various foliar and soilborne

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pathogens but also inhibited the pathogens growth to a great extent (Yalpani *et al.*, 1993; Klessig and Malamy, 1994; Dann *et al.*, 1996; Nandi *et al.*, 2003). Hence, the present study was under taken to find out the efficacy of eight pesticides and two growth hormones against pathogens causing root disease complex *viz.,* nematode (hatching of eggs and mortality of larvae) and root rot pathogens like *B. theobromae* and *F. solani* (poisoned food technique) under *in vitro* conditions using as one of the component in developing an Integrated Disease Management strategy.

Materials and methods

Four fungicides such as Dithomyl (Metayalaxyl+Mancozeb; 8+64=72% WP), Roko (Thiophanate methyl 70% WP), Dithane M-45 (Mancozeb 75% WP) and Baycor (Bitertanol 25% WP), four nematicides like Namacure (Phenomiphos 10 G), Thimet (Phorate 10 G), Nemaphos (Thionazin 10 G) and Furadan (Carbofuran 3 G) and two growth hormones (Salicylic acid and Indole 3 acetic acid) at 0.1 and 0.2% concentrations were tested under *in vitro* conditions against pathogens causing root disease complex *viz., M. incognita* (hatching of eggs and mortality of larvae) and pathogenic fungi (*B. theobromae* and *F. solani*) by poisoned food technique to assess their efficacy. Different concentration of each fungicide/nematicide was prepared using the following formula.

$$D = \frac{A \times B}{C}$$

Where, A : Required concentration (%) of the fungicide/ nematicide
B : Required quantity of solution
C : % Active ingredient available in the formulation
D : Required quantity of the product

The concentrations of growth hormones (Salicylic acid and Indole-3-acetic acid) like 0.1 and 0.2% were obtained by suitable dilution of respective appropriate concentration of stock solutions.

Testing against nematode

To study the effect of chemicals on ovicidal action, four uniform size matured egg masses (1500 eggs) were suspended in each glass cavity block containing 5 ml solution of 0.1 and 0.2% concentrations of different chemicals along with a control (distilled water) and kept for incubation at 28±2°C for 10 days. After incubation, the hatched larvae were counted every alternate day. The hatching percentage and percentage inhibition over the control were calculated and categorized in to following 5 grades (Naik, 2006).

Grade	Inhibition of pathogens	Degree of effectiveness
I	Nil	Non inhibitor
II	00.1 - 025%	Poor
III	25.1 - 050%	Moderate
IV	50.1 - 075%	Good
V	75.1 - 100%	Strong

For studying the larvicidal action, several egg masses were kept in glass cavity blocks containing 5 ml of sterile distilled water. The cavity blocks were incubated at 28± 2°C overnight for hatching. 100 freshly hatched larvae were added to 5 ml solution of 0.1 and 0.2% chemicals separately in three replications along with control (larvae in distilled water) for comparison. Observations were recorded on mortality of larvae after 48 and 72 hrs in terms of dead larvae to calculate the mortality percentage. The larval mortality was judged by transferring the immobilized larvae into distilled water for an hour to confirm their death. The degree of effectiveness of each chemical was categorized into 1-5 grades.

Testing against pathogenic fungi

The inhibitory effect of chemicals on mycelial growth of both the pathogenic fungi (*B. theobromae* and *F. solani*) was assayed by poisoned food technique (Sharvelle, 1961). For this, the calculated quantity of each chemical at 0.1 and 0.2% concentrations were mixed in 100 ml of lukewarm Potato Dextrose Agar medium separately and poured into the Petriplates under aseptic condition. A four mm mycelial disc from 7 days old culture of both the pathogenic fungi was placed at the center of the plate separately for each chemical and concentration. A control was also maintained without amendment of chemical. Three replicates were maintained for each treatment. The plates were incubated at 28±2°C for 7 days. After incubation, the effectiveness of each chemical was determined measuring the radial growth (mm) of the fungal pathogens in the treated plates and comparing with the growth in the control. Inhibition percent was calculated by the formula of Vincent (1947) and effectiveness of each chemical was categorized into 1-5 grades.

$$\text{Inhibition (\%)} = \frac{\text{Radial growth in control(mm)} - \text{Radial growth in treated}}{\text{Radial growth in control}}$$

Results

The results on ovicidal (hatching of *M. incognita* eggs) and larvicidal (mortality of larvae) properties of chemicals

Table 1. Comparative efficacy of different chemicals on ovicidal action against *M. incognita* (hatching of eggs) as assessed under *in vitro* conditions

Sl. No.	Chemicals	Concen-tration (%)	No of larvae hatched /1500 eggs on Days after					Total larvae hatched	Average hatched larvae	Inhibition over control (%)	Inhibition grade
			2	4	6	8	10				
1	Control (D.W.)	--	520	314	256	164	110	1364	272.8	-	-
2	Dithomyl (Metayalaxyl + Mancozeb)	0.1	275	220	199	176	143	1013	202.6	25.7	III
		0.2	256	205	187	169	82	899	179.8	34.1	III
3	Roko (Thioph-anate methyl)	0.1	262	228	190	169	133	982	196.4	28.0	III
		0.2	255	207	173	138	103	876	175.2	35.8	III
4	Dithane M-45 (Mancozeb)	0.1	242	209	190	164	137	942	188.4	30.9	III
		0.2	222	199	173	156	100	850	170.0	37.7	III
5	Baycor (Bitertanol)	0.1	302	257	206	154	58	977	195.4	28.4	III
		0.2	288	258	189	148	35	918	183.6	32.7	III
6	Nemacure (Phenomiphos)	0.1	198	162	120	98	85	663	132.6	51.4	IV
		0.2	152	132	108	72	35	499	99.8	63.4	IV
7	Thimet (Phorate)	0.1	171	145	106	88	63	573	114.6	58.0	IV
		0.2	140	110	89	50	34	423	84.6	69.0	IV
8	Nemaphos (Thionazin)	0.1	174	108	83	69	61	495	99.0	63.7	IV
		0.2	147	100	76	54	31	408	81.6	70.1	IV
9	Furadan (Carbofuran)	0.1	185	152	65	0	0	402	80.4	70.5	IV
		0.2	121	68	36	0	0	225	45.0	83.5	V
10	Salicylic acid	0.1	198	175	96	0	0	469	93.8	65.6	IV
		0.2	127	89	72	0	0	288	57.6	78.9	V
11	Indole 3 acetic acid	0.1	196	160	126	109	88	679	135.8	50.2	IV
		0.2	172	151	111	84	58	576	115.2	57.8	IV

	SE ±	CD at 5 %
Days (A)	0.45	1.26
Treatments (B)	0.67	1.88
Concentration (C)	0.28	0.80
A × B × C	2.14	4.19
CV %	2.56	

against nematode presented in Tables 1 & 2 clearly indicate that the concentration of 0.2% was found to be more toxic than 0.1%. Among all the chemicals, Carbofuran (nematicide) and Salicylic acid (growth hormone) were able to suppress the hatching of *M. incognita* eggs to a maximum extent of 83.5 and 78.9%, respectively at 0.2% concentration over the control. In both the cases, the maximum hatching was noticed on the second day and minimum on the sixth day (Table 1). Further, these two chemicals also caused higher larval mortality upto 80.0 and 76.0%, respectively (Table 2) at 0.2% concentration after 72 hrs of exposure. Though there was a significant difference between Carbofuran and Salicylic acid with respect to suppression of hatching and increase in larval mortality, they showed strong nematicidal activity and based on the extent of inhibition they were rated under class V as 'strong inhibitors'. The rest of the chemicals exhibited moderate to good nematicidal properties in suppression of egg hatching and increasing larval mortality. They were rated as 'moderate' to 'good' inhibitors belonging to classes III and IV. However, among the 3 chemicals, Dithane M-45 was not found effective against nematode.

With regards to the pathogenic fungi (*B. theobromae* and *F. solani*), among all the chemicals under study, Mancozeb, Carbofuran and Salicylic acid inflicted maximum inhibition of mycelial growth at 0.2% concentration (Table 3). They inhibited the mycelial growth of *B. theobromae* by 77.0, 76.0 and 75.9% while in the case of *F. solani*, the growth was inflicted to an extent of 80.0, 77.8 and 75.5%, respectively over the control. The values of the above three chemicals did not show any significant

Table 2. Comparative efficacy of different chemicals on larvicidal action (larval mortality) against *M. incognita* as assessed under *in vitro* conditions

Sl. no.	Chemicals	Concentration (%)	Duration			
			Mortality after 48 hrs		Mortality after 72 hrs	
			Mortality (%)	Inhibition grade	Mortality (%)	Inhibition grade
1	Control (D.W.)	-	7.0	-	9.0	-
2	Dithomyl (Metayalaxyl + Mancozeb)	0.1	19.0	II	26.0	III
		0.2	28.0	III	35.0	III
3	Roko (Thiophanate methyl)	0.1	20.0	II	26.0	III
		0.2	30.0	III	37.0	III
4	Dithane M-45 (Mancozeb)	0.1	21.0	II	28.0	III
		0.2	32.0	III	36.0	III
5	Baycor (Bitertanol)	0.1	17.0	II	23.0	III
		0.2	25.0	II	30.0	III
6	Nemacure (Phenomiphos)	0.1	33.0	III	52.0	IV
		0.2	41.0	III	58.0	IV
7	Thimet (Phorate)	0.1	37.0	III	56.0	IV
		0.2	48.0	III	67.0	IV
8	Nemaphos (Thionazin)	0.1	37.0	III	54.0	IV
		0.2	46.0	III	63.0	IV
9	Furadan (Carbofuran)	0.1	55.0	IV	68.0	IV
		0.2	69.0	IV	80.0	V
10	Salicylic acid	0.1	48.0	III	52.0	IV
		0.2	52.0	IV	76.0	V
11	Indole 3 acetic acid	0.1	34.0	III	46.0	III
		0.2	45.0	III	54.0	IV

	SE ±	CD at 5%
Treatments (A)	0.44	1.26
Duration (B)	0.18	0.54
Concentration (C)	0.22	0.63
A × B × C	1.04	2.09
CV %	4.56	

difference. Thus, these three chemicals proved their strong fungicidal activity and can be grouped under class V as 'strong inhibitors'. The remaining chemicals were effective only as 'moderate' to 'good' against both the pathogenic fungi under classes III and IV. However, among the above three chemicals, Mancozeb was not found effective against nematode.

Discussion

In the present investigation, among the 10 chemicals screened, Mancozeb had the maximum and strong fungicidal efficacy against both the pathogenic fungi (*B. theobromae* and *F. solani*). However, it did not exhibit any strong activity against nematode, which clearly proves its fungicidal action only. Whereas, Carbofuran being a nematicide/insecticide and Salicylic acid being a growth hormone, strongly inhibited the nematode and both the pathogenic fungi indicating that having anti nematode and anti fungal properties. Carbofuran prepared by treating by 2,3-dihydro-2, 2-dimethyl-7-benzofuranol with methyl isocynate, might be toxic to both nematode and pathogenic fungi for inhibiting them. Various workers have reported the effectiveness of Carbofuran against root knot nematode in mulberry (Govindaiah and Sharma, 1994; Sharma *et al*., 1998; 2001; Ramakrishnan and Senthilkumar, 2003) and is also used for the control of disease complex caused by the association of nematode and soilborne fungal pathogens in certain crops (Sitaramaiah and Kumari, 1997; Mahapatra and Swain, 1999; Singh and Goswami, 2001; Haseeb *et al*., 2005; Shreenivasa *et al*., 2005). The present findings are also in conformity with the results of various workers who reported that certain

Table 3. Effect of different chemicals on pathogenic fungi as assessed under *in vitro* conditions

Sl. no.	Chemicals	Concentration (%)	Radial growth of the pathogenic fungi (mm)					
			B. theobromae			*F.soani*		
			Growth	Decrease (%)	Inhibition Grade	Growth	Decre-ase (%)	Inhibition Grade
1	Control (D.W.)	-	90.00	-	-	90.00	-	-
2	Dithomyl (Metayalaxyl + Mancozeb)	0.1	57.15	36.5	III	47.40	47.3	III
		0.2	47.67	47.0	III	37.35	58.5	IV
3	Roko (Thiophanate methyl)	0.1	57.95	35.6	III	60.15	33.2	III
		0.2	48.00	46.6	III	50.99	43.3	III
4	Dithane M-45 (Mancozeb)	0.1	27.90	69.0	IV	31.45	65.1	IV
		0.2	20.70	77.0	V	18.00	80.0	V
5	Baycor (Bitertanol)	0.1	65.40	27.3	III	63.59	29.3	III
		0.2	55.60	38.2	III	53.25	40.8	III
6	Nemacure (Phenomiphos)	0.1	65.71	26.9	III	55.42	38.4	III
		0.2	60.15	33.2	III	45.90	49.0	III
7	Thimet (Phorate)	0.1	63.15	29.8	III	66.56	26.0	III
		0.2	53.50	40.5	III	57.95	35.6	III
8	Nemaphos (Thionazin)	0.1	66.73	25.9	III	60.75	32.5	III
		0.2	62.42	30.6	III	53.35	40.7	III
9	Furadan (Carbofuran)	0.1	30.60	66.0	IV	32.99	63.3	IV
		0.2	21.60	76.0	V	19.95	77.8	V
10	Salicylic acid	0.1	33.80	62.4	IV	35.25	60.8	IV
		0.2	21.65	75.9	V	22.00	75.5	V
11	Indole 3 acetic acid	0.1	47.85	46.8	III	59.40	34.0	III
		0.2	41.65	53.7	IV	51.10	43.2	III

	SE ±	CD at 5%	SE ±	CD at 5%
Concentration (A)	0.44	1.26	0.44	1.26
Treatment (B)	0.18	0.53	0.23	0.67
A × B	0.62	1.78	0.78	2.24
CV %	2.11		2.72	

phytohormones/ chemical elicitors were found to be effective in reducing the growth and incidence of many fungal and nematode diseases/pathogens in certain crops including mulberry (Reuveni *et al*., 1994; Subramanian and Kumar, 1997; Vidhyasekaran, 1998; Nandi *et al*., 2000; Sahu, 2003).

Thus, the above observations under *in vitro* studies suggest that chemicals *viz*., Carbofuran and Salicylic acid exhibited strong inhibitory properties against nematode and root rot fungi, can be utilized as one of component for further refinement to formulate an integrated technology to manage the root disease complex in mulberry.

Acknowledgements

The authors are highly thankful Mrs. M. Rekha, Scientist-C (Statistics), CSRTI, Mysore for rendering help in statistical analysis.

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