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Biological Control of Meloidogyne incognita using Mycorrhizal Fungi in Central Sudan

Elnour, A. E. [1], O.O. Elbashir [2] and G. A .A. Elbadri [3]

[1] Gezira Research Station, Agricultural Research Corporation, Pant Pathology section, Wad Medani Sudan.

[2]Geziera University, Plant Pathology Center, Wad Medani, Sudan.

[3]Agricultural Research Corporation, Crop Protection Research Center. Plant Pathology section, Wad Medani Sudan. Current address Agriculture house Company, Riyadh, KSA.

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ABSTRACT

There are many pests and diseases damaging both the quality and quantity of tomato production. Plant parasitic nematodes are one of them. They represent an important constraint on the delivery of global food security. Damage caused by plant-parasitic nematodes has been estimated at US$ 80 billion per year. Effects of three concentrations of mycorrhizal fungi were studied for control of Root Knot Nematode. Different tomato accessions were screened for Root-Knot Nematode susceptibility**.** All the tested concentrations of mycorrhizal fungi on nematode infected plants were produce positive effects i.e. nematode numbers were highly decreased compare to control which also appear as improvement of the growth of infected plants at various degrees. Among three tested concentrations, generally, middle concentration 5 ml of mycorrhiza is most effective followed by high one 6 ml of mycorrhizea and the lowest effect produced by lower concentration 4 ml of mycorrhizea. Mycorrhizea fungi appear as most effective control measures of root-knot nematode in tomato.

INTRODUCTION

Plant parasitic nematodes cause loss of 10-% of the whole loss in the crop causing billions of dollars of crop losses across the globe annually. (Cuvaca et al., 2026, Rady et al., 2021). Even this however, is an under estimate as nematode damage can be easily overlooked. Root knot nematode is sedentary endoparasites that induce the formation giant cells in the roots, from which nematode feed to complete its life cycle (Baldacci-Cresp et al., 2015, Jaouannet and Rosso 2013). The availability of water and nutrients to the plant decreases while the giant cells are located close to the root systems xylem and phloem. (Siddqui et al., 2014). Morphological and biochemical alterations induced by nematode parasitism cause abnormal growth of plants, nutrient deficiency, symptoms, roots with galls, forking and other deformations (Palomares-Rius , 2017, Moens et al., 2009). It is estimated that total annual crop losses of 14.6% is caused by plant-parasitic nematode in tropical and sub-tropical climates (Nicol et al., 2011). Annual worldwide agricultural losses caused by plant-parasitic nematodes are estimated to be $137 billion with $13 billion in the United States (Elling, 2013, Howland and Quintanilla, 2023). Chemical control remains the predominant strategy among available approaches for nematode management. In recent years, a new generation of synthetic nematicides with distinct biochemical targets and improved selectivity has emerged (Yan et al. 2026). Health and hazards concerns which have elicited close security by regulatory agencies resulted in increasing restriction or prohibition of use nematicides (Kumar and Donthi, 2024, Osman and Viglierchio, 1998). Natural products seem to avoid environmental problems caused by synthetic pesticides and many researchers are trying to identify effective

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natural products to replace synthetic pesticides (Adam et al. 2024, Mfarrej and Rara, 2019, Kim et al., 2005). The high cost and inconsistent effect of synthetic nematicide, together with their scarcity and ill- effect on an environment and public health, have increased augmented interest on alternative methods of the management for plant parasitic nematodes (Abd-Elgawad 2021, Viaene and Abawai, 1998). There are many pests and diseases damaging both the quality and quantity of tomato production. Plant parasitic nematodes are one of them. They represent an important constraint on the delivery of global food security. Damage caused by plant-parasitic nematodes has been estimated at US$ 80 billion per year (Nicol et al.,2011). Numerous studies have been conducted to determine the damage potential of Meloidogyne species on several vegetable crops including tomato, and different management strategies have been proposed. With the phase-out of methyl bromide, in particular, the problem of Meloidogyne spp. on tomato gained new inters (Seid et al., 2015). However, these were not compiled and presented in a way to help different stakeholders. Thus, the objective of this study was controlling root-knot nematodes by using Mycorrhizea fungi.

MATERIAL AND METHODS

Nematode sample collection

Root samples of tomato, eggplant and okra showing typical symptoms of root knot nematode were collected from 3 states in Central Sudan Geziera (Bida, Fadasi, and Dinagela), and Sinar (Grisli, Aradiba and Kageak), Kharoum (Shambat, Elgili, Wad Ramli and Elbambonab), (Table1). Collect samples were brought to the laboratory to extracts the causal nematode.

A total of 3-5 roots from each plant were selected and cutting roots .Then, the infected roots were put into polythene bags, labeled and brought to the laboratory of plant pathology in Agricultural Research Corporation. All the samples after investigation were found to be Meloidogyne incognita since this species was found the most dominant in Sudan(Yassin, 1986).

Table (1). Roots samples collected from different location for females' extraction:

State	Location	Plant species (local name)	Scientific name
Gezira	Bida	Egg plant	Solanum melongena
	Eldnagila	Okra	Abelmoschus esculentus
	Fadasi	Egg plant	Solanum melongena
Sennar	Grisli	Tomoto	Solanum tubersoum
	Aradiba	Tomoto	Solanum lycopersicum
	Kageak	Okra	Abelmoschus esculentus
Khartoum	Shambat	Tomato	Solanum lycopersicum
	Elgili	Egg plant	Solanum melongena

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	Wad Ramli	Egg plant	Solanum melongena
	Elbanbonab	Tomato	Solanum lycopersicum

The effect of Mycorrhizea on the nematode infection

Mycorrhizea treated on healthy tomato plants previously infected with root knot nematode M. incognita extractions to assess the efficacy of mycorrhizea on reduction of nematode infection. To assess the effect of mycorrhizea on nematode infection, disease rating index, number of eggs, galls of nematode. Number of colonies of mycorrhizea. Fresh and dry Weight of shoot and root and length of infect root were recorded.

Nematode extractions

Nematode eggs were extracted from heavily galled roots as described by (Kim et al., 2001). Roots were washed, cut into 1-2cm segments and maceration in blender for 20 seconds. The macerate was filled into a flask containing 500ml of 1.25% Sodium hypochlorite (Naocl), solution and shaken for 3 minutes to liberate eggs from the gelatinous matrix. Eggs were separated from plant debris by passing the egg suspension successively through sieves of 200µm, 100µm and 25µm mesh size, remove excess chlorine, the eggs from the 25µm sieve were washed several times with tap water. Eggs from the 25µm sieve were then collected in tap water. To confirm the pathogenicity of the extracted nematode (egg) inoculation tomato is needed.

Preparation of tested plants

Seeds of tomato variety Beto-86 were maintained in polyethylene bags and stored at 4ºC. They were surface sterilized using 1.25 % Sodium hypochlorite for 20 minutes and rinsed thoroughly in sterile distilled water and were grown in trays in commercial peat-based substrate. When plants reached the 4-5 leaf stage they were transplanted into 2:1 mixture of clay and sand sterilized soil. Plants were watered adequately throughout the experiment period. Tested plants were inoculated at three weeks old after transplanting.

For inoculation, the eggs were suspended in tap water the total number of eggs per ml were around. Experiments were conducted in a greenhouse in Geziera Research Station Wad Madani, Sudan. The treatments were arranged in a complete randomized block design with three replicates. Observations of the infected plants and rating is done as follows**.**

Nematode Rating Index

The nematode gall rating index was rated on a 0 – 10 scale with 0 = n galls and 10 = completely galled (Salazar, and Schroeder, 2018). Numbers of galls and eggs masses induced were counted under microscope. For the counting of eggs roots were immersed in an aqueous solution of phloxin B (0.15g/1) for 15 min to stain the egg masses. The number of root galls per root system was counted and prevalence determined on the fooling scale: 0=0.1=1-2.2=3-10.3=11-30, 4=31-100 and 5= greater than 100 galls. The number of egg masses per root system was counted and prevalence determined on the following scale: 0=0.1=1-2.2=3-10.3=11-30, 4=31-100 and 5= greater than 100 egg masses (Tylor and Sasser, 1978).

At the end of experiments, the plant fresh, dry shoot and root weight, number of galls and eggs per plant and galling index was scored.

The experiment was conducted two times at different seasons. Experiments were conducted in Random Complete Block Design (RCBD). Data were then analyzed using Mstat program. Treatment means were separated using Duncan's multiple range tests. Statistical differences referred to in the text are significant at $P<0.05$.

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Arubecular Mychorrhizal fungi (AMF)

Collection, isolation, and counting of fungi spore

Mycorrhizea spp which used during this investigation was isolated from grasses (Cynodon dactylon) in Gezira research station, Wad Medani, using sieves with different size from (250 µm, 180 µm, 63µm and 45µm). Using a fine ruler, stereomicroscope, diameter of the ocular field is 4x magnifications for a comfortable field of view (diameter 5 mm). The area of the field was 19.6 mm (Radius = 2.5 mm). Using plastic petri dishes for counting, enough water added to have complete coverage of the base, diameter (85 mm), area of the base calculated 5672 mm. from this datum, and the area of the ocular field, total number field in the dish is : 5672/ 19.6= 289. Extract spores and use sieves to separate out as much root and organic detritus, added the spore suspension to a petri dish and then randomly rotate the dish to spread out the spores, then place spore extraction in a test tube, dilute 1:1 with water while overtaxing, remove specific volume (10 ml) transfer to petri dish, and recount.(Giovanetti et al., 1980).

Inoculum preparation

Inoculation of tomato seedling with mycorrhizal fungi was carried out according to (Prsanthi et al., 2016), in which 4 ml (1000 spores), 5 ml (1250 spores) and 6 ml (1500 spores) means 250 spores /ml were placed 5 cm below surface soil after transplanting. Different parameters were asses to explain the effect of mycorrhizea on nematode infection these parameters include.

Plant root length and Mycorrhizal root lengths

In the experiment, all of the roots of an experimental plant collected and measured and then grid-line intersect method used to measure mycorrhizal root length, the roots colonized by mycorrhizal fungi (Giovanetti, et al., 1980). (cover table to percentage colonization when divided by root length), and the total length of roots produced by the plant. Several small samples are removed from the main root mass after blotting, pooled, and fresh weight is measured **(FW1),** the remaining roots of the sample are weighted (**FW2),** staining root sample in 0.05% ink , place in paper envelopes and dry for at least 48 hr. at 62C. Measure dry weight of large sample (**DW2**) (Giovanetti et al., 1980).

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Spread roots in plastic petri dish in which a grid with 0.5*0.5

A square is affixed to the base. Collecting the following data:

(i) Total number of intersects between lines and roots (R1).

(ii) Total number of intersects where the root is mycorrhizal (R2)**.**

The data above data is used to calculate plant root length and mycorrhizal root length of the total sample based on the assumption that distribution of mycorrhizae in the small pooled sample reflects the distribution of mychorrhize throughout the entire root system.

Dry weight of sample stained to measure colonization (DW1)

This calculation is performed setting up a proportion and solving for x (DW1), as follows:

DW2/FW1= x/FW1

(FW2)(x)= (FW1) (DW2)

X or DW1= (FW1) (DW2)/FW2

Mycorrhizal Root Length in Large (Unstained) sample (R3)

This calculation is an estimate on the actual measurement of mycorrhizal root length in the small stained sample (R2). Another proportion is set up and solved for x (R3). As follows:

R2/DW1=x/DW2

(DW1)(x)= (R2)(DW2)

X or R3= (R2) (DW1)/ DW1

Total Mycorrhizal Root Length = R2+R3

Total plant root length can be estimated by substituting R1for R2 in the same set of calculation. Method, (Giovanetti et al., 1980)

Stained Roots were spread in a plastic petri dish in which a grid with 1×1 cm squares was affixed to the base, and the following data was Collected :
(i) total number of intersects between lines (Vertically V and horizontally H) = roots(R_1)
(ii) total number of intersects where the root is mycorrhizal (R_2)

Then R_2/R_1= % of AMF colonization in root.

RESULTS

Effect of Mycorrhizae in root-Knot Nematode infection

All the tested concentrations of mycorrhizal fungi (4, 5 and 6 ml) were negatively affected Meloidogyne incognita culture compared to the control. The reactions against tested nematode varied from one

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concentration to another. Generally, the mycorrhiza inhibited the nematode forming galls and the nematode number of infected plants. (Table 2).

Table (2). Infection %, shoots and roots fresh and dry weight of nematode infected plants with three concentrations of mycorrhizea.

Concentration of mycorrhizae	Infection percentage	Fresh weight of shoots	Dry weight of shoots	Fresh weight of roots	Dry weight of roots
4 ml	4.111b	19.12a	2.867a	5.922a	1.133b
5 ml	4.000b	19.59a	2.922a	6.267a	1.278b
6 ml	4.333b	19.23a	2.911a	5.789a	1.133b
control	9.222a	16.94b	2.067b	6.611a	2.000a
Cv%	18.05	5.96	15.0	14.63	18.39
S.E±	0.956	1.243	0.399	0.809	0.065

Values in the same Colum followed by different letters significantly different according to Tukeys multiple range tests ($p < 0.05$).

Concentration of mycorrhizae	Colonization of mycorrhizae	Nematode percentage	Mycorrhizae root length	Total plant root length	Number of galls/ plant	Number of eggs/ plant
1 ml	62.91b	34.71b	10.81a	15.51a	620.6 b	290.9 b
2 ml	89.24a	34.82b	10.71a	16.03a	600.9 b	275.9 b

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3 ml	73.42b	36.21b	9.578a	13.859a	590.5 b	280.7 b
control	0.000c	62.48a	0.000b	1.85 b	734.7 a	328.5 a
Cv%	13.03	8.7	31.0	29.5	30.1	25.2
S.E±	4.419	13.36	2.362	3.465	2.892	1.683

Table (3). Number of mycorrhizea colonies, percentage Root-knot nematodes, mycorrhizea root length, and total length plant roots and number of galls eggs / plant root.

Values in the same Colum followed by different letters significantly different according to Tukeys multiple range tests ($p< 0.05$).

All the tested concentrations of mycorrhizal fungi (4, 5 and 6 ml) were negatively affected Meloidogyne spp compare to the control. The reactions against tested nematode varied from one concentration to another. Generally, the mycorrhiza inhibited the nematode forming galls and the nematode number of infected plants. All concentrations of mycorrhizea (4, 5 and 6 ml) used showed significant reduction on the infection by nematode during 2015- 2016. All parameters measured here were show significant difference compare to untreated plants. These parameters include rating index, fresh and dry shoot and root and total root length, colonization of both nematode and maycorizae and numbers of eggs and galls. which recorded increasing in the control reaching 9.000 compare to 4.000 for treated. Mycorhizae produced increasing the fresh weight of shoots from 19.23g to 16.94. significant increasing in shoots dry weight 2.86g which was higher than control plants recorded 2.0g only, fresh and dry root weight of treated plants were decreased from 5.7g to 1.1g while increased in the control 6.6g – 2g (table 2), mycorrhizea colonization showed significant increasing in treated plants reached 89%.Compare to zero in control, nematode percentages significantly reductions on mycorrizal plant reached62%, while control recorded 34%.for treated plants were found between 34 to 43% compared to the control which 62%, total numbers of plant roots containing mycorrhizea were found10 cm. However, the treated plants length 16 cm compared to the control plants length 8 cm, number of eggs showed significant reduction reached 590 egg/plant while increasing in control 734.7, number of galls showed reduction in forming galls reached 275/plant compare to increasing in control 328.5/plant (table 3).

Table (4). Infection %, Fresh and Dry weight shoots and roots of the infection plants with three concentration of mycorrhizea Second season.

Concentration of mycorrhizae	Infection percentage	Fresh weight of shoots	Dry weight of shoots	Fresh weight of roots	Dry weight of roots
1ml	3.889a	20.34a	3.067a	6.289b	1.546b

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2ml	4.000b	21.10a	3.400a	6.267 ab	1.493b
3ml	3.778b	20.73a	2.756ab	6.367b	1.667b
control	9.333a	18.00b	2.144b	7.556a	2.411a
Cv%	21.28	6.15	24.99	16.90	26.15
S.E±	1.248	1.518	0.504	1.280	0.217

Values in the same Colum followed by different letters significantly different according to Tukeys multiple range tests ($p< 0.05$).

Table (5). Number of mycorrhizea colonies, percentage root-knot nematodes, mycorrhizea root length, total length of the plant roots and number of galls & eggs / plant root, second season.

Concentration of mycorrhizae	Colonization of mycorrhizae	Nematode percentage	Mycorrhizae root length	Plant root length	Number of galls / plant	Number of eggs/plant
1ml	69.26c	38.32b	10.63a	15.99a	590.8 b	299.9 b
2ml	90.93b	38.60b	12.87a	19,04a	600.7 b	280.6 b
3ml	75.96b	39.11b	12.44a	17.20a	580.5 b	295.0 b
control	0.000a	63.20a	0.222b	3.00b	650.8 a	390.9 a
Cv%	15.66	8.14	25.98	28.31	29.5	30.5

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S.E±	61.415	13. 362	5.514	13.670	3.465	3.627

Values in the same Colum followed by different letters significantly different according to Tukeys multiple range tests ($p< 0.05$).

During this season all treated plants showed significant reduction in gall formation and infection by nematode ranging from 1- 4 compared to high recorded for control which reached 9, for both fresh and dry weight were significantly increased compere to control, i.e. fresh weight reach 18 g while treated plants showed 21 g, dry weight was increased3.4g for the treated plants while decreased in untreated plants weight 1- 2g,fresh and dry weight of the treated plants roots showed significant reduction in their weight compared to the control roots weight, colonization of mycorrhizea was showed increasing in treated plants retched 90 compared to the control zero, nematode percentage was showed significant reduction in treated plants reached 38% compare to control 63%. The total length was increased in the treated plants (17 -15 cm) compared to the control plants which was only 3 cm (Table, 2).

DISCUSSION

A number of genera and species of nematodes are highly damaging to a great range of hosts, including foliage plants, agronomic and vegetable crops, fruit and nut trees, turfgrass, and forest trees (Williams et al., 2017). Tomato is most important vegetable crop worldwide. Meloidogyne as parasitic nematode among restricting tomato production factor regarding their damage of the root system inflicting infection and deform the function of the normal root system to absorb water and nutrients, in addition they help soil borne pathogen to invade the plant root system. Continued monocropping practiced by commercial farmers in protected greenhouses (Matlala et al., 2025). provides the ideal environment for crop growth but makes it more vulnerable to infection by plant-parasitic nematodes. Many control measures were used to minimize the losses caused by such nematode like e.g. Rotation, Resistant Varieties, Cultural and Management Practices and nematicdes. Each of these measures shows degree of control with many disadvantages. Using of natural component as control agent is appear more safety unrespect of their names applied ranging from biological to natural control. Combination of plant inoculation with a commercial mycorrhizal formulation with half or full fertilizer application rates was evaluated for the effects on plant growth and yield and mycorrhization occurrence throughout two consecutive field tomato crops in southern Italy (Candido, et al.,2013). Arbuscular mycorrhizal fungi (AMF) are obligate root symbionts that can protect their host plant against biotic stress factors such as plant-parasitic nematode (PPN) infection (Schouteden et al., 2015) . The AMF symbiosis also leads to an altered root exudation composition and level (Hodge, 2000; Jones et al., 2004), which can in turn impact the PPN in the rhizosphere in terms of hatching, motility, chemotaxis, and host location (Schouteden et al., 2015). In our experimental data mycorrchizae reveal very good enhance of treated tomato plans through both decreasing the rate of infection and improve the plant growth. Inoculation of the plants in the nursery with AMF allows sufficient time to establish the symbiosis before transplanting and prior to pathogen exposure same result were founded by (Nerva et al., 2023 Talavera et al. 2001). The seedlings of tomato were positively response to association with mycorrhizae, showing growth increment, these results reinforced the previous studies with the same combination of host and AMF Anjos et al., (2005). The decrease in plant shoot development associated with nematode parasitism usually is related to the interruption of water and nutrients translocation by the giant cells same as Cofcewicz et al., (2001). The nutritional benefit promoted by mycorrhizea on tomato plants contributed for increasing the resistance or tolerance to M. javanica m. javanica same as found by Cofcewicz et al., (2001). The establishment of mycorrhizal prior to nematode contact was beneficial for tomato plants conferring conditions for improved plant growth in the presence of the pathogen.

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