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Full Length Research Paper

The potential of four non traditional legumes in suppressing the population of nematodes in two Ghanaian soils

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Most African farmers could not afford the purchase of pesticides to control crop pest, alternatives such as the use of phytochemicals are most useful. For best results, sound recommendations are needed on the use of different antagonistic plants in controlling pest. In Ghana few reliable data are available on the potential of antagonistic plants to control nematode population. A field trial was conducted in the forest and forest savanna transitional zones of Ghana to evaluate the potential of four leguminous crops in suppressing nematodes population. *Mucuna pruriens, Crotalaria spectabilis* and *C. retusa* were antagonistic but *Phaseolus vulgaris* was a favourable host to the pest. *M. pruriens* did not gall and no egg masses found on roots. *C. spectabilis* and *C. retusa* recorded insignificant galling indices of 2.6 and 1.8 respectively. However, *P. vulgaris* was significantly affected by *Meloidogyne incognita* as expressed by the significantly higher gall and egg mass indices. In addition, nodulation was greatly reduced in *P. vulgaris*. Consequently, significant higher population of nematodes (an average of 600 J2/g^{-1}root) was recovered from *P. vulgaris* whilst lower population (an average of 50 J2/g $^{-1}$root) was recovered from *M. pruriens*. The growth and yield of okra following *M. pruriens* resulted in a significant 48% yield increase over the control and *P. vulgaris* treatments. *M. pruriens* recorded 31 and 38% yield increase over *C. spectabilis* and *C. retusa* respectively.

Key words: Agricultural production, antagonistic plants, legumes, *Meloidogyne incognita*.

INTRODUCTION

Agricultural production, world wide, is beset with a myriad of constraints. In sub Saharan Africa, the two major constraints are low and declining soil fertility and pest infestation. The low and declining soil fertility arises from continuous cultivation where the levels of soil replenishment are too low to mitigate the process of nutrient mining (IFDC, 1998). Plant parasitic nematodes are important pests that cause economic yield losses of crops, (Orion, 2000; Fourie et al., 2001 and Koenning et al., 2001) especially in the tropics where environmental conditions and cropping systems favour their development (Luc et al., 2005). They have extensive host ranges (Kratochvil, 2004; Dickson and De Waele, 2005; Quénéhervé, 1995), thus making their control extremely difficult. Root-knot nematodes cause root galling that is often accompanied by stunting, chlorosis, and wilting of the host plants. Total crop failure can occur when nematodes population exceed economic threshold level (ETL) (Sikora and Fernandez, 2005) . Nematicide application is the single most effective management strategy against nematodes (Thomas, 1996). However, chemical usage presents environmental problems (Bell, 2000). The use of antagonistic plants in agricultural production offers tremendous potential as nematicidal principles have been identified in many higher plants (Chitwood, 2002). Legume family (Fabacea) contains members with considerable agricultural utility. They are desirable rotational and cover crops. By fixing atmospheric nitrogen, these legumes play a key role in

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restoration of degraded soils (Peoples and Craswell, 1992; Thomas et al., 1997). Evaluating legumes for nematode management stimulate greater exploitation of the legumes in cropping systems.

MATERIALS AND METHODS

The studies were conducted in 2004 and 2005 at two sites, Ejura (07°24 N, 01°21 W) in the forest savanna transition zone and Fumesua (01°28 N, 06°41 W) in the forest zone. Composite soil samples from rhizosphere region at a depth of 15 cm were taken from each site at the beginning and end of the study. At each site, soil was collected and bulked from three locations, air dried and sieved to pass through a 2 mm mesh. Soil reaction (pH) was determined by using the glass electrode pH meter (Radiometer Analytical, Lyon, France) in water at 1:1 (soil: water) ratio. Organic carbon (%C) was determined by the dichromate- oxidation method of Walkley-Black (Walkley and Black, 1934). From these values, the organic matter (%OM) content of soil was computed using a correction factor of 1.72 (Nelson and Sommers, 1996). Total nitrogen (%N) was determined by the Kjeldahl method (Pereira et al., 2006).

Tomato bioassay

Tomato bioassay was performed prior to the establishment of the legumes. Soil samples at a depth of 20 cm were taken randomly from twenty plots of 5 x 4 m by walking in a zigzag fashion. From each plot, four samples were taken making a total of eighty. The soil samples were put into eighty plastic pots each measuring 5 kg. One seedling each of a two–week old tomato, *Solanum lycopersicum* L. (cv. Tiny Tim) which had been germinated on tissue paper in a 9 cm petri dish was transplanted. The pots were arranged in a randomized complete block design in a green house. The plants were grown for 8 weeks and root gall index was determined (Netscher and Sikora, 1990).

Treatments and experimental design

Experiments were conducted with five treatments namely; *C. retusa, C. spectabilis, M. pruriens, P. vulgaris* and weeded fallow. The experiments were replicated four times and arranged in a randomized complete block design (RCBD) . Seeds of these legumes were directly sown in plots of four, 5 m rows 1 m apart. Hand weeding was done three times during the growth period. Fallow plots were weeded whenever the other plots were weeded. Three months after sowing, shoot biomass of the leguminous crops was determined using the quadrat sampling method. Shoots in four 50 x 30 cm quadrats were taken per plot. Root galling, nodule score, root length, number of egg mass index per 5 g and J2 g 200 cc of soil were also recorded.

Cultivation of okra

Three healthy seeds of *Abelmoscus esculentus* (L.) Moench were sown 50 cm apart in four 5 m rows on the plots previously where legume was grown. Seedlings were thinned to one three weeks after planting. Hand weeding was done three times during the crop season. A second crop was sown after the harvest of the current crop. Observation was recorded on: fresh weight of root, fresh and dry weight of stem, yield, gall index, number of egg mass per 5 g root and J2 per 200 cc soil root. Okra roots, one per plant were randomly sampled and extracted for eggs according to the procedure of Hussey and Barker (1973).

Statistical analyses

Where experiments were conducted and repeated at separate locations and in different years, the mixed model (REML) approach was used in the analysis of data. Nematode count data was log transformed, ln (x + 1) and indices based data were square root transformed, (x + 0.5) to comply with the assumption of normal distribution. Continuous data such as yield of treatments was however not transformed. Two-way and one-way analyses of variance (ANOVA) were used for two or more factor and single factor experiments for randomized complete block design. Means were separated using the Standard Error Difference (SED) test ($P \leq 0.05$). All statistical analyses were performed using GenStat Release 8.1 (Lawes Agricultural Trust, VSN International).

RESULTS

Chemical analysis of soil

There were no differences in the initial levels of soil chemical properties at the two locations (Table 1). Significant interaction was however observed between the treatments and the soil chemical properties after the cultivation of the legumes. At both locations, *M. pruriens* significantly increased the levels of organic carbon, nitrogen, and organic matter. The weeded fallow on the other hand recorded decreased levels of organic carbon and nitrogen but increased the organic matter content of the soil at Ejura. At Fumesua, weeded fallow treatment decreased only the nitrogen content of the soil. *P. vulgaris, C. spectabilis* and *C. retusa* did not change the chemical status of the soil after cultivation (Table 2).

Tomato bioassay

Root-knot nematodes were distributed in soil from all the plots at both locations. There were significant differences between the plots assigned to the different treatments. At Ejura, the highest gall index recorded was 4.5 while the lowest was 1.9. Similarly, at Fumesua, the highest gall index was 3.5 and the lowest was 1.3. In a related experiment, a mixed population of root-knot nematodes (*Meloidogyne arenaria-incognita* complex) was identified from the plots using the Sequence Characterized Amplified Region - Polymerase Chain Reaction (SCAR – PCR) method.

The reaction of leguminous cover crops to root-knot nematode infection

The response of the cover crops to infection by *Meloidogyne* species was measured by root galling and egg mass intensity. Highly significant differences ($P < 0.01$) were observed in these variables at both sites

Table 1. Initial chemical properties of soil.

	Ejura				Fumesua			
Plot no.	pH	C %	N %	OM%	pH	C %	N%	OM%
1	0.85	6.0	0.69	0.04	5.2	0.97	0.06	0.91
2	0.80	5.9	0.56	0.05	5.5	0.92	0.06	0.78
3	0.73	6.1	0.58	0.05	5.2	0.89	0.05	0.65
4	0.69	5.9	0.66	0.08	5.5	0.93	0.07	0.76
5	0.75	6.1	0.65	0.07	5.0	0.68	0.07	0.80
P- value		0.748				0.276		
SED		0.09				0.14		

Table 2. Effect of leguminous crops on some chemical properties of soil†.

Treatment	Ejura				Fumesua			
	pH	C %	N %	OM%	pH	C %	N %	OM %
M. pruriens	6.1	1.03	0.09	1.77	5.1	1.64	0.14	2.82
P. vulgaris	6.0	0.74	0.07	1.27	5.4	1.37	0.12	2.37
C .retusa	6.2	0.92	0.08	1.58	5.2	1.03	0.09	1.87
C. spectabilis	6.0	0.81	0.07	1.39	5.5	1.09	0.10	2.12
Fallow	5.5	0.61	0.05	1.06	5.1	1.00	0.06	1.47
P- value		< 0.01				< 0.01		
SED		0.01				0.02		

†Figures are changes in initial measurements after the cultivation of the cover crops, except pH values which are absolute values.

Table 3. Gall index GI (0-10), egg mass index EI (0-10) and susceptibility index GI x EI of leguminous crops at Ejura and Fumesua.

Treatment	GI‡	EI‡	GI × EI	GI‡	EI‡	GI × EI
M. pruriens	0*	0*	0*	0*	0*	0*
P. vulgaris	7.0 (2.7)	7.9 (2.8)	55.2 (7.4)	6.9 (2.6)	8.0 (2.8)	55.6 (7.4)
C. retusa	2.6 (1.6)	3.7 (1.9)	9.7 (3.1)	2.5 (1.6)	3.0 (1.7)	7.6 (2.7)
C. spectabilis	1.8 (1.3)	2.7 (1.6)	4.9 (2.2)	2.0 (1.4)	3.3 (1.8)	7.0 (2.6)
P- value		<0.001			<0.001	
SED		(0.1)			(0.2)	

‡ (x + 0.5) transformed data used in analysis in parenthesis.Figures not used in analysis.

respectively (Table 3). Similarly, a strong interaction was observed between the treatments (leguminous crops) and gall, egg mass and susceptibility indices. *M. pruriens* did not gall and no egg mass was found. The susceptibility indices of the *Crotalaria* species were significantly low at the two locations. However, *P. vulgaris* recorded significantly higher susceptibility indices; 11 times and 8 times greater than *C. spectabilis* at Ejura and Fumeusa respectively. Populations of second stage juveniles (J2) per gram root of the cover crops were significantly different ($P < 0.01$). Juveniles per gram of root in *P. vulgaris* were approximately 13 times more than in *M. pruriens* (Table 4).

The fresh, dry shoot and fresh root weights of the cover crops were not significantly different ($P > 0.05$) at the different ecological zones. The fresh, dry shoot and fresh root weights of *M. pruriens* (76.4, 54.1 and 50.8 g) at Ejura were not different from (82.0, 58.6 and 51.9 g) at Fumesua. Similarly, *P. vulgaris* recorded (76.2, 53.1 and 84.2 g) at Ejura which were not different from (81.7, 59.7 and 79.0 g) at Fumesua. The weights of the *Crotalaria* species were also not different.

Root length of the cover crops was measured to determine the extent to which the active ingredients in the root could affect nematodes in the soil system. The cover crops ability to nodulate freely at both locations was also

Table 4. Numbers of second stage juveniles (J2) g^{-1} root of leguminous crops at Ejura and Fumesua.

Treatment	J2	J2§	J2	J2§
M. pruriens	64	(1.8)	47	(1.7)
P. vulgaris	657	(2.8)	628	(2.8)
C. retusa	158	(2.2)	124	(2.1)
C. spectabilis	114	(2.0)	100	(2.0)
P- value	<0.001			
SED	(0.05)			

Data are means of four replications.
§ln(x + 1) transformed data used in ANOVA in parenthesis.

Table 6. Nodule score (0-5) of leguminous crops at Ejura and Fumesua.

Treatment	Nodule score	Nodule score‡
	Ejura	Fumesua
M. pruriens	2.9 (1.7)	2.7 (1.6)
P. vulgaris	0.4 (0.5)	0.4 (0.4)
C. retusa	1.2 (1.0)	1.7 (1.3)
C. spectabilis	1.8 (1.2)	1.3 (1.1)
P- value	<0.01	
SED	0.2	

Data are means of four replications
‡ (x + 0.5) transformed data used in ANOVA in parenthesis.

Table 5. Root length of leguminous crops at Ejura and Fumesua.

Treatment	Root length (cm)	Root length (cm)
	Ejura	Fumesua
M. pruriens	96.3	87.5
P. vulgaris	82.0	63.8
C. retusa	64.3	24.0
C. spectabilis	51.5	19.8
P- value	<0.01	
SED	5.4	

Data are means of four replications.

studied as an index to nitrogen fixation. Significant differences in treatments (P < 0.01) were observed at both locations (Tables 5 and 6). However, there was no interaction between treatments and location. There was no difference in nodulation between *C. spectabilis* and *C. retusa* but the *Crotalaria* varieties recorded significantly higher nodulation than *P. vugaris* and lower than *M. pruriens*.

Effect of leguminous cover crops on yield of okra

There were highly significant differences (P < 0.01) regarding gall, egg mass and susceptibility indices of okra which followed the cultivation of leguminous cover crops at both locations in both years of experiment (Table 7). Okra cultivated on the plots previously sown to *P. vulgaris* recorded significantly higher (P < 0.01) gall, egg mass, susceptibility indices and J2 g^{-1} root of 59, 52, 79 and 77% than *M. pruriens* which recorded the lowest 33, 25, 51 and 46% than the weeded fallow treatment. Gall, egg mass, susceptibility indices and J2 g^{-1} root of okra from *M. pruriens* plots were not significantly different from *C. spectabilis* plots. However, okra grown on plots previously sown to *C. spectabilis* recorded significantly lower indices and population per gram of root than the weeded fallow treatment.

Highly significant differences were observed in fresh and dry shoot and fresh root weights of okra cultivated on plots previously sown to leguminous cover crops (P < 0.01) at both locations in both years. Fresh shoot weight (77.1g) of okra planted on plots previously cultivated with *M. pruriens* was significantly higher than (54 and 59 g) recorded from plots previously sown to *C. retusa* and *C. spectabilis* respectively. Similarly, dry shoot weight of 53.2 g from *M. pruriens* plots was significantly higher than 35.3 and 42 g from *C. retusa* and *C. spectabilis* plots respectively. A similar trend was observed in fresh root weight of okra as a weight of 19.4 g from *M. pruriens* plots was significantly higher than 15.5 and 15 g from *C. retusa* and *C. spectabilis* plots respectively.

Highly significant differences were observed amongst treatments regarding the yield of okra (Table 8). The yield of okra from plots previously cultivated with *M. pruriens* was the highest and exceeded the yield of okra from weeded fallow plots by 48%. Similarly, yield from plots previously grown with *C. spectabilis* out yielded that of weeded fallow by 25%. There was no difference between the yield of okra from weeded fallow and that of *P. vulgaris* plots. The performance of *C. retusa* and *C. spectabilis* were the same.

DISCUSSION

M. pruriens consistently increased the levels of nitrogen and organic matter at both locations probably due to its high biomass and higher mineralization rate. The decline in organic carbon and nitrogen levels in the weeded fallow is not unexpected. A number of tropical legumes have been used to suppress root-knot nematode population densities. For instance *Mucuna deeringiana* was used to control a variety of root-knot nematode species and races in green house and micro plots in the United States (Rodriguez et al., 1992; McSorley and Gallaher, 1992) and in Puerto Rico (Vicente and Acosta, 1987). In Martinique, *M. pruriens* was effectively used to

Table 7. Effect of earlier cover crop treatment on *Meloidogyne* spp. gall index (GI), egg mass index (EI), susceptibility index (GI x EI) and J2 g^{-1} root of okra at Ejura and Fumesua in 2004 and 2005.

Treatment	GI‡	EI‡	GI × EI	J2 g^{-1}§
M. pruriens	2.5 (1.5)	3.4 (1.8)	9.0	98 (1.9)
P. vulgaris	6.1 (2.5)	7.1 (2.7)	43.5	432 (2.6)
C. retusa	3.9 (1.8)	4.2 (2.0)	13.6	217 (2.2)
C. spectabilis	3.0 (1.7)	4.2 (2.0)	13.2	141 (2.1)
Weeded fallow	4.1 (2.0)	5.3 (2.3)	21.3	234 (2.3)
P- value	(<0.001)	(<0.001)	<0.001	(<0.001)
SED	(0.06)	(0.06)	2.1	(0.03)

Data are means of four replications.
‡ (x + 0.5) transformed data used in ANOVA in parenthesis.
§ ln (x + 1) transformed data used in ANOVA in parenthesis.

Table 8. Effect of cover crops on the yield (kg/ha) of okra in 2004 and 2005 at Ejura and Fumesua

Treatments	Predicted mean
Mucuna pruriens	3648
Phaseolus vulgaris	1872
Crotalaria spectabilis	2527
Weeded fallow	1885
Crotalaria retusa	2257
P- value	<0.001
SED	334.1

Data are means of four replications.

control *M. incognita* and *Rotylenchulus reniformis* in vegetables in polytunnels (Quénéhervé et al., 1998). In the current study, *M. pruriens* significantly reduced *Meloidogyne arenaria- incognita* complex. In addition, roots of *M. pruriens* did not gall neither were egg masses found in the root system. This observation is consistent with the work of McSorley et al., (1994). This antagonistic activity of *Mucuna* might be due to the production of phytoalexins by the roots (Vargas et al., 1996). The *Crotalaria* species were also effective in reducing nematode population. According to Fassuliotis and Skucas (1969) the nematicidal compound in the root of *C. spectabilis*, monocrotaline, is responsible for suppression of nematode density. *P. vulgaris* on the contrary stimulated population increase, roots were severely galled and significantly higher egg mass index was recorded. *P. vulgaris* was considered highly susceptible to the pest under the conditions of this study which was consistent with the observation of (Sikora et al., 2005). Okra cultivated on *M. pruriens* plots recorded significantly low gall, egg mass and susceptibility indices. The most remarkable potential became evident in J2 g^{-1} root where populations were 77 and 58% lower than those on *P. vulgaris* and weeded fallow plots respectively. The residual effect of *M. pruriens* did not only control mixed population of root-knot nematodes and increased plant growth but also resulted in significant yield increase. *M. pruriens* out yielded the control and *P. vulgaris* treatments by 48 and 49% respectively. There was no difference in yield between *P. vulgaris* which was considered highly susceptible to the *Meloidogyne arenaria-incognita* complex and the weeded fallow (control) treatment.

Conclusion

The study has shown that *M. pruriens, C. spectabilis* and *C. retusa* were all effective in suppressing nematode population densities. *M. pruriens* however could be the most preferred management candidate for the following reasons: In addition to all the benefits accrued from inclusion of legumes in cropping systems, *M. pruriens* provides excellent hay for livestock, and its seeds are used as feed supplement after processing (Maasdorp et al., 2001). More importantly, *M. pruriens* is used for food in some African countries (Rachie and Roberts, 1974). Fallowing also causes some challenges in nematode management, hence a pulse crop without antagonistic effect can be included to maintain the population in the field.

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