EVALUATION OF ANTAGONISTIC PLANT MATERIALS TO CONTROL SOUTHERN ROOT KNOT NEMATODE (*Meloidogyne incognita*) IN TOMATO

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ABSTRACT

Marigold ((Tagetes patula), crotalaria (Crotalaria juncia), rapeseed plant (Brassica rapa) and oat(Avena sativa) as antagonistic plant and tomato (Lycopersicon esculentum), CL1104 variety as control, were evaluated to determine the effect on southern root knot nematode population in pot experiment at Tsukuba, Japan in year 2010. Completely Randomized Design (CRD) with three replications was used. Initial juvenile population was 30.0±2.8 per 20g soil. The juvenile nematode population was counted 63 days after transplanting and biomass of plants was incorporated in soil. Population was again counted 11 days and 13 day after biomass incorporation. At final count of Juvenile nematode population, it was found highest in tomato (66.6 /20g soil). Highest control was achieved in marigold (2.3 juvenile/20g soil) followed by crotalaria (3.0 juvenile/20g soil) . Marigold and crotalaria followed by oat in rotation with tomato were observed as best antagonist plants to control Southern root knot nematode.

KEY WORDS: Antagonistic plants, tomato, Nematode infested soil, Southern root knot nematode, juvenile, Nepal.

INTRODUCTION

Root knot nematode (*Meloidogyne* spp.) is distributed worldwide in areas with warm or hot climate and short or mid-winter and in greenhouse. They attack more than 2000 species of plants including almost all cultivated crops and on an average reduce the crop production by 5% in the world (Agrios, 2005a). Among the cultivated vegetables, tomato, eggplant, okra, peppers, gourds and melons are highly susceptible to root knot nematode. In tomato and eggplant 20-40% loss is caused by root knot nematode (Bridge and Starr, 2007).

Tomato is major vegetable consumed in Nepal which occupies fifth position in terms of area coverage after cauliflower, cabbage, radish and onion. Tomato alone covers 117,043 ha land with the average productivity of 17.1 mt/ha (VDD, 2011). Cultivation of tomato using plastic house is one of the profitable enterprises of farmers of Nepal. Several small scales as well as commercial farmer are engaged in tomato cultivation inside plastic house in most of the mid-hills of Nepal.

The area of tomato under plastic house is increasing year after year. Due to the continuous cropping of tomato problem of various diseases and insects are becoming severe, among them, root knot nematode (*Meloidogyne* spp.) is the major one identified through farmers field survey.

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The incidence of root knot nematode was first recorded in Nepal by Amatya and Shrestha in the year 1969 on tomato, eggplant, okra and chilli, among several species *Meloidogyne incognita* is the dominant species responsible for excessive loss in tomato (Bhardwaj and Hogger, 1984). Root knot nematode is also responsible for increasing severity of other disease and pest. Fusarium wilt of several plant increases in incidence and severity when the plants are affected by root knot nematode. Similarly Verticillium wilt, Pythium damping off, Rhizoctonia and Phytopthora root rot are increased due to root knot nematode infection (Agrios, 2005b).

In Nepal nematode resistant tomato varieties are unavailable. The problem is seen from the nursery stage and huge damage occurred during flowering and fruiting period. Due to limited land available for cultivation and investment of huge amount of money for the construction of fixed rain shelter type of plastic house (more than NRs 25,000 form75 m^2area) most of the farmers are not able to shift land every year.

Several plants are reported as antagonistic to the nematodes. Lespedeza (*Lespedeza* spp.), common vetch(*Vicia sativa*), velvet bean(*Mucuna pruriens*), lupine(*Lupinus perennis*), castor(*Ricinus communis*), rapeseed plant(*Brassica rapa*), giselba mustard(*Brassica hirta*), andmarigold (*Tagetes patula*) are the major green manuring crop having property to control root knot nematode (Crow et al, 1995). The different plant antagonists like rape seed (*Brassica campestris, B. napus*) and Sudan grass (*Sorghum vulgare* var sudanense) contains glucosinolate and cyanogenic glycoside compounds having pesticidal property on nematode (Santo et al., 1991). Marigold can suppress 14 genera of plant-parasitic nematodes including root-knot nematodes (*Meloidogyne* spp.) (Suatmadji 1969). Crotalaria is a poor host of many plant parasitic nematodes including *Meloidogyne* Spp. (Wang et.al. 2002a). Oat (*Avena sativa*) is known for resistance from pest to disease and grown to break life cycles of soil borne pest and diseases. Oat possesses secondary metabolites that impart resistance to nematode (Osbourn, 2003).Nematode suppressive crops prevent the buildup of damaging levels of nematodes naturally without nematicides (Gazaway, 1998). Use of low cost technology to suppress nematode population for the better yield of tomato is needed in Nepalese condition. We have diverse range of plants that are reported as nematode suppressive. Thus use of such plants for controlling root knot nematode in tomato without costly nematicides may one of the possible options for small scale tomato growers. The main objective of this experiment is to evaluate effect of selected antagonistic plants to control southern root knot nematode.

METHODOLOGY

EXPERIMENTAL SETUP

This experiment was carried out in pots (35cm diameter).Plant pathology glasshouse of TBIC was used from May to October 2010. In each treatment 4 plastic pots were used. Completely randomized design (CRD) was used. Following

five treatments with three replications were used (Table 1). Altogether 60 pots containing nematode infested soil were used for the experiment.

Table 1. Treatment details

Treatments	Scientific name	Varieties	Source
Rapeseed plant	Brassica rapa	Kyoto fusimi	Takii seed
Crotalaria	Crotalaria juncea	Nemaking	Yukigirusi
Marigold	Tagetes Patula	Dierin	Takii seed
Oat	Avena sativa	Tachibuki	Takii seed
Tomato (CL1104)	Lycopersicon esculentum	CL 1104	TBIC

MATERIALS

Following materials were used during experimental works

- Garden balsam as susceptible host
- Nematode infested soil
- Nematode suppressive plants and tomato plant as treatment
- Pot(35 cm diameter) filled with 6 liter field soil
- Media soil for rising seedlings
- Plastic pot (6 inch diameter) for growing balsam as host
- Plastic pot(6 cm diameter) for raising seedling of treatment and tomato plants

Preparation of nematode infested soil

Garden balsam was seeded as the host plant for nematode. About 30 kg nematode infested soil was obtained from National Agricultural Research Center (research team for detection of plant pathogens and nematodes) located in Tsukuba. Initial nematode population of infested soil obtained from the research station was counted (5 juvenile per 100g soil).60 pots of 6 inch diameter were filled with 500g infested soil in each pot.30 days old one balsam seedlings in each pot were transplanted. Gall formation in balsam root was observed within 25 days after transplanting. Infested soil with galled balsam roots was collected; mixed, heaped and nematode population was again counted. Following the similar procedure, large quantity of nematode infested soil was prepared to carry out experiment. Two liter infested soil per pot was used for carrying out experiment.

Cultivation of treatment plants

Seed of antagonist plants and tomato were seeded in cell tray made form plastic for raising seedling and transferred into 6 cm diameter pot after 15 days. In each experimental pot five seedlings of marigold (35 days old), oat (25 days old), rapeseed plant (25 days old) and crotalaria (25 days old) were transplanted. One tomato plant (25 days old CL 1104 variety) was planted in control pot. Plants were cultivated form July 24 to October 9.Watering and weeding according to need was done.

Sampling

Soil sampling was carried out from the pot within15 cm depth because distribution is greater in or around the root of susceptible plants (Agrios, 2005 c). Soil was mixed properly, sieved and 20±0.08 g soil was used for counting nematode population from each treatment. One sample from each treatment pot (15 soil sample from the three replications) was taken for counting nematode in each measurement. Altogether three times sampling on three different dates were done.

Counting nematode population

Baernmann funnel method was used for the isolation of juvenile nematode from soil which is common method (Bridge and Starr, 2007 b). After 24 hours juvenile nematode population was counted using 1 ml water collected through Baernmann funnel. Compound microscope was used for observation. Nematode population before setting treatment was counted. First counting was done 63 days after transplanting (DAT) then plant biomass of these pots were incorporated and nematode population was counted after 11 days from same pot. Final counting was done 76 days after transplanting.

DATA ANALYSIS

The obtained data were tabulated and statistically analyzed. ANOVA program developed by Dr.Yamada, Mitate (Technical advisor, JICA, Tsukuba) was used. Turkey's honestly significant difference (HSD, reference?) test was used for comparing means .Turkey's HSD test determines which of three or more sample means are significantly different after ANOVA has indicated that all population means are equal and is application under the condition of equal sample size and assumption of ANOVA fulfilled.(LeBlanc,2004).

RESULTS AND DISCUSSIONS

NEMATODE POPULATION BEFORE SETTING TREATMENT

Nematode populations of infested soil were counted before planting antagonists and tomato plant.30±2.8 Juvenile root knot nematodes in 20g soil were observed before setting treatment.

NEMATODE POPULATION IN DIFFERENT TIME PERIODS

The effect of days and treatment on nematode population is given in Table 2. Population of juveniles counted at three different date (63, 74 and 76 DAT) were observed as non -significant. The treatment effect for controlling nematode population was significant. While comparing the nematode population, soil with marigold treatment showed best control (2.4 juveniles/20g soil) followed by crotalaria and oat (4.2 and 6.7 juvenile per 20g soil). Rapeseed plant was almost

ineffective for controlling nematode. The population of juvenile was observed highest in nematode susceptible tomato (63.3 juvenile/ 20g soil).

Table 2. Days and treatment wise nematode population

Days of count	Treatments	Juvenile nematode population(20 g soil)
63 days		20.6 a
74* days		19.7 a
76** days		19.3 a
	Tomato (CL 1104)	63.3 a
	Rapeseed plant	22.7 b
	Marigold	2.4 d
	Crotalaria	4.2 cd
	Oat	6.7 c
HSD for days A (0.05)		4.1
HSD for treatments B (0.05)		6.2

*11 days of biomass incorporation in soil (63 days of growing+11 days

** 13 days of biomass incorporation in soil (63 days of growing +13 days)

The interaction effect of days and treatment on nematode population counted at different date is presented in Table 3. Juvenile nematodes were counted at three different dates to compare the effectiveness of antagonist plants over time period. The population of juvenile nematode in nematode susceptible tomato variety (CL1104) was almost became more than double (66.6 juvenile) within 76 days. Tomato (CL1104) is one of the suitable hosts for Southern root knot nematode contributing favorable environment for increasing population. In this experiment, almost all the rapeseed plants died within 35 days after transplanting due to excess heat in greenhouse (more than 400c for about 20 days).So the effect of rapeseed plant as antagonist was found non-significant. The final nematode population in rapeseed plant was 20 juveniles per 20 g soil.

In first count (63 DAT) best control was observed in case of marigold (2.6 juvenile/20 g of soil) followed by crotalaria (6.6 juvenile /20 g soil). Plant biomass was incorporated in soil for about 11 days to check effect of biomass incorporation for controlling nematode population. No significant effect of biomass incorporation on reducing juvenile population was observed in all treatment. In last count (76 DAT) best control was observed in marigold (2.3 juvenile/20 g soil) followed by crotalaria (3.0 juvenile/20g soil) and oat (4.6 juvenile/20 g soil).

The best control of nematode was obtained through growing marigold as cover crop. Wang et al. (2007) mentioned allelopathy phenomenon of marigold responsible for reducing nematode population. He explained α- therthienyl as most toxic compound with nematicidal, insecticidal, fungicidal, antiviral and cytotoxic activities. He found nematicidal activity in root of growing marigold

plant but not in leaf and root extract. In this experiment, biomass incorporation did not showsignificant effect which is also supported by the above statement of Wang et al.

Table 3. Interaction effect of days and treatment on nematode population

Treatments	Nematode population in 20 g soil (initial)	Nematode population in 20 g soil (63 days)	Nematode population in 20 g soil (74 **days)	Nematode population in 20 g soil (76***days)
Tomato (CL 1104)		60.3 a	63.0 a	66.6 a
Rapeseed plant	30±2.8 b*	24.3 b	24.0 b	20.0 bc
Marigold		2.6 d	2.3 d	2.3 d
Crotalaria		6.6 cd	3.0 d	3.0 d
Oat		9.3 cd	6.3 cd	4.6 d
HSD A X B (0.05)		14.4	14.4	14.4

*11 days of biomass incorporation in soil (63 days of growing+11 days)

**Average nematode population counted three times from each replication randomly before setting treatment

Mean represented by same letter are non- significant at 5% level of significance

Crotalaria was observed as good antagonistnext to marigold Because in comparison to control, 90 percent reduction in population of southern root knot nematode population was observed at 5% level of significance. The suppression effect of nematode by crotalaria depends upon the method of use (by which means? explain).Most crotalaria pre-plant cover crop followed by incorporation of biomass into soil is reported as effective for controlling root knot nematode by several researchers. So in this experiment crotalaria was used as pre plant cover crop. According to Sharma and Scholari (1984), Crotalaria can suppress *Meloidogyne* spp. better than nematicide when planted as pre-plant cover crop. As reported by several authors crotalaria acts as non-host, release antagonistic chemical, provides niche for antagonistic flora and fauna, and traps nematode (Wang et.al, 2002b). Similar effect was reported by Wang et.al. (2002c) when incorporated in soil but in this experiment the effect of biomass incorporation was not observed. One of the possible causes of this may be shorter period of incorporation into soil.

Oat has multiple benefits. It is commonly grown as green manure winter crop in many countries.)Oat also showed good result for controlling southern root knot nematode as compared to rapeseed but it is not much effective as compared to marigold and crotalaria. According to Valenzuela and Smith (2002) oat helps to control weed in field, add biomass in soil, which has allelopathic properties for nematode and plant itself, is resistance from root knot nematode.

Population density and duration of growing antagonist plant in field should be sufficient to control the Southern root knot nematode population. For effective control, root of the antagonist plant should cover upper 15 cm layer of infested soil during cultivation period. Marigold followed by crotalaria and oat were confirmed as good antagonist plants for reducing juvenile southern root knot nematode population in soil.

The juvenile root knot nematode population counted at two different time periods(63 DAT and 76 DAT) without biomass incorporation were compared with the population of juvenile before setting treatment (Figure 1).The rate of multiplication of nematode in case of root knot susceptible tomato (CL1104) was very fast. Within 63 days 60.3 juvenile per 20 g soil were observed and reached up to 66.6 juvenile after 76 days from initial level (30 juvenile).These levels(63 days and 76 days) were significantly increased from initial population. In oat it was reduced to 4.6 juvenile from initial level. In marigold and crotalaria the rate of decrease in nematode population was fast. In marigold final population was reached to 2.3 juvenile from the initial level of 30.0 juvenile followed by crotalaria (check the spelling and make uniform) (3.0 juvenile). In rapeseed plant no significant difference in population before and after treatment was observed. Rapeseed plants almost acted as the bare field and populations in two counts were 24.3 and 20 juveniles per 20 g soil.

In tomato plant (CL 1104) the juvenile population within 76 days was increased by 122% than initial(30 juvenile/20g soil). In marigold and crotalaria population levels were reduced by 90% and 92% respectively. Oat reduced population level by 83% as compared to initial population.In rapeseed plant which almost acted as fallow land; population level was reduced by 33.3% than initial.

If fallowing is done in the field, long term fallowing is needed to reduce the nematode population provided that there is no susceptible host. From these observations it is clear that growing antagonist plant in infested soil significantly reduces population level of southern root knot nematode within specific time period.

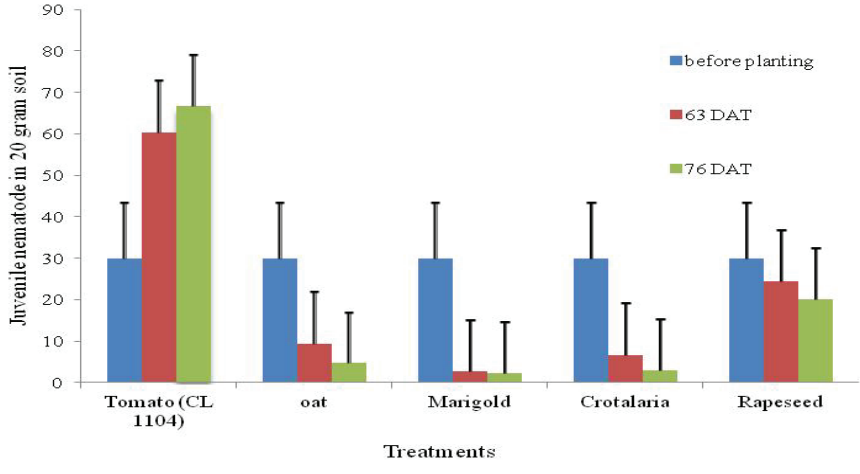

Figure 1. Comparison of juvenile nematode population before and after treatments

CONCLUSIONS

Better control of southern root knot nematode was achieved through the use of marigold followed by crotalaria and oat under the experiment in pot. These crops

have multiple benefits. In some countries farmer sell marigold flowers and also earn the money. Oat is commonly used as green manuring crop in winter. Crotalaria is good green manuring crop that fix nitrogen and add organic matter in soil. Field evaluation should be done for further confirmation of effectiveness under field condition in different climatic conditions. From this experiment marigold followed by crotalaria and oat were identified as good substitute of chemical nematicides to suppress population of southern root knot nematode in soil.Selection of proper variety, planting density and durations under different climate and soil are important factors to use such plants.

Off-season tomato cultivation using plastic house is major income generating activities for the small scale farmers of Nepal. Due to continuous cropping of tomato, problem of southern root knot nematode (*Meloidogyne incognita*) is epidemic in the districts like Kaski,Palpa, Parbat,Syangja and Kathmandu valley which is the major cause of reducing productivity of off-season tomato. Without using costly, environment polluting nematicides, root knot nematode is manageable through yearly rotation of tomato with marigold or crotalaria at minimal cost. So through the adoption of this practice small scale as well as commercial farmers can manage southern root knot nematode in the plastic house.

ACKNOWLEDGEMENT

I would like to thank government of Nepal, MoAD and Japan International Cooperation Agency (JICA) for providing opportunity to take part in “vegetable cultivation technology for small scale farmers” training. I would also like to express thanks to the technical advisors and instructors of JICA,Tsukuba for providing continuous support and guidance .

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