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EVALUATION OF ORGANIC MATERIALS FOR INHIBITION OF NEMATODE REPRODUCTION IN SOYBEAN

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ABSTRACT

Synthetic pesticides have been found to increase the yield of agricultural products over tenfold. However, the effects of pesticide misuse around the world include costly environmental pollution and disruption of the balance of nature in addition to their high cost and non-availability. Hence the need for search for alternative pesticide formulations to avert these undesirable effects. A study was conducted to evaluate the efficacy of Neem (*Azadirachta indica* A. Juss) leaf powder and organic fertiliser for management of *M. incognita* in soybean (*Glycine max)* production. A screenhouse experiment was conducted to test the efficacy of neem leaf powder (NLP) and organic based fertiliser (OBF), alone and a combination of 50% each of the NLP and OBF against root galling and *Meloidogyne incognita* multiplication on soybean and their potency in grain yield improvement. The experiment was laid out in completely randomised design with three replications. Although all treatments were effective in reducing root galling and nematode reproduction, NLP applied alone gave the highest reduction (92.59 %) in gall formation and nematode multiplication (82.07 %), followed by NLP + OBF and then OBF alone. Increases in grain yield ranged from 103.08 to 108.46% in response to organic soil amendment. Integration of the two organic substances is a better and more promising strategy since it reduced the quantity of organic fertiliser that had to be transported to farmer's field by 50%.

Key Words: *Azadirachta indica*, *Glycine max*, *Meloidogyne incognita*, organic fertiliser, root-knot nematodes

RÉSUMÉ

Il a été révélé que les pesticides synthétiques augmentent le rendement des produits agricoles au delà de dix fois ; malgré les effets de l'utilisation abusive de pesticides dans le monde entier, y compris la pollution environnementale et la perturbation de l'équilibre de la nature en plus de leur coût élevé et la non-disponibilité. D'où la nécessité de formulations pesticides de remplacement pour éviter ces effets indésirables. Une étude avait été menée afin d'évaluer l'efficacité de la poudre de feuilles de Neem (*Azadirachta indica* A. Juss) et des engrais organiques dans la gestion de *M. incognita* dans la production de soja (*Glycine max)*. Deux expérimentations en serre avaient été menées afin de tester l'efficacité de la poudre de feuille du neem (NLP) et de l'engrais à base organique (OBF), seul et en combinaison de 50 % pour chacun de NLP et OBF contre le galle racinaire et la multiplication de *Meloidogyne incognita* du soja ainsi que de leur efficacité dans l'amélioration du rendement en grain. L'expériementation avait été conduite dans un dispositif complétement aléatoire avec trois répétitions. Bien que tous les traitements étaient efficaces pour la réduction de la galle racinaire et la reproduction des nématodes, lorsque appliquée NLP seule, avait donné la réduction la plus forte baisse (92.59 %) dans la formation de galle et la multiplication des nématodes (82,07 %), suivie par NLP + OBF, et ensuite OBF seul. L'augmentation dans le rendement en grain varié de 103,08 à 108,46 % en réponse à l'amendement organique du sol. L'intégration de ces deux substances organiques est la meilleure et la plus prometteuse stratégie car il réduit de 50 % la quantité d'engrais organiques qui doit être transportés au champ de l'agriculteur.

Mots Clés: *Azadirachta indica*, *Glycine max*, *Meloidogyne incognita*, engrais organiques, nématodes à galle racine

INTRODUCTION

Although, synthetic pesticides have been found to increase the yield of agricultural products over tenfold the effects of pesticide misuse around the world are on the increase and include costly environmental pollution and disruption of the balance of nature in addition to their high cost and non-availability (IITA, 2000). Indiscriminate use of synthetic chemicals for pest control has also led to problems such as pest resistance, adverse effect on non-target organisms and health hazards to the users. Unsafe use of pesticides may result in poisoning of humans which is a problem especially in developing countries (Yudelman, *et al*., 1998).

There is, therefore, need to develop alternatives to synthetic pesticides that are effective, ecologically safe and economical (Rajapakse, 1990). As such, scientists sought and discovered several alternative and effective non-chemical means of managing pest resurgence and population. Among the known and outstanding natural strategies for reducing nematode pest problems is the use of organic materials (Akhatar and Mahmood, 1996). There is no known negative effect of organic soil amendment on non-target organisms. McSorley and Frederick (1999) observed that all genera of plant-parasitic nematodes, except *Belonololaimus,* were suppressed by organic soil amendments. Other alternatives include use of botanicals derived from very cheap and renewable resources, especially the tropical plants.

The use of botanicals in parasitic nematode control has received global attention. Toxicity of extracts of different plants on nematodes have been reported by many scientists (Onifade and Egunjobi, 1994; Hassan *et al*., 2001; Adegbite and Adesiyan, 2005; Javed, *et al*., 2007) to be comparable with synthetic nematicides (Adegbite and Adesiyan, 2001). Neem (*Azadirachta indica* A. Juss) products, including leaf, kernel and seed powders, seed extracts and oil have been reported to control several agricultural pests including plant-parasitic nematodes (Ivibijaro, 1983; Schmutterer, 1990; Akhtar and Mahmood, 1994).

Bioactive products from neem tree are effective in controlling approximately 16 species of plant-parasitic nematodes (Akhatar and Alam, 1993). Neem products may provide an alternative, sustainable and inexpensive means of managing *M. incognita* (Mi) infecting soybean pests. Although individual organic substances are known to be effective in the management of plant-parasitic nematodes, integrating the various natural means may provide a better nematode management strategy. This study was aimed at investigating the efficacy of integration of Neem leaf powder and organic fertiliser for management of *M. incognita* of soybean.

MATERIALS AND METHODS

The soil used for the experiment was collected from the Teaching and Research farms at the University of Agriculture, Abeokuta, Nigeria. The soil was sandy-loam (80 sand, 6.2 clay and 13.80% silt) with a pH of 6.10. The soil was heat sterilised using an electric soil steriliser at 65^{0}C for 90 minutes. The soil was then allowed to cool and later stored in jute sacs to rest for six weeks to regain its stability.

The experiment was carried out in completely randomised block design with six replications. Treatments included:

(i) Nematode-inoculated soybean without Neem leaf powder and Organic based fertiliser (control);
(ii) Nematode-inoculated soybean + Neem leaf powder;
(iii) Nematode-inoculated soybean + Organic based fertiliser; and
(iv) Nematode-inoculated soybean + Neem leaf powder + Organic based fertiliser.

The Neem leaves used were sourced from the University of Agriculture, Abeokuta in Nigeria. Preparation of the Neem leave powder was done by sun-drying the leaves for two weeks, after which the leaves were ground to powder. Bontrager's test (Sofowora, 1993) was employed to determine the presence of anthraquinones. A 2 g sub-sample of the Neem leaf powder was poured into 20 ml concentrated H_2SO_4 and boiled for 15 minutes before 20 ml of benzene was added and filtered. Ten mililitre of 10% ammonia solution was added to the cooled filtrate extract. A pink coloration indicated the presence of anthraquinone, the active ingredient (Azadirachtin). Chemical composition for Neem leaves was evaluated using routine analysis (Table 1). The sterilised soil was analysed for physiochemical properties (Atungwu and Kehinde, 2008).

Sowing of the seed. The soil was thoroughly mixed to ensure homogeneity. Six-litre plastic pots were filled with 4 kg of the sterilised soil. The soil was mixed with 1% (v/v) NLP or OBF or a mixture of 0.5 % each of NLP and OBF, arranged in completely randomised design. The pots were watered daily for 14 days to allow mineralisation of the organic substances incorporated. Two weeks after application of the soil amendment, four seeds of Mi-susceptible soybean variety, TGx 1019-2EN were planted per pot, but selectively thinned to one per pot six days after emergence to ensure uniform plant vigour.

Preparation of inoculum. Eggs of nematodes were extracted from 60 day old *M. incognita*-infected roots of *Celosia argentea* on which pure culture of the nematode was multiplied. The identity of the nematode was confirmed using perineal pattern described by Eisenback *et al.* (1981). To extract eggs, galled roots of *C. argentea* were thoroughly washed in cool tap-water in order to remove soil. Washed roots were mopped dried with white tissue paper and then chopped into 1-2 cm pieces. Root segments were placed into conical flasks and 0.52% sodium hypochlorite (NaOCl) solution added into each flask, corked with a stopper; and shaken manually but vigorously for 3-4 minutes to dissolve the gelatinous egg matrix (Hussey and Baker, 1973). Eggs freed from the egg sacs in the diluted NaOCl were rinsed under gentle stream of cold tap-water for 5 minutes then collected on a 500-mesh sieve. Eggs were calibrated in a 500 ml beaker for purpose of inoculating soybean seedlings.

TABLE 1. Chemical composition of neem leaf powder used for the experiment in Nigeria

Analytical value	Parameter
Na (%)	0.35
K "	2.28
Ca "	0.60
Mg "	0.56
P "	1.20
N "	0.10
m.c. "	8.90
Acidity (mg kg)	0.18
pH (H_2O)	4.60
Azadirachtin (%)	6.90

m.c. = moisture content

Inoculation. Seven day old seedlings of soybean were inoculated with approximately 5000 eggs of *M. incognita,* by pipetting approximately 5000 egg suspension into a shallow trench close to the base of each plant and covered lightly with sterilised top soil. Inoculated plants were observed weekly for plant growth and appearance of symptoms of nematode disease (chlorosis, wilting and stunted growth). At 60 days after inoculation, three replicates of plant roots were gently lifted from the soil, washed using water and manually scored for number of root galls. Juveniles *M. incognita* remaining in soil were extracted using the Whitehead and Hemming (1965) method. Eggs (second-stage juveniles) extracted from the root tissues were counted under stereomicroscope and recorded. The remaining three replicates of soybean plants were grown to maturity; harvested, threshed manually and winnowed to obtain the seed.

Data were collected on number of pods per plant, seeds per plant and grain weight. Data collected in 2006 were statistically similar to those of 2007; therefore, they were pooled before subjecting to analysis of variance (ANOVA) using Generalise Linear Model of SAS version 8.1. Statistically different means were separated using Least Significant Difference (LSD) at 5 % level of probability.

RESULTS

Table 2 depicts comparative effectiveness of organic based fertiliser (OBF) and neem leaf powder (NLP) on root gall formation and reproduction capacity of *M. incognita* in the test soybean variety. There was a significant difference (P< 0.05) in the effect of NLP and OBF applied singly or in combination compared to untreated control in all the parameter examined. The number of root galls incited by *M. incognita* on roots of TGx 1019-2EN peaked at 12.68 in the control but ranged from as low as 0.94 in NLP-treated plants to 5.46 in OBF-treated plants. Plants treated with NLP had the highest reduction efficacy (92.59%), followed by NLP + OBF (75.39%) and OBF (56.94 %). OBF or its combination with NLP was not significantly different (P>0.05) in ability to sufficiently reduce root galling in soybean as induced by Mi. Neem leaf powder contained 6.9% active ingredient, azadirachtin (Table 1).

The highest population (212.00) (Table 2) was recorded in the control treatment, while the population ditched to as low as 37.05 in NLP-treated plants representing 82.52% reduction followed by NLP + OBF (51.30%) and OBF (47.90%). Whereas the effect of NLP applied alone was statistically different (P<0.05) from OBF or NLP + OBF incorporation, there was no difference between the latter two treatments.

The reproductive capacity of the nematode was significantly (P < 0.05) affected by treatments imposed on the plants (Table 2). The highest rate of reproduction of Mi (2.90) was observed in the untreated plants, which was significantly different from the 0.52, 1.56 and 1.46 recorded in plants administered with NLP, NLP + OBF, and OBF, respectively. The rate at which the nematode reproduced was significantly (P <0.05) lower in NLP treatment than OBF or NLP + OBF treatment. The ability of the nematode to reproduce was inhibited by 82.07, 49.66, and 46.21%, in NLP, NLP +OBF, and OBF application, respectively.

Table 3 shows the effectiveness of organic soil amendment with NLP, OBF and NLP + OBF on grain yield improvement of Mi-Susceptible soybean, TGx 1019-2EN. Significant variation (P<0.05) was observed in grain yield increase due

TABLE 2. Effect of organic based fertiliser and neem leaf powder on root galling and *Meloidogyne incognita* reproduction on soybean variety, TGx 1019-2EN in Nigeria

Treatment	Galls per root system	Nematode population per plant	Reproduction factor
Untreated	12.68	212.00	2.90
Neem Leaf powder (NLP)	0.94 (92.59)	37.05 (82.52)	0.52 (82.07)
Organic based fertiliser (OBF)	5.46 (56.94)	110.46 (47.90)	1.56 (46.21)
NLP + OBF	3.12b (75.39)	103.25 (51.30)	1.46 (49.66)
Mean	5.55	11.69	1.61
LSD (0.005)	4.48	38.62	0.55

Figures in parentheses represent percentage reduction

TABLE 3. Effect of organic based fertiliser and neem leaf powder on grain production *Meloidogyne incognita*-susceptible soybean variety, TGx 1019-2EN in Nigeria

Treatment	Pods per plant	Seeds per plant	Grain yield per plant (g)
Untreated	4.10	6.20	1.30
Neem Leaf powder (NLP)	6.19 (50.98)	8.43 (35.97)	2.64 (103.08)
Organic based fertiliser (OBF)	5.95 (45.12)	8.10 (30.65)	2.71 (108.46)
NLP + OBF	6.85 (67.07)	8.69 (40.16)	2.64 (103.08)
Mean	5.77	7.86	2.24
LSD (0.005)	1.74	2.16	0.48

Figures in parentheses indicate percentage increase to application of the two organic. However, the organic base treatments were not significantly different (P<0.05). Number of pods and seeds per plant were at par under NLP, OBF, and NLP + OBF treatments. These treatments gave rise to 45.12 - 67.07, 30.65 - 40.16 and 103.08 – 108.46% increments in number pods, number of seeds and weight of grains per plant, respectively.

DISCUSSION

Effectiveness of Neem leaf powder and a novel organic based fertiliser recently developed from city refuse, applied singly and in combination, has been demonstrated. All treatments proved to be efficient in restraining soil population of the root knot nematode and root galling, with a corresponding improvement in grain yield. Both substances and their blend gave more than 100% yield increase over the control. This is indicative that the two substances, in addition to improving nutrient supply, had nematicidal properties.

Soil organic amendments have been proved to substantially increase soil health (Neher, 2001), environmental wellness (Adegbite and Adesiyan, 2005) and sustainable crop production. Hassan *et al.* (2001) reported that owing to environmental pollution and costliness of synthetic pesticides, chemical control no longer holds future in sustainable agricultural production, thus, justifying attempts by previous workers to develop non-chemical alternative control technologies using organic substances (Hassan *et al*., 2001; Adegbite and Adesiyan, 2005; Renco *et al*., 2007; Atungwu and Kehinde, 2008). Some organic substances commonly used in above treatment for *M. incognita* include Garlic and pineapple (Hassan *et al*., 2001), Neem and *Chromoleana odorata* (Adebgite and Adesiyan, 2005) and composted manure (Everts *et al*., 2006).

Soil amendments with organic materials have long been adjudged valuable in the management of plant-parasitic nematodes (Neher, 2001). In the presented work we further tested a blend of two indigenous organic substances as a means of controlling root-knot nematode populations, effective reduction of the rate of reproduction and the disease caused in soybean roots to significantly low levels.

Reduction in *M. incognita* population in this study could have been as result of nematophagous fungi in the decomposing organic materials in conformity with Linford's (1937) observations. Soil treated with the organic substance had reduced nematodes probably as a result of direct or indirect effect of the substances. Neher (2001) provided evidence that increased the nematode community diversity resulting from organic soil amendment has the potential for preventing the domination of the nematode community by a single species, encouraged the activity of nematode antagonists, and increased linkages within the soil food web. We have added neem to organic fertiliser because past experimental data on the enhancement of nematode-trapping fungi by organic matter addition lasted for only short periods of time, and does not typically exert a strong effect on nematode population densities as proclaimed by Kerry (1987).

The bulkiness of poultry manure needed for application to give visible yield improvement remained a major constraint in its adoption by resource-poor farmers in sub-Saharan countries. Our data supported the judgement that combining NLP with OBF which supplies the needed N for growth (Neher, 2001) is a logical strategy of reducing the quantity of manure needed to obtain the same increase in grain yield. NLP primarily provides Azadirachtin (Javed *et al*, 2007), an active ingredient that probably inhibits egg hatch or enhances larval mortality (Adegbite and Adesiyan, 2001) or slows down fecundity of female (Javed *et al*., 2007).

Significant reduction in nematode populations in our study could be linked to Johnson's (1957) view that decomposition of organic substances by bacteria is capable of producing compounds that are toxic to plant-parasitic nematodes; and Fakunle's (1972) findings that neem leaves are rich in tannins which may be nematostatic. It was evident from Miller *et al.* (1973) that biologically active ingredients inherent in neem leaves extract had nematicidal potentials, that are easily released by heat or bacterial degradation in soil.

CONCLUSION

It is evident that the effectiveness of NLP and OBF in suppressing nematode population in soybean is enhanced by blending the two organic substances. Since the use of synthetic nematicides by subsistent farmers is plagued with several limitations, such as prohibitive cost and lack of technical expertise in their application, among others, blending of organic substances of different origin will forestall large-readily-available supply of these materials that limit practical use of soil amendment practices for the control of pests.

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