Int. J. Indust. Entomol. Vol. 14, No. 1, 2007, pp. 57~61

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International Journal of
Industrial Entomology

Screening of Botanicals Against Root Knot Disease Complex in Mulberry (*Morus indica* L.)

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(Received 8 August 2006; Accepted 27 October 2006)

For developing an integrated eco-friendly package against root knot disease complex of mulberry caused by the association of *Meloidogyne incognita* with *Fusarium solani* and *F. oxysporum* causing serious loss in terms of leaf yield and quality during cultivation, twenty botanical extracts at 5, 10 & 20% concentrations were screened under *in vitro* conditions. Among the extracts, *Allium sativum* followed by *Lasownia inermis* were found to be effective at 20% concentration against both the virulent fungi and nematode. Both the extracts reduced the mycelial growth of virulent fungi to an extent of 76-100%, inhibited the hatching of nematode eggs by 80-90% and 76-85% larval mortality over the control. The other extracts were found either moderately or poorly effective in reducing the growth of fungi, hatching of nematode eggs and enhancing the mortality of larvae. The two effective botanical extracts, which rated as strong inhibitors against both nematode and virulent fungi, can be utilized in developing an integrated ecofriendly technology for better management of root knot disease complex in mulberry.

Key words: Botanicals, mulberry, root knot disease complex, nematode, *Fusarium* spp.

Introduction

Among the various soilborne diseases affecting mulberry [a sole food plant of silkworms (*Bombyx mori* L.)], root knot nematode (*Meloidogyne incognita*) and root rot pathogens (*Fusarium solani* and *F. oxysporum*) pose serious problems during cultivation resulting in leaf yield loss up to 20%. Though the root knot disease complex was reported in mulberry by association of nematode and root rot pathogens viz., *F. solani* and *F. oxysporum* (Nishitha Naik *et al.*, 2004), informations on the management aspects of the disease need to be generated for effective control. As the use of chemicals (fungicides/nematicides) for the control of diseases cause environmental pollution and are reported to be toxic to silkworms, there is a need to find out an eco-friendly method for the management of the disease. The use of botanicals in disease management is safe and economical alternative to the chemical method. Several workers have reported the efficacy of some botanical extracts for the control of foliar and soilborne diseases in various crops including mulberry (Philip *et al.*, 1993; Iyer *et al.*, 2004; Srinivasalu *et al.*, 2004; Sharma, 2004). Hence, the present study was taken up to screen various botanicals against root knot disease complex under *in vitro* conditions to develop an integrated eco-friendly package.

Materials and Methods

Preparation of Botanical extract

25 g fresh and healthy leaves/rhizome/flowers of 20 different plants collected from the field, were surface sterilized with 0.1% $HgCl_2$ to remove the contaminants and were washed in running water 2-3 times followed by sterile water. Extracts were prepared by grinding each in 100 ml sterile water and filtered through four-ply muslin cloth and Whatman No. 42 filter paper. Filtered extracts were centrifuged at 4000 rpm for 20 minutes and the supernatant served as stock solution (100%) for making further dilutions to conduct the present study.

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Table 1. Effect of botanicals on *Fusarium solani* and *F. oxysporum* at different concentrations (14 days)

Botanical extracts	*F. solani*			*F. oxysporum*		
	Radial growth (mm)			Radial growth (mm)		
	Concentration (%)			Concentration (%)		
	5	10	20	5	10	20
Citrus limon	87.75 (2.5%) II	67.50 (25.0%) II	63.72 (29.2%) III	90.00 (0.0%) I	90.00 (0.0%) I	58.50 (35.0%) III
Datura metel	72.00 (20.0%) II	66.25 (26.3%) III	46.48 (48.3%) III	74.25 (17.5%) II	67.5 (25.0%) II	47.25 (47.5%) III
Duranta spp.	60.75 (32.5%) III	58.5 (35.0%) III	33.75 (62.5%) IV	77.75 (13.6%) II	62.00 (31.1%) III	27.00 (70.0%) IV
Lasownia inermis	58.50 (35.0%) III	56.25 (37.5%) III	21.60 (76.0%) V	67.50 (25.0%) II	49.50 (45.0%) III	22.32 (75.2%) V
Musa paradisiaca	67.75 (24.7%) II	60.75 (32.5%) III	43.47 (51.7%) IV	85.5 (5.00) II	72.00 (20.0%) II	41.38 (54.0%) IV
Tagetus erecta	74.25 (17.5%) II	67.83 (24.6%) II	45.00 (50.0%) III	90.00 (0.0%) I	87.75 (2.5%) II	52.46 (41.7%) III
Mirabalis jalappa	60.75 (32.5%) III	59.85 (33.5%) III	47.25 (47.5%) III	72.0 (20.0%) II	62.00 (31.1%) III	56.25 (37.5%) III
Nerium indicum	76.50 (15.0%) II	67.50 (25.0%) II	56.97 (36.7%) III	90.00 (0.0%) I	90.00 (0.0%) I	52.47 (41.7%) III
Tabernaemontana coronaria	74.08 (17.6%) II	72.00 (20.0%) II	46.48 (48.3%) III	90.00 (0.0%) I	87.75 (2.5%) II	52.47 (41.7%) III
Ricinus communis	83.25 (7.5%) II	74.25 (17.5%) II	46.48 (48.3%) III	90.00 (0.0%) I	90.00 (0.0%) I	46.48 (48.3%) III
Glyricidia maculata	76.50 (15.0%) II	58.50 (35.0%) III	50.98 (43.3%) III	90.00 (0.0%) I	84.30 (6.3%) II	50.98 (43.3%) III
Bougenvilla glabra	80.00 (11.1%) II	75.00 (16.6%) II	67.50 (25.0%) II	67.50 (25.0%) II	55.00 (38.8%) III	50.00 (44.4%) III
Moringa oleifera	85.00 (5.5%) II	77.50 (13.8%) II	69.50 (22.7%) II	66.25 (26.3%)	63.50 (29.4%) III	62.50 (30.5%) III
Catharanthus pusillus	60.00 (33.3%) III	47.50 (47.2%) III	42.50 (52.7%) IV	75.00 (16.6%) II	62.75 (30.2%) III	50.00 (44.4%) III
Ixora chinensis	70.00 (22.2%) II	65.00 (27.7%) III	62.50 (30.5%) III	58.75 (34.7%) III	57.50 (36.1%) III	41.25 (54.1%) IV
Polyalthia longifolia	65.00 (27.7%) III	62.00 (31.1%) III	58.00 (35.5%) III	72.50 (19.1%) II	71.00 (21.1%) II	69.00 (23.3%) II
Zinger officinales	63.75 (29.1%) III	57.50 (36.1%) III	45.00 (50.0%) III	75.00 (16.6%) II	71.00 (21.1%) II	67.00 (25.5%) III
Allium cepa	75.00 (16.6%) II	70.00 (22.2%) II	38.75 (23.6%) II	40.00 (55.5%) IV	37.50 (58.8%) IV	34.50 (61.6%) IV
A. sativum	0.00 (100.0%) V	0.00 (100.0%) V	0.00 (100.0%) V	0.00 (100.0%) V	0.00 (100.0%) V	0.00 (100.0%) V
Curcuma longa	53.75 (40.2%) III	50.00 (44.4%) IV	45.00 (50.0%) III	55.00 (38.8%)	53.00 (41.1%) III	51.00 (43.3%) III
Control	90.00	90.00	90.00	90.00	90.00	90.00
	F test		CD at 5%	F test		CD at 5%
Between conc. (A)	**		0.44	**		0.46
Between plant ext. (B)	**		1.14	**		1.19
A × B	**		1.97	**		2.05

Roman words denoting the grading

Figures in parentheses denote per cent reduction over control

Effect of botanical extracts on mycelial growth of virulent fungi

The inhibitory effect of the botanical extracts on mycelial growth of test fungi *viz.*, *F. solani* and *F. oxysporum* was assayed by poisoned food technique (Sharvelle, 1961). Before mixing the botanical extracts in to the medium, they were kept in water bath at 45°C for 20 minutes to avoid the contamination (Jagannathan and Narasimhan, 1987). Required concentrations of 5, 10 and 20% were prepared by mixing in 95, 90 and 80 ml of autoclaved lukewarm Potato Dextrose Agar medium (the total composition of the medium was 100 ml) separately for each test fungus and concentration. The medium was poured into the 90 mm Petriplates and after solidification of the medium; a 4 mm mycelial disc from 7 day old culture of *F. solani* and *F. oxysporum* was kept separately for each in the center of the plate. The plates were then incubated at 28±2°C for 14 days. A control was also maintained without the addition of the botanical extract in the medium. Three replications for each concentration of botanical extract along with control were maintained. After incubation, the efficacy of the botanical extract was determined by measuring the radial growth of the test pathogens and was compared with the growth in the control. The inhibition percentage was calculated by the formula of Vincent (1947) and categorized in to 5 grades.

$$I(\%) = \frac{C-T}{C} \times 100$$

Where,

I = Per cent inhibition over control

C = Radial growth of fungus in control (mm)

T = Radial growth of fungus in the treatment (mm)

Grades:

I: No inhibition - Non inhibitor

II: 0.1~25% inhibition - Poor inhibitor

III: 25.1~50% inhibition - Moderate inhibitor

IV: 50.1~75% inhibition - Good inhibitor

V: 75.1~100% inhibition - Strong inhibitor

Effect of botanical extracts on hatching of nematode eggs and larval mortality

To study the effect of botanical extracts on ovicidal action, four uniform size matured egg masses (1500 eggs) were suspended in each glass cavity block containing 5 ml solution of 5, 10 and 20% concentrations of different botanical extracts along with a control (distilled water) and kept for incubation at 28±2°C for 8 days. After incubation, the hatched larvae were counted. The hatching percentage and percentage inhibition over the control were calculated and categorized in to 5 grades.

For studying the larvicidal action, 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 5, 10 and 20% diluted extracts separately in three replications along with control (larvae in distilled water) for comparison. Observations were recorded on mortality of larvae after 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.

Results and Discussion

Among the different concentrations of botanical extracts, the inhibitory effect was low at 5 and 10% concentrations but inhibitory effect gradually increased with increase in the concentration up to 20%. At 20%, significantly higher inhibitory effect on radial growth of both the virulent fungi was observed as compared to 5 and 10% concentrations. Among the botanicals, the significantly higher inhibitory effect was noticed in the extract of *Allium sativum*, which completely inhibited the growth (100%) of *F. solani* and *F. oxysporum* followed by *Lasownia inermis* that reduced the radial growth of both the virulent fungi up to 76.0% at 20% concentration and degree of inhibition of both the extracts was rated under class V as strong inhibitors against test fungi (Table 1).

There was a significant reduction in hatching of eggs and mortality of larvae of *M. incognita* in all the botanical extracts over the control (Table 2). The hatching of eggs was inversely proportional to the increase in concentration of botanical extract and incubation period. The maximum suppression of hatching of eggs and increased larval mortality was observed at 20% concentration. Among the extracts, the maximum suppression of hatching was observed in *A. sativum* and *L. inermis* by 90.4 and 80.6%, respectively at 20% concentration. These two botanical extracts also enhanced the larval mortality up to 85.0 and 76.0%, respectively after 72 hrs of exposure period. The degree of inhibition of both the extracts was grouped in class V and categorized as strong inhibitors against nematode.

Botanicals like *A. sativum* and *L. inermis* have acted as strong inhibitors having nematicidal and fungicidal properties as compared to other extracts, which may be due to the presence of toxic active compounds like allicin and lawsone, respectively (Gupta and Sharma, 1991; Gupta and Shirkot, 2004; Sakarkar *et al.*, 2004). The results are in agreement with the findings of Iyer *et al.* (2004) and Srinivasalu *et al.* (2004) who reported that *A. sativum* exhibit a broad spectrum activity against various foliar

Table 2. Effect of botanicals on *M. incognita* at different concentrations

Botanical extracts	*M. incognita* (nematode)					
	Hatching of eggs (%)-8 days			Mortality of larvae (%)		
	Concentration (%)			Concentration (%)		
	5	10	20	5	10	20
Citrus limon	65.08 (30.7%) III	60.07 (36.0%) III	56.73 (39.6%) III	45.00 III	51.00 IV	58.00 IV
Datura. metel	68.02 (27.6%) III	62.87 (33.1%) III	55.53 (40.9%) III	40.00 III	46.00 III	55.00 IV
Duranta spp.	62.03 (34.0%) III	58.33 (37.9%) III	51.00 (45.7%) III	49.00 III	56.00 IV	68.00 IV
Lasownia inermis	25.40 (72.9%) IV	22.02 (76.5%) V	18.15 (80.6%) V	60.00 IV	67.00 IV	76.00 V
Musa paradisiaca	55.20 (41.2%) III	50.93 (45.8%) III	47.87 (49.0%) III	51.00 IV	56.00 IV	61.00 IV
Tagetus erecta	50.12 (46.6%) III	47.80 (49.1%) III	40.40 (57.0%) IV	58.00 IV	63.00 IV	68.00 IV
Mirabalis Jalappa	68.70 (26.9%) III	66.87 (28.8%) III	60.80 (35.3%) III	52.00 IV	60.00 IV	65.00 IV
Nerium indicum	37.11 (60.5%) IV	33.12 (54.7%) IV	28.25 (69.9%) IV	58.00 IV	62.00 IV	68.00 IV
Tabernaemontan coronaria	62.02 (34.0%) III	65.87 (29.9%) III	69.12 (26.4%) III	48.00 III	54.00 IV	60.00 IV
Ricinus communis	39.12 (58.3%) IV	35.00 (62.7%) IV	31.12 (66.8%) IV	55.00 IV	60.00 IV	65.00 IV
Glyricidia maculata	68.00 (27.6%) III	62.00 (34.0%) III	55.13 (41.3%) III	53.00 IV	57.00 IV	62.00 IV
Bougenvilla glabra	55.12 (41.3%) III	52.15 (44.5%) III	49.15 (47.7%) III	26.00 III	28.00 III	32.00 III
Moringa oleifera	58.00 (38.2%) III	53.00 (43.6) III	49.12 (47.7%) III	32.00 III	36.00 III	39.00 III
Catharanthus pusillus	56.00 (40.4%) III	50.00 (46.8%) III	42.50 (52.7%) IV	28.00 III	32.00 III	38.00 III
Ixora chinensis	56.20 (40.2%) III	53.40 (43.1%) III	48.11 (48.8%) III	30.00 III	32.00 III	38.00 III
Polyalthia longifolia	58.00 (38.2%) III	55.00 (41.4%) III	46.71 (50.3%) IV	30.00 III	33.00 III	39.00 III
Zinger officinales	42.00 (55.3%) IV	37.20 (60.4%) IV	32.00 (65.9%) IV	36.00 III	42.00 III	55.00 IV
Allium cepa	55.15 (41.3%) III	50.48 (46.2%) III	45.18 (51.9%) IV	40.00 III	45.00 III	58.00 IV
A. sativum	18.12 (80.7%) V	14.00 (85.1%) V	9.00 (90.4%) V	68.00 IV	74.00 IV	85.00 V
Curcuma longa	45.11 (52.0%) IV	40.75 (56.6%) IV	35.54 (62.1%) IV	42.00 III	53.00 IV	69.00 IV
Control	94.00	94.00	94.00	5.00	5.00	5.00
	F test		CD at 5%	F test		CD at 5%
Between conc. (A)	**		0.46	**		0.64
Between plant ext. (B)	**		1.19	**		1.65
A × B	**		2.09	**		2.86

Roman words denoting the grading
Figures in parentheses denote percent reduction over control

and soilborne pathogens in many crops like arecanut, coconut, tomato, *etc.* Sharma *et al.* (2001) observed the effect of various leaf extracts against *Pseudomonas syringae* pv. *mori* and *Xanthomonas campstris* pv. *mori* causing bacterial blight in mulberry, among which *A. sativum* showed the maximum inhibitory effect against the disease.

Some of the botanicals were found to have strong effect either against the nematode or virulent fungi. The extracts of *Duranta* spp. and *M. paradisiaca* strongly inhibited the virulent fungi but did not suppress the hatching of nematode eggs, which clearly shows their fungicidal action only while extracts like *C. longa*, *A. cepa, T. erecta*, *N. indicum* and *R. communis* strongly suppressed the hatching of eggs and enhancing the larval mortality but were ineffective against virulent fungi indicating that they are only nematicidal in action. The rest of the botanical extracts were found ineffective both against nematode and virulent fungi. Botanical extracts like *A. sativum* and *L. inermis* having anti nematode and anti fungal properties have not been tested against root - knot disease complex so far and this is the first report.

It is concluded that the extracts of *A. sativum* and *L. inermis* are strongly effective against nematode and virulent fungi, hence they can be utilized as components in developing an integrated eco-friendly management of root - knot disease complex in mulberry.

Acknowledgements

The authors are highly thankful Mrs. M. Rekha, Assistant

Director (Statistics), CSRTI, Mysore for rendering help in statistical analysis.

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