Efficacy of Neem Seed Powder on Root Knot Nematodes (*Meloidogyne* spp.) Infecting Bambara Groundnut (*Vigna subterranea*)

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ABSTRACT--- ***Notwithstanding its economic importance, the production of bambara groundnuts (Vigna subterranea) is threatened by root knot nematodes (Meloidogyne spp.). The withdrawal of synthetic nematicides, however, from local markets has increased the demand for alternative products with nematicidal or nematostatic effects. Neem seed powder was evaluated at different application rates (15, 20, 30 g) and a control at planting to determine its effect on Meloidogyne spp. and yield of Vigna subterranea cv 'Tom' during the cropping season of 2012 on Research fields of the University for Development Studies, Nyankpala, Ghana. Results from the trials indicated that neem seed powder at 30g reduced the population of the pest and increased pod yield by 20% compared to the control. Neem seed powder at 30g could therefore be employed as a viable botanical nematicide against root knot nematodes attacking bambara groundnut instead of synthetic chemicals.***

Keywords— *Azadirachta indica*, bambara groundnut, *Meloidogyne* spp. and root galling

1. INTRODUCTION

Bambara groundnut (*Vigna subterrenea* L. Verdc.) is a tropical leguminous crop that contains 4.6% oil, 16-21% protein and 50-61.3% carbohydrate (Baudoin and Mergeai, 2001). It is one of the important grain legumes consumed in Ghana (Doku, 1996) and South of the Sahara (Ocran *et al*., 1998). It ranks third to cowpea and groundnut although the seeds are highly nutritious and contain more lysine and methionine than either cowpea or groundnuts. In Ghana, for example, it is an important food security crop in the savanna and transitional agro-ecological zones (Berchie *et al*., 2011) Bambara groundnut yields are generally low in Africa, averaging between 650 and 850 kg/ha (Rowland, 1993). One of the major causes of the low yields of bambara groundnut, apart from genetic potential, is infestation of diseases and nematodes. The root knot nematode *Meloidogyne javanica* according to Gwekwerere, (1995) is one of the serious pest that threatens the crop. Heavy infestation results in yellowing of foliage, stunting, and reduction in root systems and reduced pod weight thereby affecting yield. Traditionally, the management of root knot nematodes has mainly depended on the use of synthetic chemicals. However, indiscriminate application and drifting of these chemicals can be harmful to other beneficial and untargeted organisms. The identification and use of local plant materials for the control of nematodes is a current area of research in plant Nematology (Agbenin *et al.*, 2005). This method of controlling *Meloidogyne* spp. is appealing because of the growing problem of environmental pollution arising from the use of persistent pesticides like chlorinated hydrocarbons (Agbenin *et al*., 2005). Several works on extracts from various indigenous plants and neem (*Azadirachta indica* A. Juss.) products have revealed that some of them are effective against insects and nematodes (Khanna and Sharma, 1998; Sharma, 2000). The fresh extracts of fruits, leaf bark, root and gum of neem inhibited hatching of *Meloidogyne incognita* (Siddiqui and Alam, 1985). Apart from their potential in managing nematodes, the efficacy of a plant product can be affected by its application rate. In the present study, different levels of neem seed powder were evaluated to identify the optimum level at which it can be applied to manage root knot nematodes under field conditions.

2. MATERIALS AND METHODS

2.1 Study area

The trial was conducted on the research fields of the Faculty of Agriculture, University for Development Studies, in Nyankpala, Tamale (9°25'41"N, 0°58'42"W) which lies within the Guinea Savanna Zone of Ghana. Annual rainfall

ranges between 1000–1200mm with a temperature range between 21°C-32°C (SARI, 2007). The soil is moderately brown and drained sandy loam. The area is characterised by natural vegetation dominated with few shrubs.

2.2 Source and preparation of neem seed powder

Physiologically matured neem seeds were harvested from Nyankpala and dried for 3 days. The dried seeds were dehusked, winnowed and the mesocarp pounded into powder with a domestic wooden mortar and pestle. The powdered seeds were then weighed into 15g, 20g and 30g for application.

2.3 Sampling and extraction of nematodes

Soil samples were collected at two time periods; at the start of the trial before planting and at harvest of the crop with a 5 cm soil auger to a depth of 20 cm. Nematodes were extracted using the modified Baermann tray method (Whitehead and Hemming, 1965). After 24 h of extraction, nematodes were fixed in TAF (37% formaldehyde 7.6 ml, Tri-ethanolamine 2 ml and distilled water 90.4 ml) and third and fourth stage nematodes were mounted on aluminium double-coverglass slides. Root knot nematode specimens were then identified (CIH, 1978) under a compound microscope at a magnification of 100x.

2.4 Experimental set up and source of bambara groundnut Seeds

The study was laid out in a randomised complete block design with 3 replications. The field was ploughed, harrowed and the seeds planted on the prepared field. Plots were laid out in four rows of 1 m wide by 10 m long. Seeds were planted at a distance of 50x20cm with 2 seeds per hole and later thinned to a seedling per hole 14 days after sowing. Bambara groundnut cv. 'Tom' was used for the study. The seeds were obtained from farmers stock at Nyankpala. The seeds were not treated with any pesticide before sowing.

2.5 Application of treatments

The trial was laid out in a complete randomised design with three replications. The neem seed powders were applied at 2 different periods. The first application was done 1 week before planting as pre-plant and 21 days after sowing the seeds as a post plant soil amendment.

2.6 Data collection and analysis

Data on plant height were taken every two weeks, whilst plant fresh shoot weight and yield were taken at harvest. Number of days to 50% flowering was also taken. Second stage juveniles/ 200cc soil was determined at harvest. Root galling and number of egg mass on roots of bambara groundnut were scored based on Bridge and Page (1980) and Taylor and Sasser (1978) description respectively. Yield, being continuous data was not transformed but nematode count and index based data were log(x+1) transformed to improve homogeneity of variance. Statistical analysis was performed using Genstat 8.1 software in one way analysis of variance (ANOVA) in randomised blocks. Significant mean separation was determined with Fisher's least significance test at $\alpha = 0.05$

3. RESULTS AND DISCUSSION

3.1 Effect of treatments on plant height of bambara groundnut

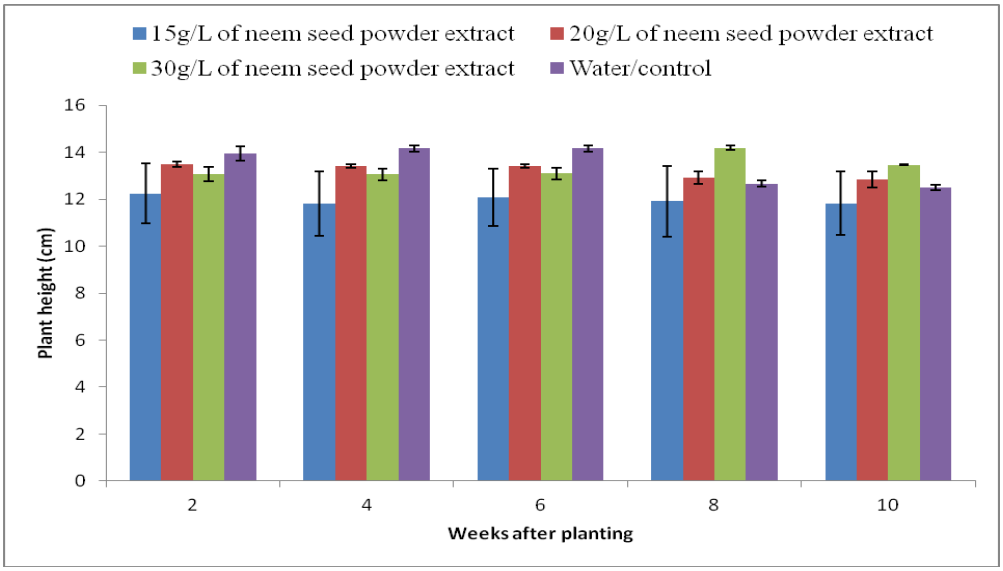

Figure 1**: Effect of neem seed powder on plant height of at two weeks interval after planting (Bars represent SEM)**

Ten weeks after applying the treatments, 30g NSP treated plants recorded the highest plant height compared to all the other treatments and the control (Figure 1). However, plants treated with 15g NSP showed no significant difference with the control plot. It was also observed that plant height declined in the controlled plots throughout the trial. This finding corroborates with the findings of Rai and Yadav (2005) who reported severe root knot nematode infestation, resulted in stunted growth. The increase in plant height of 30g NSP can be attributed to the NSP reducing activities of the pest to a minimum level which subsequently enabled the roots of the crop to absorb water and soil nutrients for the plant development.

3.2 Number of leaves

Figure 2 shows growth pattern as measured by the number of plant leaves, 30g NSP recorded the highest number of leaves at harvest. This was significantly different ($p < 0.05$) from the control plot. Similarly application of NSP at 20g per plant also increased the number of leaves compared with the control. The increase in leave number can be attributed the NSP reducing nematodes activities thereby improving on nutrient absorption by the roots for the plant's development. This agrees with Kumar and Khanna (2006) who reported that a formulation of neem based products at all concentrations was able to increase the number of leaves per plant of tomato.

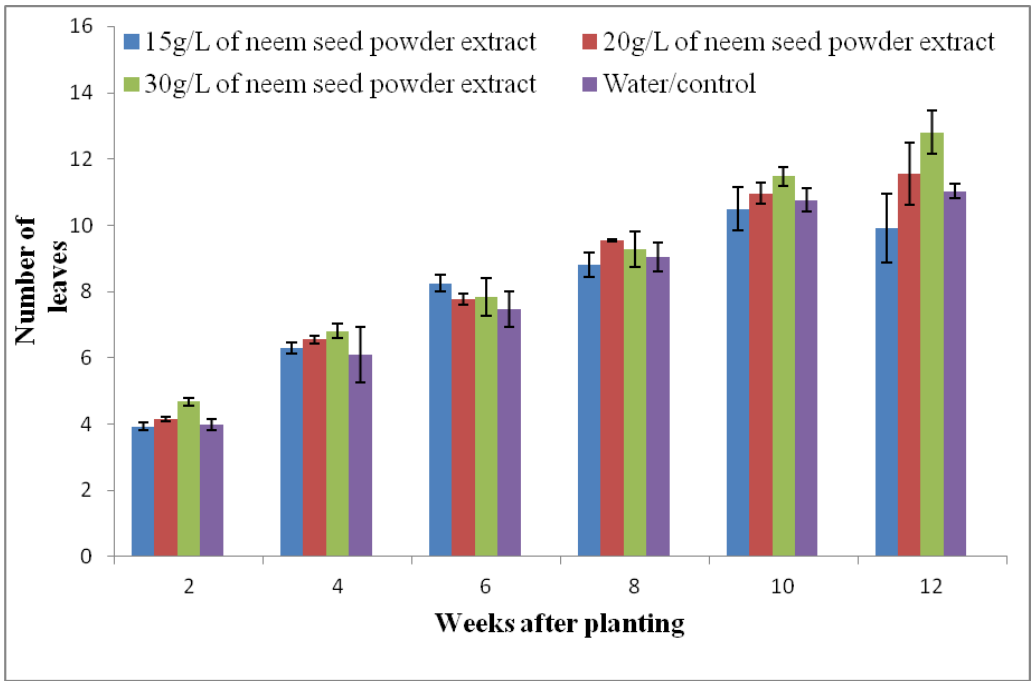

Figure 2: Effect of neem seed powder on number of leaves of at two weeks interval after planting (Bars represent SEM)

3.3 Effect of NSP on number of pods, fresh shoot weight and pod yield of bambara groundnuts

Results from the study shows that plants treated with 30gNSP recorded the highest mean number of pods at harvest (Table 1). There was however no difference between it (30g NSP) and all the other treatments and control plots. Fresh shoot weight recorded ranged between 82.40g to 107.7g for the control and 30g NSP respectively (Table 1). There was however no significant different in terms of fresh shoot weight. Yield recorded fewer than 30g NSP was found to be 20% higher than the control plot. The number of pod per plant was found to correspond with pod yield. According (Akbar *et al.*, 2010) pods produced per plant are positively correlated with yield just as yield is a function of plant population. All other things being equal, the higher the number of pods/plant, the higher the yield (Osei *et al.*, 2013).

Table 1: Effect of neem seed powder on the number of pods, fresh weight, pod weight of bambara groundnuts

Treatment	Mean number of pods/plant	Fresh weight (g)	Yield (g)
15g NSP	13.0	88.80	289.0
20g NSP	13.0	99.80	321.0
30g NSP	14.0	107.70	338.0
0g NSP (Control)	12.0	82.40	269.0
LSD (0.05)	2.9	39.70	10.05
CV (%)	12.00	22.30	17.50

3.4 Effect of NSP on root knot nematodes population, root galling and egg mass indices

The potential of NSP in reducing nematodes population and activities were exhibited in the results of the study. The control plot recorded the highest number of second stage juveniles and gall index compared to all the treatments (Table 2). Compared with all the other treatments and control, 30g NSP was able to suppress root knot nematodes population and galling significantly. Root gall index according to Kumar and Khanna (2006) is a useful parameter to evaluate the effect of neem formulations on *Meloidogyne* spp. This means that the efficacy of a neem base product to manage root knot nematode attack is dependent on its potential to minimise the pests activities which is evident in the formation of galls. The results of the study also agrees with Siddiqui and Alam (1990) who reported that the application of nimbin (neem based product) as seed dressing significantly reduced root knot nematodes population on tomato.

Table 2: Effect of neem seed powder on root knot nematodes population, root galling and egg mass indices on roots of bambara groundnuts

Treatments	Root knot nematodes pop/200cc soil	Mean root gall index (0-10)	Mean egg mass index (1-5)
15g NSP	103 (3.0)	3.33	1.66
20g NSP	72 (2.9)	2.33	2.00
30g NSP	42 (2.6)	2.00	1.33
0g (control)	1714 (4.2)	4.67	2.66
LSD (0.05)	79.2(0.2)	0.49	0.49
CV (%)	8.70	12.10	14.30

Figures in parenthesis are transformed values

4. CONCLUSION

The study found that applying 30g neem seed powder per plant as a pre and post planting soil amendment was able to suppress root knot nematodes population, reduce galling and subsequently improved growth bambara groundnut.

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