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POSSIBILITY OF THE BIOCONTROL OF *MELOIDOGYNE JAVANICA* USING THE FUNGUS *TRICHODERMA HARZIANUM* UNDER GREENHOUSE CONDITION

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Abstract

In recent decades, the biological control of the diseases caused by plant pathogens using other microorganisms has attracted the attention of researchers. In the present study, we assessed the biological control of root knot nematode *Meloidogyne javanica* in cucumber using three isolates of the fungus *Trichoderma harzianum* under greenhouse condition. The roots of the cucumber seedlings were inoculated with fungal spore suspension and 300 active second-instar larvae of the nematodes. One million spores/mL from the fungus was used for each plant. In this experiment, we assessed the effects of *Trichoderma harzianum* according to the diameter of the gall, number of the root knots, mean weight of the wet and dry roots and aerial parts, number of the egg masses per each plant, and number of the eggs per each egg mass. The isolates of the fungus reduced the severity of the disease and number of the galls in the roots. Moreover, increasing of the mortality rate in the larvae and decreasing of the hatching rate of the eggs were compared to the control samples. The results showed, the changes in the entire diseased plant which indicated that *M. javanica* could be controlled in cucumber by *Trichoderma harzianum.*

Keywords: Biological Control, Root-knot Nematode, Antagonism, Cucumber

Introduction

The plant parasite nematode, *Meloidogyne javanica* is the second most common species in the world, which is of paramount importance in terms of diversity and frequency in Iran (Akhiani *et al*., 1984). This nematode parasites the roots of plants and its second-instar larvae causes root infection and galls (Sharon *et al*., 2010). The male nematode is worm-shaped, and the female nematode is pear-shaped. The female nematode produces eggs after mating or even without mating with the male nematode, and the eggs are placed in a gelatin mass for preservation. All or some of the eggs may be laid inside or outside the root tissues through winter. In the spring, the eggs hatch, and the second-instar larvae attack to the host plant. The symptoms of the disease caused by the nematode mostly appear on the roots in the form of knots, knot-like protuberances, and dry decay. In younger plants, these symptoms may lead to death, while it appears as short height, reduced the plant growth, yellowness, and cause wilting under higher temperature in older plants.

Today, the biological control of root-knot nematodes has become a priority with the aim of reducing the problems of chemical pesticides, which pose great risk to the life of humans and pollutes the environment. According to the literature, introducing various antagonists, including fungi, to the soil and rhizosphere could minimize the disease burden to the below of economic threshold (Maleki Ziarati *et al*., 2006). Biocontrol is a phenomenon with various mechanisms, including antibiosis, competition for space and food, and loss of iron adsorption through producing siderophore, lubricant enzymes and parasitism, increasing the growth of the plant, and inducing resistance (Sikora *et al*., 2005).

Fungal antagonists, such as *Trichoderma harzianum,* have remarkable application in the control of plant pathogens, including *Rhizoctonia* and *Fusarium*. This fungus is among the contributing factors that could reduce the population of *M. javanica* through the parasitizing of egg, larvae, and adult female nematode. Furthermore, this fungus induces resistance in the plant and changing the levels of enzymes such as superoxide dismutase, catalase, peroxidase, polyphenol oxidase, and other compounds (e.g., phenols) in the plant (Harmen *et al*., 2006). Colonization of the surface of the roots by antagonists, especially *Trichoderma harzianum,* could reduce the direct attack of pathogens through inducing systemic resistance.

Several biocontrol mechanisms have been proposed for *Trichoderma harzianum* in natural conditions, so that the biocontrol effect of an isolate could be the result of a set of mechanisms, which directly affects the pathogen, increases the resistance of the plant to the pathogen, and the mutual interaction of the host and pathogen. Use of *Trichoderma harzianum* is mainly focused on the direct effect of the fungus on the pathogen. Under such circumstances, the fungus destroys the target microorganism before penetration into the plant tissue through producing hydrolytic

enzymes, toxins, and antibiotics. *Trichoderma harzianum* could generate semi-phyto hormones such as cytokinin, zeatin, giberline, indole acetic acid, ethylene, which increase the growth of the plant and enhance food absorption. On the other hand, it stimulates inductive and systemic resistance in the plant, which in turn reduces the rate of the disease.

The production of various enzymes and elimination of pathogens before penetration into the host are among the foremost mechanisms of *Trichoderma.* Some of the most important enzymes are chitinases, proteases, lipases, and glucanases, which destroy the cell wall of the target cells. In various species of *Trichoderma,* there are several important exochitinase and endochitinase types. By breaking glycoside bonds in linear chitin polymers, this enzyme destroys their structure (Benitez *et al*., 2004).

The present study aimed to evaluate three isolates of *Trichoderma* and the possibility of their biocontrol ability in the eggs and second-instar larvae of the root-knot-producing by *M. javanica* under greenhouse conditions.

Materials and Methods

Preparing the root-knot population

To prepare the pure population of the nematode, we used the eggplants contaminated with the root-knot nematode collected from an egg mass. The samples were separately placed near a four-leaf seedling of the susceptible cucumber cultivar of RAMI F-1 in a pot containing 1500 grams of sterilized soil in greenhouse conditions. After three months, the nematodes were extracted from the contaminated roots using 1.5% sodium hypochlorite based on the method proposed by Hussey and Baker (1973). Morphological methods were used to identify the nematode. To do so, cross-sections were obtained from the ends of the female nematode based on the method proposed by Hartman and Sasser (1987), and their characteristics were assessed.

Preparing the Trichoderma isolates

Three pure isolates of various *Trichoderma* species were obtained from the phytopathology department of Karbala, Oman, and Kufa universities, which were cultured and preserved on PDA medium.

Preparing the pots and planting cucumber

To do this experiment, 48 pots were purchased in size two. One seed of cucumber was planted in each pot. The experiment was totally random with eight treatment samples. For each treatment sample, we considered three repetitions of the susceptible cultivar and three repetitions of the resistant cultivar.

Inoculation of nematode to cucumber seedlings

Each pot contained 1500 grams of sterilized soil (one part sand, one gravel, and one peat). Afterwards, 3000 second-instar larvae of the nematode were added to the pots.

Inoculation of the fungus to the cucumber seedlings

For the inoculation of various isolates of *Trichoderma* in the cucumber seedlings in the greenhouse, 350 grams of millet seeds were added to the suitable flasks and sterilized at the temperature of 121°C for 15 minutes. Following that, 10 pieces of two-millimeter discs provided from the fresh cultivates of the fungal isolates were added to each flask, which were preserved at the temperature of 28°C for 15 days for the growth of the fungus. During this period, the contents of the flasks were mixed properly. 15 grams of the fungus grown on millet seeds was eventually added to each pot. After two days, one cucumber seedling was planted in each pot, and kept at the temperature of 28±1°C in the greenhouse. The treatment controls had no inoculum. In this experiment, we used two susceptible cultivars of RAMI F-1 and YAKTA F-1, considering three repetitions of the susceptible cultivar and three repetitions of the resistant cultivar for each *Trichoderma* isolate.

Evaluation of damage and efficiency of biological control

To determine the pathological indexes, we assessed some factors such as the number of the galls, eggs and egg masses per 2 cm of the root, larvae per 100 grams of the soil surrounding the root, and also reproductive parameters. To do so, the contents of each pot were placed in separate trays, and the soil surrounding the root was gently washed. The samples were transferred to the laboratory, and the mentioned parameters were counted.

Statistical analysis

Data analysis was performed in SPSS version 22 using one-way analysis of variance (ANOVA) and the mean values were compared using Duncan's test.

Results and Discussion

Evaluation of the morphological and morphometric features of the second-instar larvae, adult female nematode, and cross-sections of the cuticle network at the end of the nematode, as well as the results of the differential hosts, indicated that all the studied species were *M. javanica.*

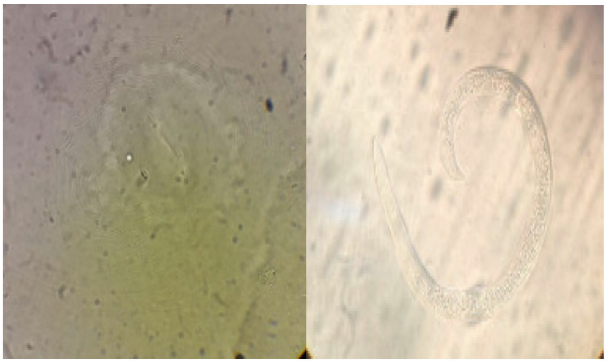

Figure 1 : Second-instar larvae of the root-knot nematode (right) and female perineal pattern at the end of body (left)

The length of the body of the second-instar larvae was within the range of 402-560 µm. The head was not distinct from the body in the larvae and it was straight (12.15-13.5 µm). In addition, the knots were horizontally wide, without protuberance.

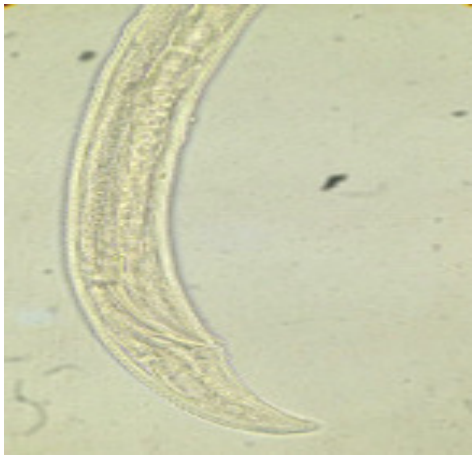

Figure 2 : The tail section of an adult root-knot nematode

The biocontrol effects of the *Trichoderma* isolates obtained from Oman, Kufa, and Karbala against root-knot nematode *M. javanica* were investigated beside the cucumber roots under greenhouse conditions. The results of the inoculation of 3000 second-instar larvae per each plant and 15 gram from fungal inoculum in each pot showed significant differences between the wet and dry weights of the cucumber roots, as well as the length of the aerial parts of the inoculated plants, compared to the plants inoculated with the root-knot nematode. In this regard, the most significant increase was observed in the length of the aerial parts of the plants treated with the isolate of Oman in the unique resistant cultivar. Moreover, the treatment of the nematode inoculated plants with the *Trichoderma* isolate from Kufa resulted in the more significant increase of the dry and wet weight of the plants inoculated with the root-knot nematode (Figure 3).

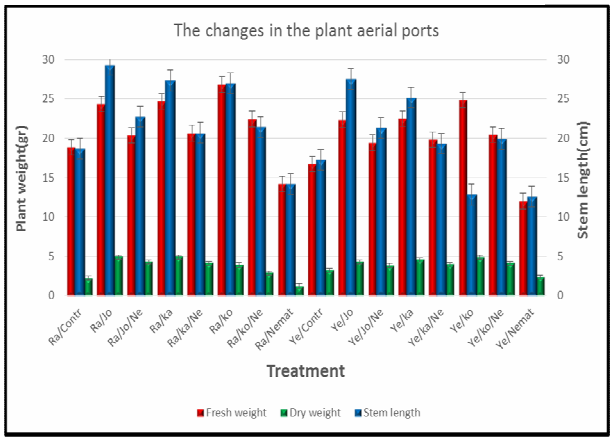

Figure 3 : Mean changes in the aerial parts of nematode infected plants treated by *Trichoderma* isolates.

The results of the infected plants treated by *Trichoderma isolates* showed the increasing of the root wet and dry weight and also the length of the cucumber roots. In addition, the most significant increase was observed in the wet and dry weight of the infected plants which were treated by Kufa *Trichoderma* isolate, while the most significant increase in the length of the root was observed in the infected plants treated by Oman isolate in the susceptible cultivar of RAMI (Figure 4).

With the colonization of the rhizosphere, *Trichoderma* prevented the expansion of the nematode into the internal tissues of the roots, thereby increasing the growth of the treated plants. This is consistent with the results obtained by Harman *et al*. (2004). The effects of *Trichoderma* on the wet and dry weight of the root and aerial parts indicated that the increased weight of the roots of the plants inoculated with the fungus was due to t5533he decreased number of the galls on the root of cucumber, which is in line with the results of the experiments conducted by Sharon *et al*. (2001).

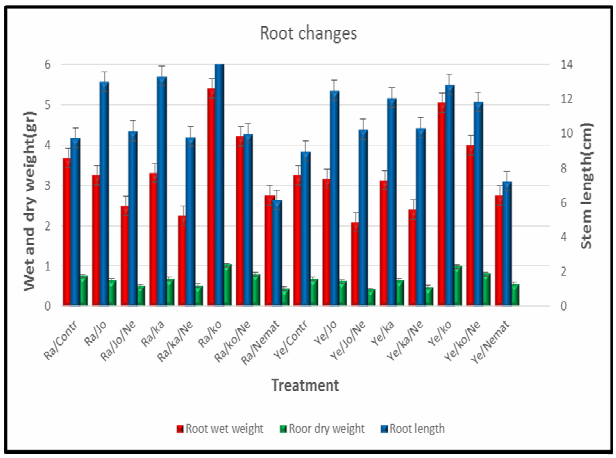

Figure 4 : Mean changes in dry and wet weight and the length of the infected root treated by *Trichoderma isolates*

According to the results of ANOVA table regarding to the treatment samples in 5% probability level *Trichoderma* had a significant effect on the reduction of the growth indexes of the root-knot nematode (number of the galls, mass eggs in each cyst, and female nematode in each gall). Furthermore, *Trichoderma* could significantly decrease the number of the galls, egg masses, and female nematode in each gall (Figure 5).

The most significant reduction was observed in the number of the galls and egg masses in each gall on the infected plants treated by Kufa *Trichoderma* isolate in the unique cultivar.

Trichoderma battles with pathogens through competition, antagonistic properties, and producing enzymes and toxins. In addition, this fungus has an exclusive function against nematodes through producing anti-nematode compounds and directly affecting second-instar larvae, as well as reducing the adsorption of nematodes by the roots, which in turn limits their penetration. *Trichoderma* also induces resistance mechanisms in the plant against the attack of nematodes, thereby decreasing their pathogenicity (Sharon *et al*., 2001).

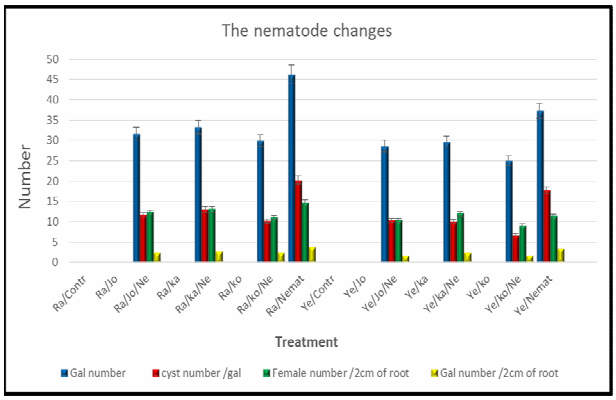

Figure 5 : The number of gall, cyst and female of root knot-nematode in cucumber plants treated by *Trichoderma isolates*

In a study in this regard, Spiegel and Chat (1998) reported that using various isolates of *Trichoderma* in soil could reduce the gall index in the infected plants with root-knot nematode. In addition, Windham *et al*. (1989) have claimed that the *T. koningi* isolate in soil could effectively decrease the nematode eggs of *Meloidogyne* spp.

In another research, Sharon *et al*. reported the increased growth of treated tomato plant with *Trichoderma,* making a reduction in the number of galls in the roots of the infected plants. In the present study, it seemed that *Trichoderma* could prevent the penetration of nematode into the roots through stimulating defense mechanisms, which is in congruence with the results obtained by Sharon *et al*. (2003), Windham *et al*. (1989) and Sahebani *et al*. (2015).

In another study made by Pandey *et al*. (2003), various isolates of *Trichoderma* were used in chickpea plant. According to their findings, nematode control was associated with the decreased number of galls and the final population of the nematodes, as well as the significantly reduced number of the eggs and second-instar larvae per each gram of the root, in all the plants treated with the isolates of *T. viride*. Furthermore, the findings of Al-Ameiri (2009) demonstrated that the treatment of plants with *Trichoderma isolates* caused a significant reduction in the number of the galls in the roots contaminated with root-knot nematode compared to the controls.

The findings of the current research confirmed the ability of *Trichoderma isolates* to control root-knot nematodes. In addition, the present study indicated that *Trichoderma* could decrease the pathogenic factors associated with root-knot nematodes compared to the controls. Therefore, it could be concluded that the application of this antagonist in soil could increase the efficiency of nematode control.

In conclusion, it is recommended that various isolates of *Trichoderma* could be applied as controlling agents for *M. javanica* in cucumber under greenhouse conditions. This fungus acts as an inductive agent in defense compounds, thereby stimulating the defense system of the plant.

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