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EFFECT OF MARIGOLD LEAF EXTRACT AND CAPTAFOL ON FUNGAL PARASITISM OF ROOT KNOT NEMATODE EGGS-KENYAN ISOLATES

by
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Summary. Marigold leaf extract and captafol significantly inhibited fungal parasitism of *Meloidogyne incognita* and *M. javanica* eggs. Captafol treated eggs were the least parasitised. The highest concentration of extract had the second most significant inhibitory effect on egg parasitism. No significant difference in fungal egg parasitism of the two nematodes was detected in all treatments except at the lowest marigold extract concentration. Fungal parasitism of *M. javanica* eggs was significantly higher at this extract concentration. *Fusarium solani* and *F. oxysporum* differed in their ability to parasitize eggs. The former fungus was more parasitic than the latter.

Although certain plant parts or extracts have nematicidal properties that inhibit nematodes (Siddiqui and Alam, 1988; Zaki and Bhatti, 1990), effects of leaf extracts on activity of fungal antagonists have only been partially investigated (Owino *et al.*, 1991).

This paper reports on the effects of marigold (*Tagetes patula* L.) extracts and captafol (fungicide) on fungal parasitism of *Meloidogyne javanica* (Treub) Chitw., and *Meloidogyne incognita* (Kofoid *et* White) Chitw. eggs, and the parasitic potentials of *Fusarium solani* Sacc. and *F. oxysporum* Schlecht against *M. javanica* eggs. Captafol was included as a treatment because it has a broad range of action against fungi but has no effect on nematodes (Van Bezooijen, 1979).

Materials and methods

Marigold leaf extract was prepared by cominuting 30 g of leaf pieces in a blender containing 80 ml of double distilled water and then centrifuging and filtering. The extract was arbitrarily termed as standard (ST) and this was diluted with distilled water to provide ST/4 and ST/10 solutions. All solutions were passed through 0.22 µm millipore filters and stored at 4°C before use on the following day.

Two experiments were conducted. In expt. 1, effect of marigold leaf extract and captafol on fungal parasitism of *M. incognita* and *M. javanica* eggs were determined on water agar (WA). Egg masses of *M. incognita* and *M. javanica* were collected from tomato (*Lycopersicon esculentum* Mill) cv. Moneymaker grown in fungi/nematode infested soil collected from different fields in Nairobi.

Egg masses of each species were homogenized in distilled water and passed through 0.250, 0.150, 0.075, and 0.025 mm sieves. Eggs retained on the 0.025 mm sieve were washed five times with distilled water and re-suspended in 20 ml of distilled water. One ml of the suspension containing approximately 1000 eggs was poured onto a Petri-plate containing 1.5% water agar (WA) medium supplemented with 0.1% Chloramphenicol solution (Sikora *et al.*, 1990). Ten plates each received 1 ml of marigold extract of concentrations ST/10, ST/4 or ST or 1 ml of captafol. Control plates received 1 ml of distilled water.

Treatments were arranged in a randomized design and incubated at 25°C for 14 days (Freire and Bridge, 1985). After the incubation period, 100 eggs per plate were randomly scored and the proportion parasitised determined under the stereo-microscope at x 100 magnification (Kerry and Crump, 1977). Egg parasitism was confirmed by the presence of fungal mycelium inside the eggs.

F. solani and *F. oxysporum* were frequently isolated from parasitised eggs using procedures described by Freire and Bridge (1985). Individual eggs were aseptically transferred on to new WA Petri plates and icubated for 4 days to allow fungal growth. Pure fungal cultures were obtained by aseptically transferring each fungal colony to new WA

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plates using a sterile platinum loop. Fungi were cultured on potato dextrose agar medium (PDA) in 9 cm-diameter Petri dishes for 10 days at 20°C.

In another experiment, the effect of leaf extracts and captafol on nematode egg parasitism by *F. solani* and *F. oxysporum* was assessed. Fungi-free eggs were obtained from tomato cv. Moneymaker grown in sterile sand. PDA plugs (9 mm diam.) were cut from the peripheries of 10-day-old fungal colonies with a cork borer and placed at the centre of 1.5% WA in Petri-dishes. Each plate was inoculated with a single plug and incubated at 20°C until the whole agar surface was covered with the mycelium of the particular fungus. This took 11 to 18 days. One ml of sterile distilled water containing 1000 eggs was then spread on to each fungal-covered WA surface. Ten plates received 1 ml of marigold extract of concentrations ST/10, ST/4, ST or 1 ml of captafol (1 ppm). Controls had 1 ml of distilled water or had extract or captafol but no fungus. The plates were incubated for 2 weeks at 25°C and egg infection was evaluated as described before.

Results and discussion

Captafol and marigold extracts had a significant (P = 0.05) inhibitory effect on parasitism of *M. javanica* and *M. incognita* eggs on WA (Table I). Parasitism of both nematodes decreased with an increase in extract concentration. Captafol-treated eggs were the least parasitized. A significantly greater proportion of eggs were infected in control plates not treated with the extract or Captafol. In general, egg parasitism was similar for the two nematode in all treatments.

Although the parasitic potential of *F. solani* and *F. oxysporum* decreased significantly with an increase in leaf extract concentration (Table II), captafol significantly suppressed fungal egg infection than all extract treatments. In general, the two pathogens differed significantly in their response to marigold extract but not to Captafol. *F. solani* infected more eggs than *F. oxysporum* in most cases.

The significantly lower rate of parasitism of eggs treated with marigold extract (Table I) demonstrates inhibitory effect of these substance on egg parasitism.

This could be due to the antifungal compounds present in fresh marigold tissues (Baker, 1981). Since egg parasitism is initiated with the formation of penetration pegs (Stirling and Mankau, 1979), it is possible that the active ingredients in the extract inhibited formation of these organs. Since chitinolytic enzymes and toxins are also associated with egg infection (Stirling and Mankau, 1979), it is possible that the marigold extract inhibited or altered the mode of action of these biological chemicals.

In general, *F. solani* infected more eggs than *F. oxysporum* (Table II). This suggests differential tolerance of these isolates to active ingredients in marigold leaves, or inherent variation in their parasitic capabilities against *M. javanica* eggs. Since variations in activity of different strains of same or different fungal species do occur (De Leij and Kerry, 1991; Kuc and Shain, 1977), further work on effects of extracts on different isolates of fungal antagonists is essential if more information on fungal-extract interraction is to be obtained for use in nematode management programmes.

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Table I - *Effect of captafol and leaf extracts of marigold on fungal parasitism (%) of root-knot nematode eggs* [a].

Treatments	Percent fungal parasitism	
	M. incognita	M. javanica
Control	65.2 e	61.3 e
Leaf extract concentration [b]		
ST/10	32.2 d	19.8 c
ST/4	18.3 c	16.9 c
ST	10.2 b	7.3 b
Captafol (1 ppm)	1.4 a	1.9 a

[a] Values are means of 10 replicates. Means followed by different letters within a column or row are significantly different (P = 0.05) according to Duncans Multiple range test. b) See text.

Table II - *Effect of captafol and leaf extract of marigold on parasitism (%) of* M. javanica *eggs by* F. oxysporum *and* F. solani *on water agar* [a].

Fungus	Percent fungal infection				
	Leaf extract concentration b)				Captafol (1 ppm)
	0 (No extract)	ST/10	ST/4	ST	
Control (No fungus)	1.1 a	0.4 a	0.2 a	0.3 a	0.0 a
F. solani	63.1 e	34.4 d	18.3 c	16.8 c	1.8 a
F. oxysporum	58.3 e	19.9 c	8.3 b	3.6 a	1.2 a

[a] Values are means of 10 replicates. Means followed by different letters within a column or row are significantly different (P = 0.05) according to Duncans multiple range test. b) See text.

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