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ORIGINAL ARTICLE

Comparing Neem extract with chemical control on *Fusarium oxysporum* and *Meloidogyne incognita* complex of tomato

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Shervin Hadian, Kamran Rahnama, Salar Jamali, Ali Eskandari: Comparing Neem extract with chemical control on *Fusarium oxysporum* and *Meloidogyne incognita* complex of tomato

ABSTRACT

Fusarium wilt disease and root-knot nematode both are important diseases of tomato in Iran. *Fusarium oxysporum* f.sp. *lycopersici* is often found in a synergistic relationship with *Meloidogyne incognita* in north of Iran. Management of disease complexes appears to less straight forward than one might anticipate. The use of chemical is becoming less appealing because of the human and environment health implications. Also, the chemicals required are often not within the reach of farmers in most of the developing part of the world. This research is aimed at finding an alternative mode of control. Tomato inoculated with *Meloidogyne* and *Fusarium* was treated with 50 g/kg soil neem seed powder in the glass-hous. Sixty days after inoculation the plants were uprooted and root gall indices, disease severity of *Fusarium* and tomato growth parameter were determined and results have been done by SAS test and significant difference $P \leq 0.01$. Results have been shown neem seed powder significantly ($P \leq 0.01$) reduced the disease severity of *Fusarium* and root-knot nematode. All the treatments significantly improved the growth of the plants as compared to untreated inoculated plants. Carbofuran was highly effective against nematode, Bavistin against fungus, *A. indica* seed powder against both the pathogens. Neem decrease root knot index from 4.7 in control treatment (fungi+nematode) to 0.25, and also decrease disease severity from 85% in control treatment to 12%. Neem not only controlled these diseases but also cause increase in growth characters such as plant weight and length. Results suggest the possible use of neem seed powder for control of the root-knot nematodes – *Fusarium* wilt disease complex.

Key word: Carbandazin, Carbofuran, *Fusarium oxysprum*, *Meloidogyne incognita*, Neem seed

Introduction

Tomato (*Lycopersicon esculentum*, Mill.), is the most important tropical vegetable crop wildly used throughout the world. *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) is economically important wilting pathogen of tomato [14]. Root-knot nematodes (*Meloidogyne* spp.) cause severe annual yield reduction or even total crop loss, due to complexes involving plant parasitic nematodes and soil borne pathogens [8]. Among various pests and diseases, nematodes-fungus disease complex particularly of *Meloidogyne incognita* and *Fusarium oxysporum* poses a great problem to the cultivation of pulse crops by inflicting severe yield losses [11,20]. In the north of Iran, *Fusarium* wilt is often found in a synergistic relationship with *Meloidogyne incognita*.

Management of disease complexes appears to less straightforward than one might anticipate. The most obvious solution is to use chemical methods to control one of the interacting organisms and thus prevent the disease complex from occurring.

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However, it is fundamental to have prior knowledge of the interaction involved, as even low densities of fungi or nematode can result in a disease complex of significant importance [4]. Tactics for management of plant-parasitic nematodes continue to rely on nematicides for suppression of population densities. The most effective non-fumigant nematicide is Carbofuran which is highly toxic. The use of highly toxic pesticides has been criticized by the public due to potential hazards to environmental and human health [19].

Allen *et al.* [7] revealed that Benomyl at 10 µg/ml completely inhibited fungal growth of *F. solani*, *F. oxysporum* and *F. proliferatum*. carbendazim were the most effective fungicides in inhibiting mycelial growth of *F. oxysporum* f. sp. *lycopersici* [25].

The inappropriate use of agrochemicals especially fungicides, which found to pose more of carcinogenic risk than insecticides and herbicides together may give rise to undesirable side effects. Additionally, resistance by pathogen to fungicides has rendered certain fungicides infective. There may be a need to develop new management systems to reduce the dependence on synthetic pesticides. Now days, plant extracts as natural products are widely used to control diseases. Plant extracts and essential oils show antifungal activity against a wide rang of fungi [16].

The inappropriate use of agrochemicals especially fungicides were found to possess adverse effects on ecosystems and a possible carcinogenic risk than insecticides and herbicides together [22,27,21].

Moreover, resistance by pathogens to fungicides has rendered certain fungicides ineffective [32]. Due to the aforementioned considerations, there may be a need to develop new management systems to reduce the dependence on the synthetic agrochemicals. Recent trends favor the use of alternative substances derived from natural plant extracts to control diseases [31]. Several higher plants and their constituents have been successful in plant disease control and have proved to be harmless and nonphytotoxic, unlike chemical fungicides [6]. In the search for eco-friendly insecticides that can be integrated in organic pest control programs, azadirachtin has been probably best investigated and exempted from residue tolerance requirements by the US-Environmental Protection Agency, fact that implies its friendly environmental profile [15].

In this study plant extract of *Azadiracta indica* which belongs to the family *Meliaceae* commonly known as neem and also it is found throughout south of Iran was selected for evaluation of its antifungal and nematocidal activities.

Material and methods

This experiment was conducted in plant pathology laboratory and glass-house of Islamic Azad University of Gorgan, Iran on 2008.

Preparation F.oxysporum f.sp. lycopersici and M. incognita inoculums:

Pure culture of *F.oxysporum* f.sp. *lycopersici* isolated have been received from agricultural department of Ferdowsi Mashhad university of Iran on PDA(Potato Dextrose Agar) in Petri plate, in order to mass-produce pure culture of the 6 days growth fungus transferred to flasks containing sterilized wheat seeds. The flasks were incubated in incubator at a temperature of ($25\pm1c^0$) for 10days. During incubation, the flasks were shaken three times in a day.

Infested tomato root were collected from south of Iran. Root knot nematode was identified on the basis of perineal pattern female nematode. A single egg mass of the *M. incognita* isolated from infected tomato roots and placed singly in Petri plates containing distilled water. The second juveniles emerging from each single egg mass were inoculated on seedling of tomato, grown in pots containing steam sterilized soil, the plants were maintained in glass- house at ($25\pm1c^0$) for one year to allow the reproduction of nematode and this nematode culture was use for the experiment.

Preparation of Neem Seed Powder:

Neem fruits were collected from south of Iran, whole seeds of *Azadirachta indica* were first dried in sunlight for 24 h and then in oven at 70 for 1 h, were manually pounded in a large steel mortar with pestle to produce a fine Neem seed powder. Powdered form was used to allow easy decomposition of *A. indica* seeds in soil.

Experimental procedure:

Experiment was conducted in glass-house at temperature ($25\pm1c^0$) in 30 earthen pots (18 cm top diameter) filled with a mixture of autoclaved sandy loam soil (sand 70%, silt 22% and clay 8%, pH 7.5) and compost (4:1). Two leaf stage of tomato seedling were transferred to pots witch treated with (Carbofuran at 1g/kg soil, Bavistin at 1 g/kg soil, *A. indica* seed powder at 50 g/kg soil) and three pots were untreated.

Each treatment was replicated 3 times in a completely randomized block design and watered daily, after 3 days all pots except the 3 untreated pots were inoculated with 2000 freshly hatched second stage juveniles of M. *incognita* and *F. oxysporum* inoculums *with* 2%weight ratio of soil into l cm holes around the base of the plant which were then filled with soil. Uninoculated pots and nematode+fungus inoculated pots served as controls.

Recording of data:

Sixty days after inoculation the plants were uprooted and root gall indices were determined by the Taylor and Sasser, 1978 root knot index on a

scale 0-4, where 0=no infection or root galling, l=slight infection (1%-25%), 2=moderate infection (26%-50%), 3=severe infection (51%-75%) and 4=very severe infection (76%-100%) [13]. In order to determine the extent of *F.oxysporum* infection washed roots of inoculated plants were cut into 1.0 cm pieces, then treated with10% KOH solution and finally kept at 90 ^{0}C for l h. These root segments were washed again with distilled water, then acidified and stained with Trypan blue (0.5% *(V/V)* in lactophenol) as described by Philips and Hayman [24] Five stained pieces of each taproot were mounted on slides in lactophenol and presence of mycelium of the fungus was estimated. The root infection was calculated by measuring the infected portion in relation to total length of root pieces [9]. Fresh and dry root and shoot weights and height were obtained. Final nematode population in the entire soil volume was extracted by Cobb's sieving and decanting technique along with Baermann funnel and in roots by macerating 5 g root tissues in a Warring blender [29], and counted as per the procedure suggested by Doncaster, 1962 [13]. Data were analyzed by SAS test (analysis of variance) and significant differences among treatments were tested by the least significant difference test (LSD) at probability levels of 5% (LSD0.05) and 1% (LSD0.01).

Results and discussion

Application of Neem seed powder to soil inoculated with either *Fusarium* or *Meloidogyne* or both of pathogens significantly ($P \leq 0.01$) increased tomato fresh and dry shoot and root weight and also shoot height compared with inoculated and uninoculated pots that were not treated with Neem (table1).

Between all treatments Neem seed powder had the most affection on increasing of growth and height of tomato in fungus+Neem treatment. Neem increase fresh and dry weight of root in control from 1.2 and 0.56 to 1.8 and 0.78. Shoot height of fungus+Neem treatment was 26.9 that compare to control it is shown Neem could increase height of tomato. Neem seed powder not only increased weight of tomato but also increased height of that. The most fresh and dry weight have been shown in nematode +Neem seed powder treatment, that was 4.8 and 0.72. the highest shoot (29.9) was seen in fungus+neem treatment, all through neem seed powder significantly ($P \leq 0.01$) increase all growth parameter of tomato comparing to control (table1).

The suppressive effect of the treatments on nematode population both in roots and soil was highly significant as compared to untreated incubated plants. Nematode population in nematode+ fungus treatment was 2766 but Neem has decreased population to 532. The reproduction rate of *M.incognita* was significantly suppressed by all the treatments as compared to untreated inoculated plants. Reproduction rate of *M.incognita* was 1.38 in nematode+ fungus but its decrease to 0.26 by Neem. Similarly all the treatments were found to be highly effective in their ability to reduce root-knot index (RKI) when compared with untreated plants. Root-knot index was decrease from 4.7 in fungus+ nematode (control) to 0.25 in fungus+ nematode+ Neem. There was no significantly difference between Carboforan treatment and Neem seed powder on root-knot reduction, but both of that with comparing to control significantly reduce the root galls of tomato. All treatment except Bavistin significantly decreases reproduction of nematode (table2). Neem not only could decrease nematode population but also increase growth parameters of tomato.

Bavistin was highly effective in suppression of tap root colonization by fungus (15% root colonized) followed by Neem seed powder (12%) and Carbofuran (60%) respectively (table2). Neem seed powder was the most effective treatment in reducing fungi colonizes. And also, neem could decrease *Fusarium* disease severity in fungus+ neem treatment rather than nematode+fungus+neem, it is shown nematode increase Fusarium disease severity (table2). By all of this we can use Neem seed powder instead of chemical compound for controlling of these two pathogens. Neem seed powder not only reduces affection of *Fusarium* and *Meloidogyne* but also increase growth character of tomato.

Discussion:

Our result have been shown neem seed powder could have nematocidial effect and it was parallel to studing of other researchers, various neem products including neem seed, neem cake, its oil and Nimin (containing neem triterpenes) as urea coating agents, and root-dip or seed treatment with neem extracts, have been found to be nematicidal against several species of parasitic nematodes [5] attacking vegetables and legumes [13].

The ovicidal effect of Carbofuran is effective in preventing penetration of nematodes into the roots or in reducing nematode activities within the soil in our study. This may suggest that Carbofuran acts directly on the nematodes in the soil thereby preventing or limiting hatching of eggs and the movement of larvae into roots. This is in agreement with the works of [2].

In this study Bavistin was found effective in controlling root colonization by fungus. It was agree with the other research results. Bavistin inhibits the nuclear division of fungi by inactivating the spindle, which is composed of microtubules. Various scientists have also been reported, Bavistin as an important control measure against *F. oxysporum* [13].

Table l: Effect of carbofuran, bavistin and *Neem* against *Meloidogyne incognita* and *Fusarium oxysporum* disease

Treatments	Fresh weight (g)		Dry weight (g)		Shoot height
	Root	Shoot	Root	Shoot	
Control	1.29bc	4.65b	0.56b	0.67b	24c
Nematode+fungus	0.82h	3.14i	0.35e	0.46g	17.26i
Nematode+fungus+carbofuran	1.17f	4.22e	0.54cb	0.61d	22.2e
Nematode+fungu+bavistin	1.03g	3.75g	0.44d	0.53f	18.89h
Nematode+fungus+Neem	1.26dbc	4.38d	0.5c	0.64c	22.85d
Nematode	1.01g	3.2i	0.43d	0.52f	20.7f
Nematode+carbofuran	1.21def	4.48c	0.52cb	0.67b	23.18d
Nematode+Neem	1.31b	4.8a	0.56b	0.72a	25.25b
Fungus	1.20ef	3.45h	0.42d	0.58e	19.78g
fungus+bavistin	1.25cdc	4.14f	0.54cb	0.63c	23.73c
fungus+ Neem	1.8a	4.45cd	0.78a	0.66b	26.9a
LSD	0.0053	0.0738	0.53	0.016	0.54

Each value is an average of three replicate Means followed by the same letter within a columns do not differ significantly at $p < 0.01$according to LSD test SAS.

Table 2: Effect of carbofuran, bavistin, Neem on root-knot development, reproduction of *Meloidogyne incognita* and infection of *Fusarium oxysporum*

Treatments	Final nematode population (PF)	Reproduction factor RF=PF/PI	Root-knot index (RKI)	Disease index (%)
Control	0h	0f	0c	0g
Nematode+fungus	2766b	1.38b	4.7a	85a
Nematode+fungus+Carbofuran	519g	0.25e	0.4c	60b
Nematode+fungu+Bavistin	2744c	1.37b	3.7b	15d
Nematode+fungus+ Neem	532f	0.26e	0.25c	12e
Nematode	2864a	1.43a	5a	0g
Nematode+carbofuran	936d	0.46c	0.25c	0g
Nematode+Neem	1100e	0.43d	0.4c	0g
Fungus	0h	0f	0c	50c
fungus+bavistin	0h	0f	0c	6f
fungus+ Neem	0h	0f	oc	5f
LSD	1.35	0.013	0.52	1.35

Each value is an average of three replicate Means followed by the same letter within a columns do not differ significantly at $p < 0.05$ according to LSD test SAS

In the present study Neem seed powder reduced either the *Fusarium* wilt, number of nematode galls or the interaction resulted by combination between *M.incognita* and *Fusarium oxysporum* f.sp *lycopersici* and improved the shoot dry weights of tomato plants. Results showed significant suppression of both *M. incognita* and *F. oxysporum* by Neem seed powder. To cope with this, *A. indica* seed powder and other bio agents may be applied. It is clear from the results that besides chemicals, *A.indica* seed powder was sufficiently effective against both the pathogens, this may be due to presence of active principles and toxic chemicals in *A. indica* seed powder [26].

Application of Neem seed powder to soil inoculated with either Fusarium or both pathogens increased tomato shoot and root weights significantly compared with inoculated and uninoculated pots that were not treated with Neem. Wilt severity and vascular discolouration of tomato were reduced for Neem treated pots. Root galling index, number of eggmasses per gram of root and final nemtode population in soil decreased significantly for Neem treated plants compared to the untreated plants [4]. Results of present experience is agree with Agbenin et al., 2004 reports, Neem seed powder increased root and shoot weights and heights and decreased root galling index and presence of mycelium on root.

Finding in this study confirmed that Neem seed powder can be used as natural fungicides to control *Fusarium oxysporum* to reduce the dependence on the synthetic fungicides and it was agree with the result of Aba Alkhail, 2005.These results of the present investigation are clear indication for potential of neem to control *Fusarium*, Joseph *et al* [18] showed neem in all consideration (5%, 10%, 15%) has fungicide potential. Paul and Sharma [23] repoted the aqueous extract of *A.indica* inhibited vigorously the growth of soil born pathogenic fungi.

Neem seed reduces nematode population and it was effective in causing larval mortality, it was agree with *Adegbite* and *Adesiyan*, 2005 reports.

Conclusion:

It has been concluded from present research that certain plant extracts are a source of cheap and effective nematicides of root knot nematodes and fungicide of *Fusarium*, also it doesn't has human and environment health implications. *Meloidogyne incognita-Fusarium oxysporum* disease complex can cause severe losses in tomato. Although chemicals viz. carbofuran and bavistin showed a significant effect in decrease of diseases but *A. indica* seed not only increase of growth Parameters but also control

disease complex and it can be a good replacement of chemical control.

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