Effective stage of African marigold (*Tagetes erecta* L.) in reducing the root-knot nematode (*Meloidogyne incognita*) Kofoid and White population in a tomato (*Solanum lycopersicon*) crop in Ghana

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ABSTRACT

The aim of the study was to investigate the effective stage of the African marigold, *Tagetes erecta*, in suppressing root-knot nematode populations in Ghana. Two experiments were conducted in the plant house-/laboratory in 1999 and 2000 at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. In the first and second experiments, different stages of *T. erecta* was investigated for nematicidal activity and hatching suppression of *Meloidogyne incognita* respectively. Data on nematodes were log transformed and subjected to analysis of variance using Genstat 8.1.Population of second stage juveniles recovered from tomato protected by *T. erecta* was significantly lower than unprotected tomato ($P< 0.05$). Of the 1000 eggs of *M. incognita* inoculated to tomato plants, 1273 juveniles were recovered from the control treatment, while just 62 juveniles were recovered from eight-week old tomato plants protected by *T. erecta*. Similarly, root gall index of tomato protected by *T. erecta* was significantly lower at eight weeks, which corresponded with the reduction in population at the same stage of growth. Fresh shoot and root and dry shoot and root weights of tomato plants protected by *T. erecta* were higher than unprotected plants. Root diffusates of *T. erecta* demonstrated significant hatching suppression activity against *M. incognita* eggs. The control treatments recorded 91 and 93 % hatching while ten-week old plants recorded 14 and 15 % hatching, representing significant hatching reduction of 85 and 84 % respectively. The effective stage of *T. erecta* in controlling *M. incognita* corresponded with eight to eleven weeks after germination.

Key words: Control, Ghana, nematodes, *Solanum lycopersicum, and Tagetes erecta*

INTRODUCTION

Phytoparasitic nematodes are persistent pests of cultivated crops (Chitwood, 2002). The root-knot nematodes, *Meloidogyne* (Goeldi) species are the most economically important (Williams-Woodward and Davis, 2001). Globally, over 90 species of the genus, *Meloidogyne* have been described (Sikora and Fernandez, 2005). In Ghana, *M. incognita* (Kofoid and White) is the most damaging nematode pest associated with vegetable production (Hemeng, 1980). *M. incognita* therefore is a limiting factor in the vegetable industry and the successful management of the pest would improve yield of vegetables significantly.

Crop rotation and the use of chemicals have been the major management strategies to control plant parasitic nematodes. While crop rotation is perceived as an environmentally friendly option, it has a major shortcoming. The strategy is not effective in reducing the populations of nematodes having extensive host ranges such as *Meloidogyne* species (Sikora and Fernandez, 2005). That makes selection of suitable rotation crops extremely difficult.

Most synthetic nematicides in use today lack broad-spectrum activity (Chitwood, 2002). Also, repeated use of some nematicides can lead to a condition known as enhanced degradation resulting from the selection of soil microflora capable of metabolising the active ingredients (Cabrera *et al.*, 2005). Perhaps, more importantly, it is generally perceived that chemicals are

environmentally unfriendly, contribute to groundwater contamination and dangerous to farmers who use them incorrectly. For these reasons, chemicals such as methyl bromide, which is highly effective, are being withdrawn from use (Anon, 2000; Thomas, 1996).

Plants antagonistic to nematodes are those considered to produce anti-helminthic compounds with different modes of action (Pandey *et al.*, 2003). The production and active release of toxic substances while the crop is growing is usually responsible for control (Sikora *et al.*, 2005). Marigolds, *Tagetes* species which exude polythienyls have been proven to be nematicidal. Rotations of *T. patula* or *T. erecta* provided economic control of *Pratylenchus penetrans* on tobacco for two successive years (Reynolds *et al.*, 2000). Gnanapragasam (1986) proved that *Tagetes* spp. reduced population densities of nematodes in Sri-Lanka. Osei (2000) reported that *T. erecta* reduced the population of *M. incognita* to below threshold levels on tomato in microplots in Ghana.

The antagonistic plant, African marigold, *Tagetes erecta* L. has been extensively used to control plant parasitic nematode populations with varying degrees of successes (Bridge, 1996; Wang *et al.*, 2001; Chitwood, 2002). However, to be most beneficial in rotation systems, the stage of the plant at which control is effective must be known. The aim of the study was to investigate the effective stage of the African marigold, *Tagetes erecta,* in suppressing root-knot nematode populations in Ghana.

MATERIALS AND METHODS

Two experiments were conducted in 1999 and 2000 at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. In the first experiment, fifty-two plastic pots each measuring (27 x 21 x 15 cm) were filled with 7 kg of steam-sterilized soil. A black soil collected from an old refuse dump was mixed with river sand in a ratio of 3:1. The pots were placed on raised plat-forms in the plant house. Three seeds of the African marigold, *T. erecta* L. were sown at stake in each of 48 pots. Three weeks after sowing the *T. erecta,* seedlings were thinned to one plant per pot and a single tomato seedling cv. Power were nursed individually on steam-sterilized soil for three weeks and transplanted 8 cm away from the potted *T. erecta* plants. *M. incognita* was identified through perineal patterns (Jepson, 1987). The eggs used as inoculum were extracted from plant house cultures of *M. incognita* built on tomato roots by shaking for 3 min. in 0.05% NaOCl solution (Stanton and O'Donnell, 1994). The tomato seedlings (four pots /treatment) were inoculated with 1000 *M. incognita* Kofoid and White eggs each at transplanting time through five small holes made in a circle 5 cm from the tomato seedlings. Inoculation was done with a graduated pipette from a concentrated solution of eggs. This was the first treatment designated "Three weeks old *T. erecta* protected tomato". The following week's treatment was designated "Four weeks old *T. erecta* protected tomato". The same procedure was adopted on hebdomadal basis for the rest of the pots. The last four pots served as control which had only tomato seedlings and inoculated with 1000 eggs each and designated "Tomato without *T. erecta*". Each treatment was allowed to grow for 8 weeks. The 13 treatments were arranged in a completely randomized design (CRD) and replicated four times.

The experiment was conducted in November 1999 and repeated in March 2000. Soil was sampled from the rhizosphere region of tomato in pots and thoroughly mixed together. Juveniles were extracted from 200 cm^3 sub samples of the soil using a modified Baermann funnel method. Counting of juveniles was under a stereo microscope at magnification 100x. Root gall index of tomato was on a scale of

0-10 (Netscher and Sikora, 1990) where 0 = roots with no galls and 10 = maximal degree of galling. Fresh and dry shoot and root weights of tomato were recorded to 0.1 g.

In the second experiment, forty-eight plastic pots were each filled with 7 kg soil. The medium and the experimental procedure were the same as the previous experiment. Three *T. erecta* seeds/pot were sown at stake. The seedlings were thinned to one plant/pot one week after germination. Three weeks after germination, harvesting started. *Tagetes erecta* seedlings in the four pots were uprooted and washed under tap water to remove adhering soil particles in the laboratory. The washed roots were cut into 1 cm pieces and 5 g root each weighed to 0.1 g. The 5 g root was tied in a clean white gauze material which was placed in a 9-cm Petri dish and 20 ml of distilled water added. Each was properly labelled and allowed to stay for three days for diffusates to leach from the root pieces. On the third day, *M. incognita* eggs were isolated using the NaOCl method described in experiment 1. One hundred eggs each were counted under the microscope and transferred into the preparation for hatching to take place. *M. incognita* juveniles (J2) which hatched within 7 days (McLeod *et al.*, 2001) were counted at magnification 100x. The purpose was to identify the stage of *T. erecta* that was most effective in inhibiting hatching of *M. incognita* eggs. The first four Petri dishes represented the first treatment and designated "Three weeks old *T. erecta*". This procedure continued on weekly basis for twelve weeks and the last four Petri dishes had only 20 ml of distilled water and 100 eggs and designated control. The experiment, which was conducted in May 2000, was repeated in August of the same year.

Data on nematodes (juveniles, root gall index and egg hatch) were log transformed, {log (x +1)}, before analysis to comply with the assumption of normal distribution. Data were subjected to analysis of variance (ANOVA) using Genstat 8.1. (Lawes Agricultural Trust, VSN International) statistical package and treatment means were separated by using Duncan's Multiple Range Test (DMRT).

Table 1: Effective stage of *Tagetes erecta* in reducing population of *Meloidogyne incognita* juveniles (J2) in Ghana

Treatment	November 1999		March 2000	
		J2		J2
Three weeks old *T. erecta* protected tomato	765	(2.8 i)*	790	(2.9 h)
Four weeks old *T. erecta* " "	757	(2.8 i)	764	(2.8 h)
Five weeks old *T. erecta* " "	420	(2.6 g)	400	(2.6 f)
Six weeks old *T.erecta* ""	461	(2.7 h)	466	(2.7g)
Seven weeks old *T. erecta* " "	394	(2.6 g)	384	(2. 6 f)
Eight weeks old *T.erecta* " "	62	(1. 8 a)	58	(1. 8 a)
Nine weeks old *T.erecta* ""	74	(1.9 b)	76	(1.9 b)
Ten weeks old *T.erecta* ""	180	(2.3 c)	173	(2.2 c)
Eleven weeks old *T. erecta* "	224	(2.4 d)	234	(2.4 d)
Twelve weeks old *T. erecta* " "	330	(2.5 f)	332	(2.5 e)
Thirteen weeks old *T. erecta* " "	297	(2.4 e)	296	(2.5 e)
Fourteen weeks old *T. erecta* " "	403	(2.6 g)	399	(2.6 f)
Tomatoes without *T. erecta* (control)	1273	(3.1 j)	1290	(3.1 i)
P – value		(< 0.05)		(< 0.05)
Mean		434		436
SED		0.02		0.03
Cv (%)		0.4		0.5

Means within a column with different subscripts differ significantly ($p < 0.05$); Data are means of four replications;* log transformed { log (x +1) }; SED =Standard error difference; Cv = Coefficient of

variation.

Table 2: Root gall index (RGI) of *Tagetes erecta* protected tomato plants in Ghana

Treatment	November 1999		March 2000	
	RGI		RGI	
Three weeks old *T. erecta* protected tomato	8.5	(0.9 d)* 8.0	(0.9 e)	
Four weeks old *T. erecta* " "	7.8	(0.9 d)	7.0	(0.8 d)
Five weeks old *T. erect* " "	5.0	(0.7 c) 4.0	(0.6 b)	
Six weeks old *T. erecta* " "	4.8	(0.7 c) 4.5	(0.7 c)	
Seven weeks old *T. erecta* " "	4.5	(0.6 b) 4.0	(0.6 b)	
Eight weeks old *T.erecta* " "	2.3	(0.5 a)	2.5	(0.4 a)
Nine weeks old *T.erecta* " "	3.5	(0.5 a)	3.8	(0.6 b)
Ten weeks old *T.erecta* " "	4.0	(0.6 b) 4.0	(0.6 b)	
Eleven weeks old *T. erecta* " "	4.0	(0.6 b)	4.0	(0.6 b)
Twelve weeks old *T. erecta* " "	5.0	(0.7 c) 5.5	(0.7 c)	
Thirteen weeks old *T. erecta* " "	5.5	(0.7 c)	5.3	(0.7 c)
Fourteen weeks old *T. erecta* " "	5.5	(0.7 c)	5.3	(0.7c)
Tomato without *T. erecta* (control)	9.5	(1.0 e)	9.8	(1.0 f)
P – value		(< 0.05)	(< 0.05)	
Mean	5.4		5.2	
SED	0.06	0.06		
Cv (%)		5.5		4.6

Means within a column with different subscripts differ significantly ($p < 0.05$)
Data are means of four replications
* log transformed { log (x +1) }
SED = Standard error difference; Cv = Coefficient of variation

RESULTS AND DISCUSSION

Results of the first experiment indicated that a significantly higher ($P < 0.05$) population of 1,273 juveniles / 200 cm^3 of soil was recovered from the control treatment compared with 8 weeks old *T. erecta* protected tomato which recorded a mean of 62 juveniles / 200 cm^3 (Table 1). This represents 95% percent more juveniles found on control (tomato) plants than the 8 weeks old *T. erecta* protected tomato plants. The above results were supported by the root gall indices of tomato (Table 2). An approximate mean index of 10 was obtained from the control whereas the lowest mean index of 2 was recorded 8 weeks after planting *T. erecta*. Thus, the 8 weeks old *T. erecta* reduced the infection rate of tomato by 80%.

Similar results were obtained for fresh and dry shoot and root weights in grams. Significant differences ($P < 0.05$) in both fresh and dry shoot weights were observed between the control and the other treatments. However, the most noticeable difference occurred between the control treatments (5 g and 0.4 g) and the eight-week old *T. erecta* protected tomato (8.3 g and 1.1 g) respectively. Similar differences in root weights were observed between the control treatments (0.8 g and 0.3 g) and the eight-week old treatments (1.6 g and 0.6 g) respectively (Table 3).

From the second experiment, eight to eleven weeks old *T. erecta* demonstrated significant ($P<0.05$) hatching suppression potential (Table 4.). The control treatment recorded 91 and 93% hatching for the two time periods respectively, while eight to eleven weeks old *T. erecta* plants recorded (24, 21, 14 and 16%) and (25, 22, 15 and 16%) respectively.

After eleven weeks of growth, hatching inhibition was reduced and this could be

attributed to the dilution of the active ingredients in *Tagetes* as the plant was aging.

In the current study, eight-week old *T. erecta* significantly reduced *M. incognita* population and also recorded the lowest infection rate. However, highly significant hatching suppression was recorded by ten and eleven-week old *T. erecta*. These findings corroborated the observation of Bridge (1996) that *T. erecta, T. patula* and *T. minuta* reduced the population levels of root-knot nematodes. Alam *et al.* (1990) observed that the active ingredients ∝-terthienyl (2, 2'-5-2''-terthienyl) and 5-(3-buten-1-ynyl)-2, 2-bithienyl, present in *T. erecta* were responsible for nematode control. Marigolds are not only antagonistic to root-knot nematodes; they are also effective against other nematode species. Forage pearl millet and marigold as rotation crops with potatoes resulted in fewer root lesion nematodes and increased potato yields than rotations with rye (Ball-Coelho *et al.*, 2003). Also, rhizobacteria living in association with marigold roots are suppressive to root lesion and other nematodes (Stuarz and Kimpinski, 2004). Ploeg and Maris (1999) observed that the suppression of marigolds on *M. incognita* is temperature dependent. Galling occurred on two normally resistant cultivars at 30^{0}C, and most cultivars were ineffective when grown at 15^{0}C or lower. Ploeg (1999) demonstrated that the efficacy of marigold-based nematode control was a function of the marigold cultivar used and the biological and environmental parameters in a given agro-ecosystem. Apart from the Asteraceae, *Tagetes* spp., nematicidal properties have been identified in some tropical legumes.

In Martinique, Quénéhervé *et al.* (1998) used *Mucuna pruriens* to effectively control *Meloidogyne incognita* and *Rotylenchulus reniformis* in vegetables in polytunnels. McSorley *et al.* (1994) reported that *Mucuna deeringiana* and *Crotalaria spectabilis* were free of root-knot nematode galling in microplot trials.

Controlling nematodes at the embryonic stage would be profitable as inoculum levels would reduce for crops to have head on start in growth. In Ghana, the African marigold is commonly cultivated for its aesthetic value. However, Reddy *et al.* (1986) reported that, marigold is used in poultry feed to improve yolk colour in eggs. The use of marigold is environmentally acceptable and its incorporation into the farming system does not require special skill and technique.

CONCLUSIONS

The African marigold, *T. erecta* controlled the population build up of root-knot nematodes, *M. incognita*. The effective stage of the antagonistic plant in controlling root-knot nematodes corresponded with eight to eleven weeks after germination. In a rotation system involving *T. erecta* therefore, the following crop could be cultivated after eleven weeks of the cultivation of *T. erecta*.

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Table 3: Effect of *Tagetes erecta* on fresh and dry shoot and root weights (g) of tomato at 8 weeks in Ghana

Treatment	Fresh shoot weight (g)	Dry shoot weight (g)	Fresh root weight (g)	Dry root weight (g)
Three weeks old *T. erecta* plus tomato	5.6 f	0.5 g	1.1 e	0.4 c
Four weeks old *T. erecta* " "	6.6 e	0.6 f	1.3 d	0.5 b
Five weeks old *T. erecta* " "	6.6 e	0.7 e	1.4 c	0.5 b
Six weeks old *T.erecta* " "	7.8 c	1.0 b	1.6 a	0.6 a
Seven weeks old *T. erecta* " "	7.9 c	1.0 b	1.6 a	0.6a
Eight weeks old *T.erecta* " "	8.3 a	1.1 a	1.6 a	0.6 a
Nine weeks old *T.erecta* " "	7.9 c	1.0 b	1.5 b	0.6 a
Ten weeks old *T.erecta* " "	8.1 b	1.0 b	1.6 a	0.6 a
Eleven weeks old *T. erect* " "	8.0 b	1.0 b	1.5 b	0.6 a
Twelve weeks old *T. erecta* " "	7.9 c	1.0 b	1.5 b	0.5 b
Thirteen weeks old *T. erecta* " "	7.7 cd	0.9 c	1.5 b	0.5 b
Fourteen weeks old *T. erecta* " "	7.7 cd	0.8 d	1.4 c	0.5 b
Distilled water (control) " "	5.0 g	0.4 h	0.8 f	0.3 d
P – value	<0.05	<0.05	<0.05	<0.05
Mean	7.3	0.8	1.4	0.5
SED	0.14	0.03	0.03	0.01
Cv (%)	0.75	3	2	5

Means within a column with different subscripts differ significantly ($p < 0.05$), Data are means of four replications, Data with similar letters are not significantly different, SED = Standard error difference; Cv = Coefficient of variation.

Table 4: Effective stage of *Tagetes erecta* in suppressing hatching of *Meloidogyne incognita* eggs in Ghana

Treatment	May 2000 No. of eggs hatched	August 2000 No of eggs hatched
Three weeks old *T. erecta*	83 (1.9 g)*	84 (1.9f)
Four weeks old *T. erecta*	80 (1.9 g)	81 (1.9 f)
Five weeks old *T. erecta*	77 (1.9 g)	79 (1.9 f)
Six weeks old *T.erecta*	76 (1.9 g)	76 (1.9 f)
Seven weeks old *T. erecta*	68 (1.8f)	69 (1.8 e)
Eight weeks old *T.erecta*	24 (1.4 d)	25 (1.4 c)
Nine weeks old *T.erecta*	21 (1.3 c)	22 (1.3 b)
Ten weeks old *T.erecta*	14 (1.1 a)	15 (1.2 a)
Eleven weeks old *T. erecta*	16 (1.2 b)	16 (1.2 a)
Twelve weeks old *T. erecta*	28 (1.5 e)	28 (1.5 d)
Thirteen weeks old *T. erecta*	25 (1.4 d)	26 (1.4c)
Fourteen weeks old *T. erecta*	27 (1.4 d)	29 (1.5 d)
Distilled water (control)	91 (2.0 h)	93 (2.0 g)
P – value	(< 0.05)	(< 0.05)
Mean	48	49
SED	0.03	0.03
Cv (%)	2.6	0.8

Means within a column with different subscripts differ significantly ($p < 0.05$), Data are means of four replications, * log transformed { log (x +1) }, SED = Standard error difference; Cv = Coefficient of variation

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