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BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE (*MELOIDOGYNE INCOGNITA*) IN SWEET PEPPER USING NEMAX AND BIO NEEM

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ABSTRACT: The root-knot nematode (RKN) *Meloidogyne incognita*, is a devastating parasite to sweet pepper and is responsible for yield losses up to 15%. RKN is usually controlled by applying synthetic nematicides which have severe negative effects on human health and the environment. These nematicides have been banned from use and producers now seek alternative eco-friendly nematode management strategies. A greenhouse study was conducted to determine the optimum effective doses of NeMax (sesame extract) and Bio Neem (neem extract), and to compare them with Vydate L to evaluate their efficacy at controlling RNI in sweet pepper. From the results, all nematicides caused a reduction in *R. similis* population density. NeMax at high rates (80 ml/L) was comparable to Vydate L at reducing RKN by over 80% in the roots ($P \leq 0.05$). Bio Neem at recommended rates (15 ml/L) was effective at reducing RKN by 52% in the roots and its efficiency in the soil was comparable to NeMax. Vydate L had the fastest knockdown rate of all nematicides but the continuous nematoxic and nematostatic effects of NeMax and Bio Neem prolonged the reoccurrence rate of RKN. The advantages of using these bio-nematicides and their effects on sweet pepper production are discussed.

Keywords**:** Bio-nematicide, *Meloidogyne incognita,* neem, sesame, sweet pepper.

Introduction

In Trinidad, sweet pepper (*Capsicum annuum* L.) is an important crop that is produced for local markets and regional export (Ramdial and Rampersad 2010). This crop is adversely affected by a complex of fungi, viruses, insects and nematodes, the latter being an important limiting factor in greenhouse production (Calipas 2002). Several nematode species adversely affects sweet pepper but the root-knot nematode (RKN) *Meloidogyne incognita*, is considered the most devastating and is responsible for yield losses up to 15% (Ploeg 2009; Noling 1999). Second-stage juvenile (J2) RKN feeds on the roots causing large galls or "knots" to form throughout the root system. Severe infections result in reduced yields and can also affect consumer acceptance of the crop (Mitkowski and Abawi 2003). Infected plants are usually stunted, chlorotic, easily wilted, and unproductive (Olsen 2000). Effective RKN control is therefore essential for the survival of the sweet pepper industry in Trinidad.

Root-knot nematode control in Trinidad has been based mainly on the use of non-fumigant synthetic nematicides (Rosenstock et al. 1991). These products are now prohibited due to health concerns to production workers and their negative impacts on the environment (Nagaraju et al. 2010). This has intensified the search for alternative forms of nematode control that is effective with low mammalian toxicity (Nagaraju et al 2010; Mc Sorley 1996).One alternative is the utilization of plant extracts with nematicidal activity (Mc Sorley 1996). Studies have shown that extracts from sesame and neem plants have nematoxic and nemastatic effects and can disrupt nematode movements to the roots (Hussain et al 1984; Siddiqui and Allam 1989). However, the adoption of such management strategy has been met with scepticism by sweet peppers producers, because the efficacy of these plant extracts is still inconsistent (Villanueva 2005).The objectives of this greenhouse study were to determine the optimum effective dose of NeMax (sesame extract) and to compare it with Bio Neem (Azadirachtin) and Vydate L (Oxamyl) to evaluate their efficacy at controlling RNI in sweet pepper.

Materials and Method

A greenhouse study was conducted from January to March 2012 at a commercial greenhouse (10 m X 25 m) in Cumoto, Trinidad (10.6017°N, 61.2117°W). The average monthly temperature and precipitation at Cumoto were 26.7°C and 93.98 mm, respectively for the length of the study.

Experimental Design

The study consisted of 120 sweet pepper plants spaced 45 cm apart in six beds (20 plants per bed). The experiment was arranged in a completely randomized block design with six treatments and twenty replicates, each plant being a replicate. Sweet pepper plants (cv. Aristotle) were utilized and no nematode pre-planting treatments was applied. The growing medium comprised of unsterilized Aquoxic Tropudults (55 Piarco loamy sand series) soil, with a CEC of 4.30 cmol/kg and a pH of 4.1. Treatments included three commercial nematicides: NeMax at rates of 40 ml/L, 60 ml/L and 80 ml/L, Bio Neem at 15 ml/L, and Vydate L at 25 ml/L, along with one controls (Table 1).

Plants were grown in unsterilized topsoil with a moderately high nematode presence (estimated 400 J2 RKN per 100cc soil). Approximately 80 days after planting the treatments were applied at the base of each plant using a knapsack sprayer. The application rates and frequencies of each treatment are given in Table 1. Agronomical management was done according to the protocols recommended for greenhouse sweet pepper production, which included fertilization, irrigation, pruning and training, pollination, and pest and disease management (Jovicich et al. 2004).

The number of *R. similis* in the roots and soil were estimated one week after treatment application using the extraction methodologies described by Southey (1986) for the roots, and Whitehead and Hemming (1965) for the soil. The *R. similis* density was estimated fortnightly for the roots and weekly for the soil.

Table 1: Active Ingredients, sources, application rates and frequencies of treatments used.

Treatments	Active Ingredients	Source	Application rate(s)	Application Frequency
Control	No treatment	-	-	-
NeMax	Sesame Oil	Brandt Consolidated, Inc	40ml/L 60ml/L 80ml/L	3 applications @ 7 days intervals.
Bio Neem	Azadirachtin 3000 ppm	Marketing Arm International	15ml/L	3 applications @ 7 days intervals.
Vydate L	Oxamyl	E.I. Du Pont de Nemours and Company	25ml/L	3 applications @14 days intervals.

Data Collection: Radopholus similis Population Density

Procedure

The roots were carefully washed, chopped into 1-2 cm pieces and macerated in a kitchen blender for 15 seconds. The soil was crushed and mixed thoroughly. The blender nematode filter extraction method was used to extract nematodes from 15 g of roots and the modified Whitehead tray method was used to extract nematodes from 200 ml of soil (Southey 1986; Whitehead and Hemming 1965). The extracted solutions were then decanted and passed through a 25-µm sieve. The collected nematode sample was identified and counted in three 1-ml aliquots out of a 20 ml aqueous suspension with a stereoscopic microscope using 1 ml counting dishes. All vermiform developmental stages (juveniles and adults) were counted. Nematode population densities were log (x+1) transformed prior to analysis.

Data Analysis

Differences in the nematode density were analyzed with the use of analysis of variance (ANOVA). Prior to analyses, variables were tested for homogeneity of variances and normality, and data found to be non-homogenous were either Log10 (X + 1) or square root transformed before statistical analysis. Non-transformed means were reported in Figures and only significant differences ($P \leq 0.05$) will be discussed unless stated otherwise. All statistical analysis was performed using the statistical software Minitab® 16.1.1 (Minitab Inc).

Results and Discussion

Throughout this study all nematicidal treatments caused a reduction in RKN density in sweet pepper roots and soil. This reduction varied significantly ($P \leq 0.05$) in response to different treatments. In Trinidad, synthetic nematicide such as Vydate L (Oxamyl) has set the benchmark for RKN control in sweet pepper production and was used in this study as a standard for comparing the bio-nematicides. From the results, reductions in RKN in the soil were greater in plants treated with Vydate L than the bio-nematicides (NeMax and Bio Neem) (Figure 1). This was due to the high toxicity of Vydate L which was very effective at reducing RKN populations. Vydate L was also effective at reducing RKN in the roots and was comparable to NeMax (Figure 2). McGarvey et al. (1984) reported that Oxamyl if used in low concentration appears to act as a nematostat, protecting the roots by inhibiting the orientation or feeding ability of nematodes. However, the recommended concentration for Vydate L use in most crops is high enough to be extremely dangerous to human health and can cause phyto-toxicity in many crops.

The bio-nematicides were also effective at reducing soil borne J2 RKN (Figure 1). This reduction may be due to the nematostatic effects of sesame extracts and Azadirachtin on nematodes reported by Thomson (1992). If this nematostatic effect lasts long enough, the nematodes may eventually starve and the mortality rate will increase. Therefore, the effectiveness of these nematistats depend on the length of time the nematodes remain immobilized and disoriented (Schomaker and Been 2006). However, if the concentration is suitable for RKN survivorship, the nematode can recover rapidly and become parasitic once again. At high concentration (80 ml/L), NeMax was effective at maintaining a long lasting nematostatic effect which caused an increased RKN mortality rate in the soil. A similar but less potent nematostatic effect was also observed in the beds treated with Bio Neem (Figure 1). This may be due to the low Bio-Neem concentration (15 ml/L), which may be effective for use as a preventative treatment but should be increased when used as a curative measure.

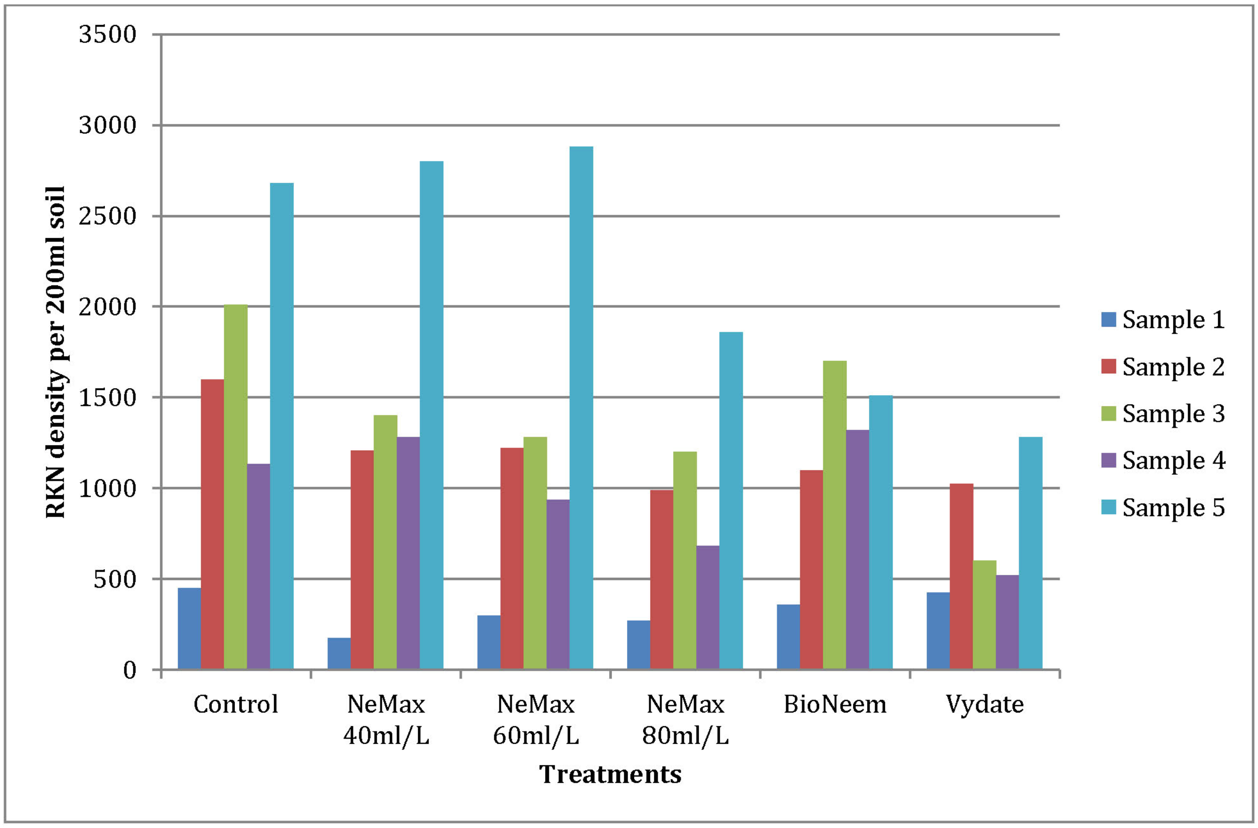

Figure 1: Effect of nematicide treatments on RKN density in the soil of sweet pepper plants

NeMax was the most effective at preventing RKN penetration into the roots of sweet pepper plants, with its efficacy being increased with higher concentration (Figure 2). This was due to the anti-feedant effects of sesame extracts and Azadirachtin on nematodes (Thomson 1992). Rehma et al. (2009) also suggested that sesame extracts was able to induce a defense mechanism in roots of plants causing an inhibition of nematodes to invade plants without directly killing the nematodes. Therefore, nematodes continue to exist within the soil but are unable to enter the roots. Proper bio-nematicide application rates and frequencies are therefore needed to maintain its anti-feedant effect as the surviving nematodes in the soil can become parasitic once again. Bio Neem also contains a similar anti-feedant effect but its efficacy at reducing RKN in the roots of sweet peppers was subpar compared to that of NeMax (Figure 2). From the results, a steep raise in RKN density was observed from sample 1 to 2 in plants treated with Bio Neem. This indicates that Bio Neem was ineffective at prolonging the reoccurrence rate of RKN which may be due to the low concentration used. The specificity of nematode control observed in bio-nematicides must also be considered, as certain plant extracts may have a higher potency or stronger anti-feedant, nematostatic and nematoxic effect on RKN that others.

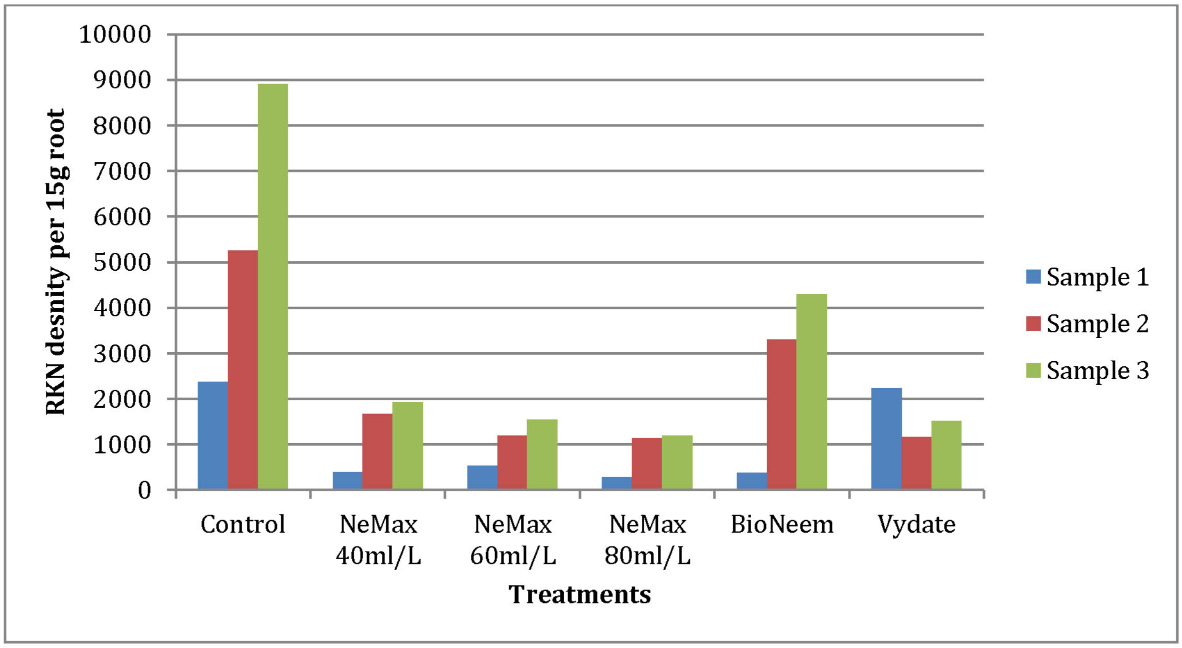

Figure 2: Effect of nematicide treatments on RKN density in the roots of sweet pepper plants

Conclusion

This study indicates that the efficacy of certain plant extracts were comparable to that of Vydate L at reducing RKN in sweet peppers. Vydate L had the fastest knockdown rate of all nematicides but the continuous anti-feedant and nematostatic effects of NeMax and Bio Neem prolonged the reoccurrence rate of RKN. NeMax, at a high rate (80 ml/L), was effective at controlling RKN in both the roots and soil. However, the high cost per application of NeMax (80 ml/L) may render the treatment impractical for use by small-scale farmers. Therefore, NeMax at high concentrations should be utilized as a curative measure or to supplement other cost effective nematode control strategies. Further research is needed to identify effective application rates of Bio Neem for use in greenhouse sweet pepper production.

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