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Combined Effects of The Earthworm, *Allolobophora longa*, The Fungus, *Fusarium oxysporum* and The Root-Knot Nematode, *Meloidogyne javanica* on Eggplant

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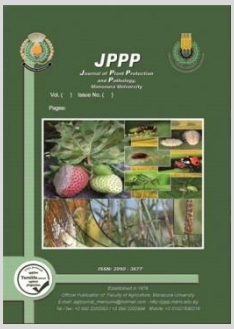

ABSTRACT

Biotic interactions among the earthworm, *Allolobophora longa*, fungus, *Fusarium oxysporum* and the root-knot nematode, *Meloidogyne javanica* infecting eggplant *solanum melongena* cultivar balady were studied under green house conditions . Multiplying nematode in all trials at the added earthworms was significantly lower than in the control (check). The highest of numbers of galls per root system and rate of nematode reproduction on eggplant were found in treatment nematode of alone, followed by treatment of *M. javanica +F. oxysporum* .The highest percentages of reductions in length and fresh weight of shoots and roots occurred on eggplant treated by *M. javanica + F. oxysporum, M. javanica + F. oxysporum* +15worms of *Allolobophora longa* where the values were close. The increase was only in fresh weight shoot with treatment *F. oxysporum* + 5worms of *A. longa* while the increase were in both length and fresh weight shoot with treatment 10 worms of *A. longa* . The highest percentage value of severity was detected by treatment of nematode + fungus . But, the lowest of severity was found in treatment of *F. oxysporum* +10 worms *A. longa*.

Keywords: *solanum melongena, Allolobophora longa , Fusarium oxysporum, Meloidogyne javanica* ,eggplant, interaction.

INTRODUCTION

Earthworms play an important environmental role to ecosystem engineers..Many works had reported effects of earthworms on plant growth, studies combining these invertebrates and soil pathogens showed numerous positive interactions. Significant decrease in the nematode population was noted in the sequential inoculation of the fungus followed by nematodes and combined inoculation of the two pathogens.The earthworm *Reginaldia omodeoi* reduced the damage caused by plant parasitic nematodes *Heterodera schachtii* on plants (Blouin *et al*. 2013).

The effects earthworm such as a reduction in the disease severity were found in the earthworm treated plants (Gámiz *et al*., 2018). The earthworm *Pontoscolex corethrurus* affect on total nematode densities mainly by increasing the density of dominant bacterial-feeding (*Acrobeloides*) and fungal-feeding (*Aphelenchus*) nematodes(Dash *et al.,* 1980). Earthworms and vermicompost have been shown to reduce of nematode infestation in banana plantations (Senapati, 1992).

In natural ecosystems, plant diseases are generally controlled by the effect of biological interactions (competition, predation and parasitism) resulting from the effects of soil ecosystem engineers (Lavelle and Spain, 2001). The present research was carried out to study the effects denisites earthworm of *Allolobophora longa* on nematode and fungus communites. Besides, earthworms which can improve plant growth and enhance the protection from the plant parasitic nematodes and fungi.

MATERIALS AND METHODS

Nematode:

A pure stock culture of the root-knot nematode, *Meloidogyne javanica* was prepared from naturally infected eggplant roots collected from an infested field. Individual egg-masses with their mature females were removed from root tissue. Each egg-mass was placed in a small glass capsule containing fresh water. The female from which egg-masses were taken preserved in 4 % formaldehyde solution in glass capsules for nematode identification. Each egg-mass was transferred to a 25 cm clay pot filled with steam sterilized sandy loam soil and growth with a seedling of tomato cv. Rutgers Inoculated pots were placed in a greenhouse and watered when needed. After two months of inoculation, infected roots were then chopped and used as sources of inoculation for other series of clean cv. Rutgers tomato seedling. By repeating this procedure enough quantities of incoulation from stock cultures were obtained on tomato cv. Rutgers.Identification of *Meloidogyne* species (Eisenback *et al*.,1981*)*.

The galled roots of eggplant *solanum melongena* cultivar balady were soaked in tap water to remove adhering soil particle. Roots were cut into small pieces of 2-cm and placed in a 1000-ml container with 200 ml of 5% NaOCl solution. The container was vigorously for three minutes dissoloveed the gelatinous matrix were extracted eggs from egg-masses. The liquid suspension was poured through a 200 –mesh sieve nested up on a 500 – mesh sieve. Eggs suspended in the solution passed through the 200- mesh sieve.Which removed the root debris and were

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on the collected by 500-mesh sieve.The eggs were collected in distilled water and preserved until inoculation (Hussey andBarker,1973).Was examined by microscope. Females were gently picked up and placed over a drop of lactophenol on a clean slide. Female posterior tail was cut with help of a sharp knife. Females were identified on the basis perineal pattern system according to(Eisenback *et al*.,1981).

Isolation,purification,identification of fungi and inoculum preparation: Okhuoya *et al*.,(2012). Plants were collected and washed with tap water adhering soil particles. Small parts of infected were surface sterilized using1% NaOCl for 2minutes,and washed with distilled sterilized water for several times.Then they were dried using sterilized filter paper and transferred into petri-plates containing potato dextrose agar medium (PDA). Plates were incubated at 28 ± 2C° for7 days. Hyphal tips of fungi were grown on PDA medium. All fungi were purified using single or hyphal tip technique cultures and identified (Barnett and Hunter 1998). The inoculums used of isolated fungi were prepared by culturing of *Fusarium oxysporum* grown in 500ml glass bottles contanining the following substrate per bottle medium and autoclaved for 30 minutes. The bottles were incubated at 25 ± 2C°for two weeks to obtain sufficient growth of the fungi.Then the inoculums were 1000coelomocytes /ml to the soil.

Earthworms:

Were collected from Al -Azhar University farms and adults with (clitellum were identified using the external morphology according to (Moreno 2004). Treatments were provided with earthworms at levels: 5, 10 and 15 individuals per pot, inoculated nematode *Meloidogyne javanica* alone, fungus *Fusarium oxysporum* alone ,combination(*Meloidogyne javanica +Fusarium oxysporum*), (*Meloidogyne javanica+ Allolobophor longa*),(*Fusarium oxysporum+ Allolobophora longa*) , (*Meloidogyne javanica + Fusarium oxysporum+ Allolobophor longa*) compared to untreated control. Pots were filled with autoclaved soil mixture of 1:1(v:v) sandy and clay. After the establishment eggplant seedling in the pots, earthworms were added on surface of the soil, nematodes *Meloidogyne javanica* and fungus *Fusarium oxysporum.* Treated pots suspension of 3,000 eggs was pipetted into three holes in the root zone of 2-week-old seedling. while fungi 1000coelomocytes /ml.per three seedling eggplant cultivar balady in pot.. Plants were inoculated with pathogens to create four replicates. The randomized complet design used in the experimental maintained at30±5C° and waterd as needed. The sixty days experiment after inoculation, plants were removed carefully from soil and the plants were observed for their plant growth. Parameters were as indicated by length and fresh weight of roots shoots were recorded. An aliquot samples 250g soil were processed for nematode extraction by method (Southey 1964). Numbers of galls , egg masses, developmental stages number, and numbers of eggs/ eggmass were counted per root system and fresh weights of the root and shoot systems as well as their lengths were determined. Eggs of ten randomly selected egg-masses of each root system were also counted.

Disease severity percentage :

Disease percentage disease index (PDI), disease plant exhibited stunting, dark brown vascular discoloration, and death as described (Paz-Lago *eT al*., 2000).

An analysis of variance (ANOVA) was applied to test (Gomez and Gomez,1984). Comparison between means of each factor were carried out using - range test (Duncan, 1955).

RESULTS AND DISSCUSSION

Results

Are presented in Table (1) and Fig (1). It was found the root-knot nematode, *M. javanica* succeeded in developing and multiplying in all the different treatments. The nematode population was significantly affected by the tested earthworms *A.longa* and fungus *F. oxysporum* as compared to treatment of nematode alone (check)under greenhouse conditions, inoculation of nematodes only (check). The highest rate of nematode reproduction on eggplant was (15.97) for nematode alone. Followed by, N+F(5.77). Median values recorded were found with N+E5 (2.78), N+E10 (2.44), N+F+E10 (2.36), N+E15(2.35), and N+F+E15 (2.08), respectively. While the lowest rate of nematode reproduction N+F+E5 (1.64) was detected with The effect of *F. oxysporum* is evaluated with various degrees, percentages of severity with the fungus only was (50.00%). The highest value of severity with N+F (58.00%), Followed by N+F+E5 (50.00%). Moderate values of severity induced by treatments N+F+ E15(30.33), N+F+E10 (25.00%).But, treatment of F+E10 recorded (8.33%). the lowest value of severity

Table 1. Effect of the earthworm, *Allolobophora longa*,the fungus, *Fusarium oxysporum* and the root-knot nematode,*Meloidogyne javanica* infecting eggplant Cultivar Balady on galling and reproduction of the nematode under greenhouse.

Treatments	No. of galls/root system	No. of nematodes in soil/pot	No.of developmental stages /root	No.of egg-masses /root	No.of eggs / egg-mass	Nematode final population	Rate of nematode reproduction(Pf/pi)	Severityof % *F. oxysporum*
N alone (Check)	253 a	240 c	90 c	136 a	350 a	47930	15.97	-
F alone (Check)	-	-	-	-	-	-	-	50.00
N+F	223 a	600 ab	90 c	88 b	189 b	17322	5.77	58.00
N+E5	104 b	200 c	176 ab	45 c	177 b	8341	2.78	-
N+E10	61 b	435 ab	176 ab	41 c	164 b	7335	2.44	-
N+E15	67 b	205 c	201 a	50 c	133 b	7056	2.35	-
F+E5	-	-	-	-	-	-	-	41.66
F+E10	-	-	-	-	-	-	-	8.33
F+E15	-	-	-	-	-	-	-	16.66
N+F+E5	50 b	470 ab	193 a	28 c	153 b	4947	1.64	50.00
N+F+E10	76 b	767 b	183 ab	43 c	143 b	7099	2.36	25.00
N+F+E15	77 b	450 a	180 ab	44 c	128 b	6262	2.08	30.33
LSD at 5%								

N= Nematode (*Melodogyne javanica*)

F= Fungus (*Fusarium oxysporum*)
E= Earthworm (*Allolobophora longa*) E5= 5 Individuals, E10= 10 Individuals and E15= 15 Individual

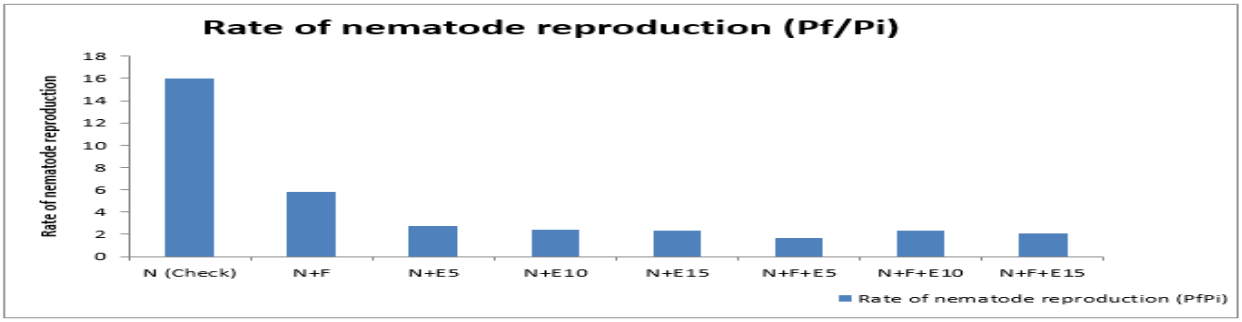

Fig. 1. Rate of nematode reproduction on eggplant as influenced by *Meliodogyne javanica, Fusarium oxysporum* and *Allolobophora longa* alone or combined under greenhouse conditions.

Plant growth parameters as indicated by length and fresh weight of shoots and roots of eggplant *solanum melongena* balady cultivar as well as percentage decrease or Increase when compared with healthy one were recorded in Table (2) and Fig. (2). Data in general indicated that in the treatment N+F plant growth criteria were highly significantly decreased in comparison to their checks. Thus the highest percentage of reductions in lengths shoots and roots lengths and fresh weights were occurred on eggplant balady cultivar (34.02 – 54.85%) and (34.89 – 70.99%), respectively. Followed by N+F+E15 (34.02 - 56.31%) and (34.29 – 67.58%) for shoots and roots lengths and fresh weights. On the other hand, the results revealed that the increased of shoot fresh weight with N+E10 (+5.73%) and F+E5 (+3.94%).while the increase in both length and fresh weight shoot with E10 (+3.09 – 68.49%). Nematode activity and inhibition of plants affected by earthworm participation indicates the importance of the soil fauna in the ecological through fauna interactions.

Table 2. Plant growth of eggplant cultivar Balady as influenced by the biointeractions of the root-knot nematode,*Meloidogyne javanica*,the earthworm, *Allolobophora longa* and the fungus, *Fusarium oxysporum* under greenhouse conditions.

Treatments	Length (cm)				Fresh weight (gm)			
	Shoot	Decr. Or	Root	Decr. Or	Shoot	Decr. Or	Root	Decr. Or
Untreated C	**32.33 ab**	**Incr. %**	**34.33 a**	**Incr.%**	**8.37 b**	**Incr. %**	**5.86 a**	**Incr. %**
F	30.00 abc	7.20	26.00 abc	24.26	7.16 b	14.46	4.60 ab	21.50
N	27.00 abc	16.49	22.00 bcdef	35.92	8.00 b	4.42	4.00 abc	31.74
N+F	21.33 e	34.02	15.50 cdefg	54.85	5.45 b	34.89	1.70 cd	70.99
N+F+E5	23.56 cde	27.13	22.00 bcdef	35.92	6.40 b	23.54	2.83 bcd	51.71
N+F+E10	25.00 bcde	22.67	24.33 bcde	29.13	5.87 b	29.87	4.83 ab	17.58
N+F+E15	21.33 de	34.02	15.00 efg	56.31	5.50 b	34.29	1.90 cd	67.58
N+E5	26.33 bcde	18.56	22.00 bcdef	35.92	6.30 b	24.73	2.20 cd	62.46
N+E10	30.00 abc	7.21	26.00 abc	24.26	8.85 b	+5.73	4.73 cd	19.28
N+E15	23.22 cde	28.18	18.33 cdefg	46.61	6.40 b	23.54	2.75 bcd	53.07
F+E5	29.33 abcd	9.28	16.33 cdefg	52.43	8.70 b	+3.94	1.60 d	72.70
F+E10	29.67 abc	8.23	25.00 bcd	27.18	7.80 b	6.81	3.60 abcd	38.57
F+E15	28.67 abcd	11.32	13.67 fg	60.18	6.87 b	17.92	3.00 bcd	48.81
E5	30.33 abc	6.19	28.33 ab	17.48	7.00 b	14.46	4. 70 ab	19.80
E10	33.33 a	+3.09	20.00 bcdefg	41.74	14.10 a	+68.46	3.93 abcd	32.94
E15	22.67 cde	29.88	12.00 g	65.05	7.70 b	8.00	2.93 bcd	50.00

$$\text{Decrease \%} = \frac{\text{Untreated plants(Control) - Treated plants}}{\text{Untreated plants(Control)}} \times 100$$

$$\text{Increas \%} = \frac{\text{Treated} - \text{control}}{\text{control}} \times 100$$

N= Nematode (*Melodogyne javanica*) F= Fungi (*Fusarium oxysporum*)
E= Earthworm, (*Allolobophora longa*) E5= 5Individuals, E10= 10 Individuals and E15= 15 Individuals. (untreated) C =Control.

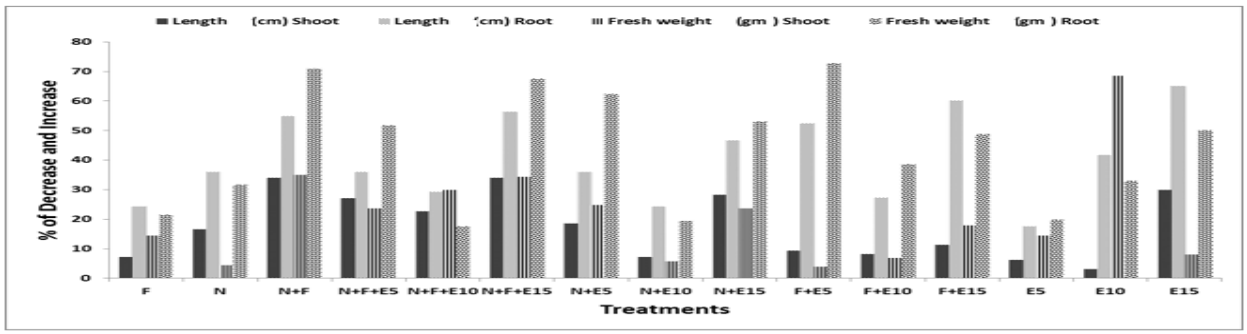

Fig. 2. Plant growth of eggplant cultivar Balady as influenced by the biointeractions of the root-knot nematode,*Meloidogyne javanica*, the earthworm, *Allolobophora longa* and the fungus, *Fusarium oxysporum* under greenhouse conditions.

Discussion

Influence earthworms on soil fungi in many ways, from changing physical and chemical state of their habitats to direct ingestion of fungal communities and dispersal of fungi propagules highly suppressive effects of the earthworms gut fluid on viability of fungal spores inoculum potential of pathogens. The observed inhibition of *Fusarium oxysporum* growth after exposure to extract coelomic fluid is interesting from an ecological point of view and can affect nematode communities in different ways, can be directly consumed and digested with plant remains and soil larvae, adults and probably eggs can be eliminated in this way. That earthworms have beneficial physical, biological and chemical effects on soils and can increase plant growth and crop yields and managed ecosystems.These beneficial effects have been attributed to improvements in soil properties and structure, to greater availability of mineral nutrients to plants, and to biologically active metabolites acting as plant growth regulators. Earthworms affects soil fertility by increasing availability of nutrients, improving soil structure and water holding capacity. It has been suggested that earthworms can increase the velocity of decomposition of organic residues and also produce several bioactive humic substances are endowed with hormone like activity that improves plant nutrition and growth plant. (Baker 2007;Blouin *et al*. 2013; Bohlen *et al*. 2004; Brown *et al*. 2004; Brown *et al*.1999; Craven *et al*. 2016; Dechaine *et al*. 2005; Eisenhauer *et al*. 2012; Groenigen,J. van *et al*. 2014; Groenigen, K. J. van. 2014;Kardol *et al*. 2015; Scheu 2003).

In the general, it is even accelerated. More diverse fungal and nematode Communities inhabited earthworm-processed substrates than were found in fresh substrates. It is generally believed that fungal hyphae and hatching eggs nematode destroyed and may be a preferred food source for earthworms.Worms probably accelerate the composting process by dispersal in the soil , and indirectly by their effects on the substrate (burrowing and casting).

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أثرالتفاعل الحيوى لكل من دودة الأرض *Allolobophora longa* وفطر *Fusarium oxysporum* ونيماتودا تعقد الجذور *Meloidogyne javanica* علي نباتات الباذنجان (الصنف بلدي)

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تمت دراسة التأثير الحيوى المنفرد والمشترك لكل من نيماتودا تعقد الجذور *Meloidogyne javanica* والفطر *Fusarium oxysporum* وديدان الأرض *Allolobophora longa* على نباتات الباذنجان *solanum melongena* (الصنف البلدي) ، ومعدلات تكاثر نيماتودا تعقد الجذور والضرر الناتج عن إصابتها بفطر الفيوزاريوم وكذلك قياسات النمو الجذري والخضري لنباتات الباذنجان . وأظهرت النتائج أن أعلي معدل تكاثر للنيماتودا على جذور نباتات الباذنجان عند إضافة النيماتودا منفردة (15.97) ، يليها عند إضافة النيماتودا مع فطر الفيوزاريوم (5.77)وكان أقل معدل تكاثر للنيماتودا عن إضافة النيماتودا مع فطر الفيوزاريوم وديدان الأرض بعدد ٥ ديدان في المعاملة هو(1,64) . وبدراسة تأثير ديدان الأرض مع الفطر والنيماتودا على نباتات الباذنجان ، أظهرت النتائج أن أعلى نسبة موت للنباتات عند إضافة النيماتودا مع الفطر (٥٨%) ، بينما كانت أقل نسبة موت أو ضررعند المعاملة بالفطر مقترنا مع ديدان الأرض بعدد ١٠ ديدان في المعاملة (٨.٣٣%). وهذا يوضح دور ديدان الأرض في خفض نسبة الموت مقارنة بالنيماتودا مع الفطر. بينما أظهرت دراسة القياسات الخضرية على نباتات الباذنجان أن أعلى معدل للنقص في طول ووزن المجموع الجذري والخضري عند المعاملة بالنيماتودا مع الفطر (٣٤.٠٢ سم – ٥٤.٨٥ جم) و (٣٤.٨٩ سم – ٧٠.٩٩ جم). بينما حدثت زيادة في وزن المجموع الخضري للنباتات عند المعاملة بالنيماتودا مع ديدان الأرض بعدد ١٠ ديدان في المعاملة (٥.٧٣ جم) ، ثم معاملة الفطر مع ديدان الأرض بعدد ٥ ديدان في المعاملة كانت (٣.٩٤ جم). بينما زاد المجموع الجذري والخضري بنسبة (٣.٠٩ سم – 46 .68 جم) عند المعاملة بديدان الأرض فقط بعدد ١٠ ديدان في المعاملة ، مما يوضح دور ديدان الأرض في زيادة كلا من المجموع الجذري والخضري للنباتات . ويتضح من الدراسة دور ديدان الأرض في خفض نسبة الموت بالنسبة لنباتات الباذنجان وكذلك زيادة النمولكل من المجموع الجذري والخضري للنباتات وقد يرجع هذا لأكتساب النباتات صفة المقاومة للنيماتودا والفطر وتحسين التربة ونمو النباتات.