The Use of *Trichoderma Viride* C. in the Management of Chilli Root Knot Disease in Nakuru County, Kenya

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Abstract

Chilli (*Capsicum annum* L.) is a sub-tropical crop grown for its pungent tasting fruits and sour leaves. Kenya is among the largest exporters of chilli. Chilli yields are largely reduced by sedentary nematodes (*Meloidogyne spp*). Chillis are prone to root knot nematodes which reduce the quality and yields. This study focused on the use of *Trichoderma viride* as a potential biocontrol agent against root knot nematodes. Investigation was done on the effect of *Trichoderma viride* at different concentrations on the plant height, leaf number, stem diameter, number of galls, nitrogen and phosphorous levels in chilli infected with root knot nematodes. Four dilutions of *Trichoderma viride* were obtained through serial dilution (100%, 50%, 25% and 12.5%). Bio-control efficacy of *Trichoderma viride* against the root knot nematodes was tested under glasshouse and field conditions. Evaluation after treatment of plants with *T. viride* isolates showed that there was no significant effect on plant heights, number of galls, stem diameter or leaf number in chilli. There was significant difference in the nitrogen and phosphorous levels when different treatments of *Trichoderma viride* were applied on chilli with root knot nematodes.

Key words: Chilli, *Solanum incanum*, *Trichoderma viride*, Root knot nematodes, *Meloidogyne* spp

Introduction

The chilli plant is unique due to its edible berries that have a characteristic pungent taste. This is attributed to the presence of an organic compound capsacain (Lee *et al.,* 2004). This compound produces heat sensations in most mammals including man. Chillis are widely distributed mainly due to their seed dispersal by birds. Birds are insensitive to capsacain and therefore feed comfortably on the chilli berries (Perry *et al.,* 2007). Chilli is attacked by root knot nematodes which reduce the yields significantly. The use of chemicals to control nematodes has been faced with challenges due to its effects on the environment. The withdrawal of methyl bromide as a soil fumigant has triggered research into alternative methods of controlling nematodes. Biological control methods in recent years have been adopted as more environmental friendly techniques of nematode control (Hafeez, 2000). Different fungal isolates have been tested for antagonism against root knot nematodes. The genus, *Trichoderma* is a common filamentous fungi found in most soils (Behzda *et al*., 2008). The myco-parasitic abilities of different strains of *Trichoderma* against

microorganisms such as nematodes have been reported (Al-Fattah, 2007). *Trichoderma harzianum* has been used to control *Meloidogynejavanica* (Baharullah, 2008).

Materials and Methods

Nematode population was multiplied in chilli (Oiseau variety from Technisem, France) over a period of 4 months. This nematode population was used as the stock in the experiment. *Capsicum annum* seeds were germinated in a seed bed under non heated greenhouse conditions for a period of one month. Heavily galled roots from infected chilli were mixed with the soil around the rhizosphere of the seedlings. Regular watering was done to give a conducive environment for nematode infestation. Galls were extracted from infected roots after three months and were used for further increase of the nematode stock as described by Hussey and Barker (1973).

Fungal colonies of *Trichoderma viride* from the Egerton University Department of Biological Sciences were multiplied on potato dextrose agar plates at 25^{o}C over a period of one week. The stock solution of *Trichoderma viride* was derived by scrapping fungal colonies and dissolving them in water. The solution was put in an orbital shaker set at 100 rpm for fifteen minutes. The concentration of the stock solution was determined using a hemocytometer. It was found to be 2.0 x 10^{6} spores per millimeter cubed and was labeled 100%. The consequent treatments were diluted to 50%, 25% and 12.5% (Pavaraj *et al.*, 2012).

The experiment was laid in completely randomized block design in the field set up and completely randomized design in the green house with three replicates in both experiments. Each replicate had six pots.

Soil that was used in the experiment was steam sterilized at 100^{o}C in a metal drum. The soil was allowed to cool and was put in plastic pots (Wang and McSorley, 2008). The soil in each pot was treated with nematodes eggs suspended in 50ml of water. There were an approximate number of 5000 nematode eggs per ml (Muthamia, 2004).

Six treatments of *Trichoderma viride* were used. Four levels of *Trichoderma viride* derived earlier were used (100% labeled T1, 50% labeled T2, 25% labeled T3, and 12.5% labeled T4). The positive control was a nematicide by the market name nimbecidine labeled T6. The active ingredient is azadiractin (0.03%) and neem oil (90.57%) applied at the rate of 6-8ml per meter squared. The negative control used was tap water labeled T5.

The treatments were applied once. Four seedlings were planted per pot one week after application of treatments. Data collection for heights, leaf numbers and stem diameter was done monthly after planting of seedlings, the data was recorded thrice. The means of root galls were collected after two months after planting to allow gall formation. The galls forming on the roots of each plant were counted. The data was recorded twice.

Chilli leaves were harvested after two months. The leaves were oven dried at 60^{o}C for 12 hours. They were then crushed using an electric grinder and suspended in water. Total nutrient analysis

was done using Kjeldahl oxidation method as used by Okalebo and Woomer (1993). The determination of nitrogen level was done by putting5 mls of an aliquot to a Kjeltec auto distillation apparatus (Model 2200). The ammonia in the distillate was collected in a receiver with excess boric acid and an indicator. Blank determination was be done by digesting reagents in place of the sample and distilling and titrating with N/70 HCl as for the samples. The percentage of Nitrogen (N) in the plant tissue was determined by this formula (Okalebo and Woomer, 1993).

$$\%N \text{ in plant sample} = \frac{\text{Corrected ml of N/70 HCl} * 0.2}{\text{Weight of sample in grams}}$$

The determination of phosphorous level was done by putting 5mls of supernatant wet-ashed digested solution to a 50ml volumetric using a pipette. Twenty ml of distilled water was added to each flask. Ten ml of ascorbic acid was added, then made to 50ml with distilled water.

The solution was allowed to stand for 1hour to permit full colour development. The standard and sample absorbance (blue colour) was measured at 880nm wavelength using a spectrophotometer (Model Pharmacia Biotech).

The total phosphorous percentage was computed as

$$\% \text{ P in plant sample} = \frac{C * 0.05}{W}$$

Where C is the corrected concentration, *means multiplication, P is phosphorous and W is the weight of the sample in grams (Okalebo and Woomer, 1993).

Means and standard errors of the means were calculated for all data.
Treatment effects were determined by one way ANOVA using SAS program (Version 9.3).

Results and Discussions

There was no significant difference in mean heights, number of leaves, diameter of stems, number of galls, recorded when different treatments of *T. viride* were used both in the field and greenhouse experiment.

There was significant difference in the nitrogen and phosphorous levels in the field experiment. The nitrogen levels increased across the two months of data collection in the 100% treatment, and the positive control. In all the others the nitrogen level reduced in the second month. During the first reading, the highest levels of nitrogen were recorded in the negative control treatment followed by the 12.5% treatment. The lowest nitrogen level was recorded in the 25% treatment followed by the positive control treatment. During the second reading the highest nitrogen level was recorded in the negative control treatment followed by the 100% treatment. The lowest nitrogen level was in the 50% treatment followed by the positive control treatment. During the first reading the phosphorous levels were highest in the negative control treatment followed by the 100% treatment. The lowest phosphorous level was recorded in the 50% treatment followed by the 25% treatment. During the second reading, the highest phosphorous levels were recorded in the

negative control treatment followed by the 25% treatment. The lowest phosphorous level was recorded in the 100% treatment followed by the positive control treatment (Table 1).

Table 1: Means of nitrogen and phosphorous levels

Treatments	N 1	N 2	P1	P2
T2	1.2ab	1.2a	151.1b	1527a
T4	1.9ab	1.5a	414.4b	1682a
T1	1.6ab	1.7a	486.7b	478a
T5	2. 7a	2.2a	1255a	3535a
T6	0.9b	1.3a	281.1b	852a
T3	0.9b	0.9a	263.3b	2034a

a *

In the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least significant Difference(LSD) test.

There was no significant difference in the nitrogen and phosphorous levels when different treatments of *T. viride* were used in the greenhouse experiment (Table 2).

Table 2: Means of nitrogen and phosphorous levels

Treatments	N 1	N 2	P1	P2
T2	1a	1.1a	297.5a	253.9a
T4	1.2a	1.1a	1320.6a	796.1a
T1	0.9a	1.2a	758.9a	849.4a
T5	1.a	1.1a	921.7a	795a
T6	1.2a	1.1a	1296.1a	825a
T3	1.1a	1.1a	252.8a	1321.1a

a *

In the column, means followed by the same letter are not significantly different from each other at P=0.05 according to Least significant Difference(LSD) test.

The lack of significant difference on the means of heights, stem diameters, leaf numbers and gall numbers would attributed to lack of sufficient chitinolytic enzyme activity. Previous studies have shown *Trichoderma harzianum* significantly reduces the soil population of *Pratylenchus*, *Xiphinema* and *Meloidogyne* in sesame. It has been reported as an effective bio-control agent against root knot and other nematodes (Parveen *et al*., 1993, Saifullah and Thomas, 1996, Khan and Saxena, 1997, Hafeez *et al.*, 2000 and Sharon *et al*., 2001). *Trichoderma viride* has been shown to have high rates of mycoparasitism such as that observed against *Meloidogyne incognita.* It is expressed in aggressive nature of isolates against the female body and egg masses. *Trichoderma* species (*Trichoderma atroviridae, Trichoderma harzianum, Trichodermarossicum, Trichoderma tomentosum, Trichoderma virens* have been used to control plant-parasitic nematodes (Hafeez *et al.*, 2000). *Trichoderma* chitinolytic enzyme systems play an important role in egg-parasitism. Significant reduction is observed in the root-knot development caused by *M. incognita*. Our experiments suggest that the chitinase activity of *T.viride* was not very high to cause a significant effect on the parameters measured. It is widely known that environmental parameters such as

abiotic (soil type, soil temperature, soil pH, water potential and such like) and biotic (plant species and variety, microbial activity of the soil) factors as well as other factors such as method and timing of applications may have influence on the biological control efficacy of *Trichoderma* isolates (Behzda *et al.,*2008). Biological control methods are recommended for disease control due to their little negative environmental impact. Organisms used in bio-control also propagate naturally in the environment so they can be replenished naturally (Poornima, 2009). *Trichoderma spp* multiplies easily in soil medium, antagonistic strains would therefore be a natural biological control for soil and root knot nematodes (Behzda *et al*, 2008)**.** *In vitro* monoculture growth rate tests, dual confrontation assays and comparison of strain specific egg-parasitic index (EPI) show that *T. harzianum* strains possess the strongest egg-parasitic ability (Parveen *et al*., 1993). Biological control is an environment friendly alternative. This therefore suggests that more evaluation is necessary in these isolates with a possibility of getting efficacy at different formulations.

High nitrogen levels in the negative control and 12.5% treatment during the first reading of the *Trichoderma* extract field experiment could be attributed to better leaf proliferation. This would be a result of low disease severity in the negative control and higher nematicidal activity of the *Trichoderma viride* extract in the 12.5% treatment. The lack of significant effect on nitrogen levels during the second reading could be as a result of low disease severity. High phosphorous levels in the negative control and 100 % treatment during the first reading of the *Trichoderma* extract field experiment could be attributed to better root proliferation. This would be a result of low disease severity in the negative control and higher nematicidal activity of the *Trichoderma viride* extract in the 100 % treatment. In the *Trichoderma* greenhouse experiment, lack of significant effect on nitrogen and phosphorous levels could be as a result of low disease severity (Muthamia, 2004).

Conclusions and Recommendations

According to the findings, the infection of root knot nematodes on chilli has significant effect on the various growth parameters in chilli. The findings show that there was significant effect on nitrogen and phosphorous uptake by root knot nematodes. This implies that the overall nitrogen and phosphorous level in the plant was affected by the presence of root knot nematodes. The nematicidal effect of *Trichoderma viride* brings out a clearer view of such effects. This implies that application of nitrogen and phosphorous based fertilizers will reduce the effect of root knot nematodes. It is recommended that various *Trichoderma* spp. isolates should be evaluated for nematicidal activity. It is further recommended that molecular characterisation should be done to determine the root knot nematode species infecting chilli. Further research is necessary to provide information on nematicidal effect of other micro-organisms.

It is recommended that various *Trichoderma* spp. isolates should be evaluated for nematicidal activity. These experiments should be carried out in different ecological zones. It is further recommended that molecular characterisation should be done to determine the root knot nematode species infecting chilli. Further research is necessary to provide information on nematicidal effect of other micro-organisms.

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