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Occurrence, Population Density and Biological Control of Root-Knot Nematode, *Meloidogyne javanica* Infecting Pomegranate Orchards in Assiut Governorate, Egypt

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Abstract:

The survey conducted to assess the incidence of root-knot nematode on pomegranate in five localities (El-Badary, Manfalout, Sedfa, Sahel-Selim and El-Fath) in Assiut governorate. Results showed that 490 out of 500 samples were infested with root-knot nematode showing 98% infestation. Maximum infestation was found in El-Badary, Manfalout and El-Fath (100%), while the minimum was observed in Sahel-Selim (94%) followed by Sedfa (96%). In growing season 2013, the highest occurrence of root-knot nematode second stage was in pomegranate orchards of Sahel-Selim locality (394 J2/100 g soil), but the lowest number was found in Sedfa county (88.8 J2/100 g soil). In 2014, the highest number of juveniles per 100 g soil was observed in El-Fath county (275.4), whereas Manfalout county was the lowest infection (134.2). Bioagents (fungi, bacteria, yeast and actinomycetes) were isolated from pomegranate rhizosphere and evaluated against root-knot nematode J2 mortality percent *in vitro*. Among 29 fungal culture filtrates, the highest effect was found in culture filtrate of 3 isolates (2, 3 and 10) with average mortality percent 8.33, 10.01 and 9.22, respectively with nonsignificant. The fungal isolate No. 3 was selected and identified as *Fusarium verticilloids*. From 17 isolates of bacteria, yeast and actinomycetes, the highest mortality was observed in case of isolates No. 8, 9, 10, 11, 12, 15 and 16 with average 24.22, 23.69, 25.59, 25.82, 26.52, 22.13 and 25.24 mortality percent respectively, with nonsignificantly difference. According to morphological and physiological characteristics, isolates No. 10, 11, 12 and 16 were identified as *Xenorhabdus beddingii, Streptomyces halstedii, Pantoea agglomerans* and *Pichia guilliermondii*.

Keywords: *Pomegranate, Root-knot nematode, population density, occurrence, bioagents.*

Introduction:

The pomegranate (*Punica granatum* L.) is an ancient fruit belongs to the family Punicaceae which includes one genus and two species. It is a widely grown horticulture crop in many tropical and subtropical countries. In Egypt, pomegranate considered one of the most important fruit trees cultivated in warm regions such as Assiut province where the climate is characterized by long hot summer and low air humidity.

Most of the pomegranate cultivated areas around the world were found infested with plant-parasitic nematodes such as Jordan (Hashim, 1983), Libya (Siddiqui and Khan,

1986), and Pakistan (Khan *et al.,* 2005 and Khan and Shaukat, 2010).

Several of the trees showed symptoms of severe decline. These included stunting, poor vegetative growth, desiccation and defoliation of branches and yellowing of leaves (often with brown necrotic tips) as suggested by Hashim (1983).

Root-knot nematode, (RKN) which comes from *Meloidogyne* species is an obligate endoparasitic nematode, which can be found in varieties of plants, considered as a host to that particular nematode. Their potential host range encompasses more than 3000 plant species and they cause great agriculture loss. (Ralmi *et al.*, 2016). It can be managed effectively by chemical treatments but many of the nematicides are expensive, pernicious to the environment and human health, so using antagonistic plants can be very attractive alternative (Mukhtar *et al.,* 2013). Due to environmental concerns and increased regulations on use of chemical nematicides, more effective management strategies for root-knot nematodes are currently being investigated (Noling and Backer*,* 1994). Among the biological control agents that have been assessed are antagonistic bacteria, nematophgous fungi and yeasts (Kiewnick and Sikora, 2005 and Karajeh, 2013).

Fungal natural products are very promising potential sources of new chemicals to manage plant-parasitic nematodes (Anke and Sterner, 1997). Adverse effect of culture filtrates of several fungi on hatching and mortality of root-knot nematodes has been reported by Mankau, 1969; Shukla and Swarup, 1971; Khan *et al.,* 1984; Nitao *et al.,* 1999, 2001; Meyer *et al.,* 2004 and Sun *et al.,* 2006).

With this background, therefore, we intend to find alternative methods to manage root-knot nematode. So this study aimed to occurrence and population density of root-knot nematode infected pomegranate orchards in five localities in Assiut governorate, isolation and manages root-knot nematode using bioagents under laboratory conditions.

Material and Methods:

1- Occurrence and population density of root-knot nematodes:

An extensive survey of root-knot nematodes associated with pomegranate orchards in Assiut governorate was undertaken during 2013 and 2014 growing seasons. A total of 500 soil and root samples were collected from five different localities (El-Badary; Manfalout; Sedfa; Sahel-Selim and El-Fath) in Assiut governorate (100 samples from each locality) cultivated with pomegranate (Manfalouty cv.).

1-1- Sampling and Nematode extraction:

Soil and root samples were collected by digging the soil surrounding the trees and mixed carefully. All samples were kept in polyethylene bags to prevent water drying and sent directly to the laboratory for nematode extraction and identification.

Each soil sample was carefully mixed and 100 g from each soil sample were successively wet-sieved through 100 and 400 mesh sieves (Goodey, 1957). The obtained suspension containing the nematodes was transferred to a Baermann pan fitted with a soft tissue paper to separate the active nematodes from the

debris and fine soil particles. After 48 hours, nematode-water suspension was collected and concentrated to 10 ml in a glass vial by using a 400 mesh sieve.

Root-knot nematodes larvae, in aliquats of 1 ml of the extracted nematode suspension, were counted by Hawksely counting slide under the research microscope.

2- Nematode stock culture:

Egg masses of *Meloidogyne javanica* (Treub) Chitwood**,** infected pomegranate roots collected from localities of El-Badary, Sahel-Selim, Sedfa, El-Fath and Manfalout counties were used to inoculate 2 weeks old healthy seedlings of tomato cv. Super Marmande. Six weeks after inoculation, plants were uprooted and examined for nematode infection and reproduction. The infected roots were used to extract nematode eggs as described by Hussey and Barker (1973).

3- Management of *M. javanica* by bioagents under laboratory conditions:

Isolation of certain bioagents from pomegranate rhizosphere:

Twenty nine isolates of fungi, twelve bacterial isolates, four yeast isolates and one actinomycete isolate were isolated from pomegranate rhizosphere of five counties (El-Badary; Manfalout; Sedfa; Sahel-Selim and El-Fath) in Assiut governorate.

Preparation of fungal culture filtrates:

One-week old fungi cultures on PDA plates (100 mm) were homogenized into potato dextrose broth (PDB) medium 1 cm PDA / 250 ml flask containing 100 ml PDB and incubated at 25˚C on a shaker (240 rpm) for 7 days. After incubation, the culture broth was centrifuged at 10,000g for 10 minutes, and the supernatant passed through a 0.2 μm filter. All culture filtrates were stored at 4˚C until used (Nitao *et al.,* 1999 and Meyer *et al.,* 2000).

3-1- Assays of fungi culture filtrates against *M. javanica in vitro:*

Second stage juveniles of *M. javanica* were surface sterilized with 0.5% NaOCl for 15s, washed with sterile distilled water three times and transferred to either culture filtrates of isolated fungi or sterile water, served as control (1 ml of J_2 / 10 ml of culture filtrate). There were 29 isolates with 3 replicats and each replicat containing about 100 J_2 according to Naserinasab *et al.,* 2011 with modification. Data were recorded on % J_2 mortality after 12, 24, 36 and 48 h of incubation at 25±2°C.

3-2- Preparation of bacterial, actinomycete and yeast isolates:

Cultures (48 hrs-old) grown on NS medium (5.0g peptone, 3.0g beef extract, 5.0g sucrose, 1000 ml distilled water and adjusted to pH7.0) (Dowson, 1957) were centrifuged at 10,000 rpm for 10 mins to separate the bioagent cells. After centrifugation, supernatants were discarded and pellets were washed by centrifugation three times with sterilized distilled water (SDW) and finally suspended in SDW (Abo-Elyousr *et al.,* 2010). The optical density (OD) of the suspension was adjusted to 0.2 (A360 nm) with the help of a UV-visible spectrophotometer (spectronic 20D) equivalent to 10^5 CFU/ml. This

concentration was used for all experiments.

3-3- Assays of bacteria, actinomycete and yeasts against *M. javanica In vitro*:

Effects of bacterial, yeast and actinomycete suspentions were evaluated against *M. javanica* J2 under laboratory conditions. For this experiment, 100 freshly hatched *M. javanica* J2 (1ml from nematode suspention) were transferred to 10 cm diam Petri dishes containing 10 ml of each (bacterial, actinomycete or yeast suspentions) (10^5 CFU/ ml) separatly. Petri dishes maintained at 25˚C in an incubator. Each treatment was replicated 3 times. Mortility percent of J2 were determined under resarch microscope at 60x magnification after 12, 24, 36 and 48 hrs of incubation. (Abo-Elyousr *et al.,* 2010).

4- Identification of bioagents using morphological and physiological characteristics:

Identification of fungal isolate was carried out by using the morphological characteristics of mycelia and spores as described by Booth (1971) and Leslie and Summerell (2006) and confirmed by Assiut University Mycological Center (AUMC) Assiut, Egypt.

Bacteria, actinomycete and yeast were identified according to their morphological cultural and physiological charactristics as recommended by Kurtzman and Fell, (1998) for identify yeasts, Bergey´s Manual of systematic Bacteriology (krieg and Holt, 1984) and Bergey´s Manual of Determinative Bacteriology 9th edition (Holt *et al.,* 1994) for identify bacteria and actinomycetes.

Results:

1- Occurrence and population density of root-knot nematodes:

A total of 500 soil and root samples were collected from pomegranate orchards in five localities in Assiut governorate (Sahel-Selim, El-Badary, Manfalout, Sedfa and El-Fath). These orchards were cultivated with Manfalouty variety. Soil and root samples were collected and processed for the nematode extraction and identication.

The results of analysis of soil and root samples are given in Fig. 1 showed that, 490 out of 500 samples were infested with root-knot (R K) nematodes showing 98% infestation. Maximum samples (100%) were found infested with the nematode in El-Badary, Manfalout and El-Fath, while minimum infestation was observed in Sahel-Selim followed by Sedfa with average percent 94 and 96, respectively.

The occurrence and population density of second stage juveniles in 100g soil was obtained and listed in Fig. 2. Data showed that, the highest occurrence of root-knot nematodes was in pomegranate orchards of Sahel-Selim locality in growing season 2013 and El-Fath locality in growing season 2014, where the average numbers of extracted juveniles from soil were 394 and 275.4 J2/100g soil, respectively. On the other hand, the lowest number of second stage juveniles counted in samples of Sedfa at 2013 and Manfalout localities 2014 with an average of 88.8 and 134.2 J2/100g soil, respectively. The average number of the second stage juveniles recovered from soil samples of El-Badary, El-Fath and Manfalout

localities in season 2013 were 229.4, 147.6 and 131 respectively, whereas in season 2014 of El-Badary, Sahel-Selim and Sedfa localities were 234.4, 134.6 and 141 J2/100g soil, respectively.

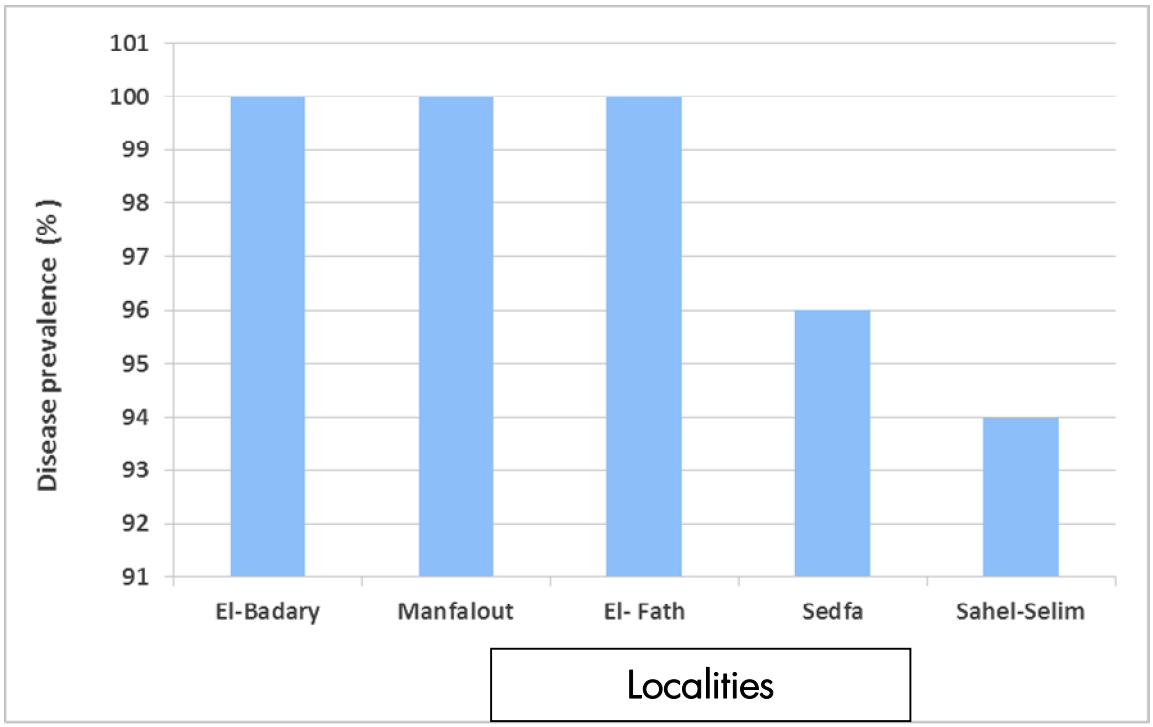

Fig (1): Prevalence of root-knot nematodes associated with Pomegranate at five localities of Assiut governorate.

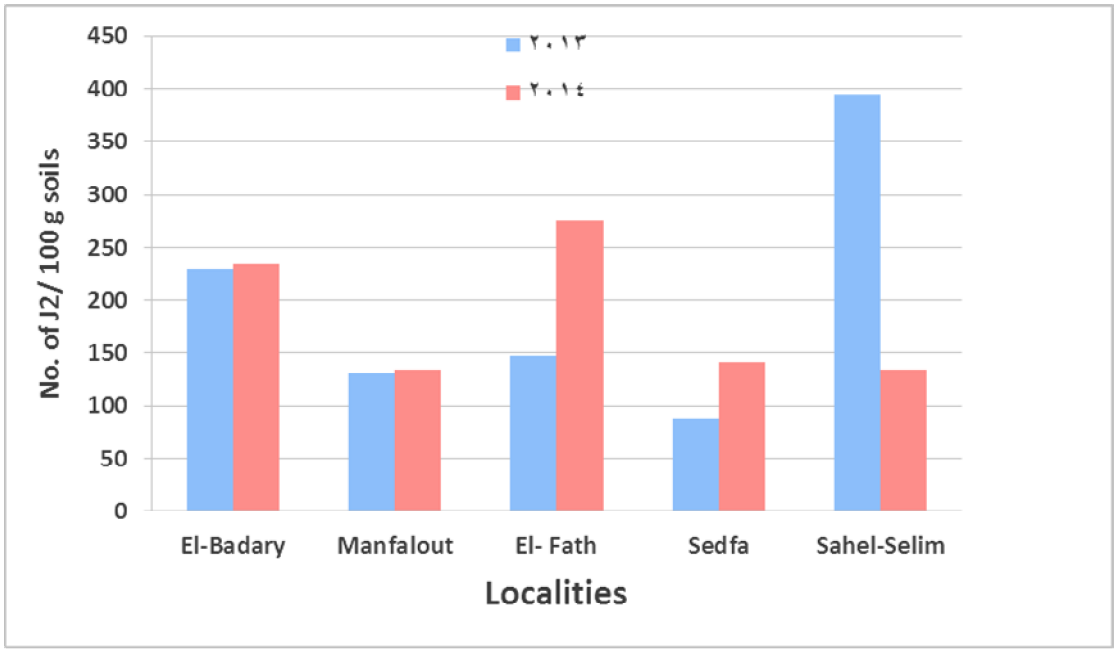

Fig (2): Population density of root-knot nematodes associated with Pomegranate orchards in Assiut governorate during 2013 and 2014 seasons.

2- Effeciency of bioagents against *M. javanica* under laboratory conditions:

In this experiment, fungi were used as fungal culture filtrate, while bacteria, actinomycete and yeasts were used as cell suspention.

2.1. Fungal culture filtrates:

Twenty nine fungal isolates were isolated from pomegranate rhizosphere. Fungi were grown on PDB medium for 7 days and their culture filtrates were investigated against *M. javanica in vitro*. Data in Table (1) showed that, there were significantly differences between the isolates. Isolates No.2, 3 and 10 were the highest effect on nematode with average mortality 8.33, 10.01 and 9.22% followed by isolate No. 6 with average 6.57%. The lowest mortality percent was observed in case of the rest of fungal culture filtrates compared with control.

The nematode mortality percent affected with exposure period. The highest effect of culture filtrate was found after 48 and 36 hrs exposure with average mortality 2.28 and 2.12, respectively, with nonsignificantly different while, the least effect was observed after 12 and 24 hrs exposure with average mortality 0.093 and 0.75, respectively with nonsignificantly different.

Table 1. Efficiency of certain fungal culture filtrates against *M. javanica* larvae *in vitro*.

Isolate No.	Locality	% of J2 mortality				Mean
		Exposure time (hours)				
		12	24	36	48	
1	Sedfa	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
2	Sedfa	0.0^{G}	0.0^{G}	16.67^{A}	16.67^{A}	**8.33^{AB}**
3	El-Fath	0.0^{G}	7.54^{CD}	16.24^{A}	16.24^{A}	**10.01^{A}**
4	El-Fath	2.78^{F}	2.78^{F}	2.78^{F}	2.78^{F}	**2.78^{C}**
5	El-Fath	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
6	Sedfa	0.0^{G}	6.06^{DE}	10.10^{C}	10.10^{C}	**6.57^{B}**
7	Sedfa	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
8	Sedfa	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
9	El-Fath	0.0^{G}	0.0^{G}	4.76^{EF}	4.76^{EF}	**2.38^{C}**
10	Sedfa	0.0^{G}	6.11^{DE}	12.91^{B}	17.85^{A}	**9.22^{AB}**
11	El-Fath	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
12	El-Fath	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
13	Sedfa	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
14	El-Fath	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
15	Sedfa	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
16	El-Fath	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
17	El-Badary	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
18	Manfalout	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
19	Manfalout	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
20	Sahel-Selim	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
21	Manfalout	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
22	Sahel-Selim	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
23	Manfalout	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
24	El-Badary	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
25	Sahel-Selim	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
26	Sahel-Selim	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
27	El-Badary	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
28	Manfalout	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
29	El-Badary	0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
Control		0.0^{G}	0.0^{G}	0.0^{G}	0.0^{G}	**0.0^{C}**
Mean		**0.093^{B}**	**0.75^{B}**	**2.12^{A}**	**2.28^{A}**	

LSD value at 5%:
Isolates (A) =3.253 Time (B) =0.8012 AB= 2.741

Bacteria, actinomycete and yeast:

Effects of certain bacteria, actinomycete and yeast on root-knot nematode, *M. javanica* larvae activity were investigated under laboratory conditions. Some antagonistic isolates were found with high effect on nematode, while the others were the least effect compared to the control.

Data in Table 2 showed that, there were a significantly differences between the tested antagonistic isolates on J2 % mortality, the highest percent of mortality was observed in case of isolates No. 8, 9, 10, 11, 12, 15 and 16 with average mortality (24.22, 23.69, 25.59, 25.82, 26.52, 22.13 and 25.24), respectively followed by isolates No. 1 (15.95), 3 (14.94), 4 (12.19), 5 (8.74), 6 (11.34), 7 (11.35), 13 (16.61), 14 (11.89) and 17 (14.62).

Data also concluded that, the mortality of nematode was attributed to the exposure periods. There were significant differentiations between the exposure periods, where the nematode exposure to 48 hrs obtained the highest mortality, followed by 36 hrs with average percent 24.65 and 19.55, respectively, while, 12 hrs was the least effect and then 24 hrs with average percent 9.643 and 14.28, respectively, compared to the control.

Table 2. Efficiency of certain bacterial, actinomycete and yeast isolates against *M. javanica* larvae *in vitro*.

Isolate No.	County	Category	% of J2 mortality				Mean
			Exposure period (hours)				
			12	24	36	48	
1	El-Badary	Bacteria	12.50	14.24	17.22	19.85	**15.95^{CDE}**
2	El-Badary	Yeast	6.857	8.207	9.40	10.85	**8.828^{EF}**
3	El-Badary	Bacteria	9.077	12.89	14.48	23.31	**14.94^{DE}**
4	El-Badary	Yeast	7.737	12.29	11.28	17.48	**12.19^{EF}**
5	El-Badary	Bacteria	4.487	7.503	10.22	12.73	**8.737^{EF}**
6	El-Badary	Yeast	9.49	10.12	10.81	14.94	**11.34^{EF}**
7	El-Fath	Bacteria	8.047	11.33	12.36	13.67	**11.35^{EF}**
8	El-Fath	Bacteria	14.88	21.18	28.90	31.90	**24.22^{AB}**
9	Manfalout	Bacteria	5.293	15.47	30	43.98	**23.69^{ABC}**
10	Manfalout	Bacteria	21.59	23.64	27.44	29.70	**25.59^{A}**
11	Manfalout	Actinomycetes	19.22	23.47	27.72	32.87	**25.82^{A}**
12	Sahel- Selim	Bacteria	11.63	18.83	27.93	47.68	**26.52^{A}**
13	Sahel-Selim	Bacteria	8.513	10.55	23.42	23.96	**16.61^{BCDE}**
14	Sedfa	Bacteria	3.03	12.86	14.25	17.41	**11.89^{EF}**
15	Sedfa	Bacteria	9.47	21.93	25.70	31.42	**22.13^{ABCD}**
16	Sedfa	Yeast	15.25	18.71	28.96	38.03	**25.24^{A}**
17	Sedfa	Bacteria	2.777	8.64	23.73	23.33	**14.62^{DEF}**
Control			3.723	5.163	8.077	10.67	**6.908^{F}**
Mean			**9.643^{D}**	**14.28^{C}**	**19.55^{B}**	**24.65^{A}**	

LSD value at 0.05:
Isolates (A) = 8.027
Time (B) =2.48
Interaction (AB) = 6.638

2.2. Identification of bioagents using morphological and physiological characteristics:

Identification of highest antagonistic isolates of bacteria, actinomycetes and yeast isolates were carried out using the morphological and physiological characteristics.

Identification fungus isolate (No. 3) was revealed to *Fusarium verticilliodes* based on the morphological feature of mycelia and spores as described by Booth (1971) and confirmed by the Assiut University, Mycological Center (AUMC).

On the basis of the obtained data and those reported by Krieg and Holt (1984) and Holt *et al.* (1994), it could stated that, all tested isolates (bacteria and actinomycetes) were identified as following, Bacterial isolate No. 10 was identified as *Xenorhabdus beddingii* and isolate No. 12 was identified as *Pantoea agglomerans* While, actinomycete isolate No. 11 was identified as *Streptomyces halstedii*,.

According to Kurtzman and Fell (1998) to describe yeast isolate No. 16 was identified as *Pichia guilliermondii*.

Discussion:

The present survey confirms the occurrence of root-knot nematodes (*Meloidogyne* spp.) associated with pomegranate orchards in Assiut governorate. The results of analysis of soil and root samples collected from pomegranate orchards in five different localities (Sahel-Selim, El-Badary, El-Fath, Sedfa and Manfalout) showed that, 490 out of 500 samples were infested with root-knot nematodes showing 98% infestation. Maximum samples (100%) infested with root-knot nematodes were found in El-badary, El-Fath and Manfalout, while minimum infestation (94%) was observed in Sahel-Selim. Root-knot nematodes were reported by several investigators as an important nematode pest attacking pomegranate orchards in different countries, (Hashim, 1983) in Jordan, Siddiqui and Khan (1986) in Libya, Khan *et al.,* (2005) and Khan and Shaukat (2010) in Pakistan.

The effect of fungal culture filtrates were examined against *M. javanica* under laboratory conditions, *Fusarium verticilliodes* culture filtrate was the highest in *M. javanica* mortality percent.

Culture filtrates of many fungi possess activity against nematodes and the nematicidal action of these culture filtrates may involve the production of toxic metabolites by the fungi (Caroppo *et al.,* 1990; Singh *et al.,* 1991; Hallmann and Sikora, 1996; Nitao *et al.,* 1999; Kusano *et al.,* 2000, 2003; Nakahara *et al.,* 2004; Kanai *et al.,* 2004; Heydari *et al.,* 2006; Hayashi *et al.,* 2007; Liu *et al.,* 2008; Du *et al.,* 2009).

Fungal natural products are very promising potential sources of new chemicals to manage plant-parasitic nematodes (Anke and Sterner, 1997).

Species of *Aspergillius, Penicillium, Trichoderma, Fusarium, Paecilomyces* and *Alternaria* are known to produce toxins and antibiotics like aflatoxins, pencillin, virdin, fusaric acid, lilacin and phyto-alternarin (Nafe-Roth, 1972; Arai *et al.,* 1973; Wheeler, 1975; Ghewande *et al.,* 1984). Adverse effect of culture filtrates of several fungi on hatching

and mortality of root-knot nematodes has been reported by Mankau, 1969; Shukla and Swarup, 1971; Khan *et al.,* 1984; Nitao *et al.,* 1999, 2001; Meyer *et al.,* 2004; Sun *et al.,* 2006).

Few investegations of the effect of Fusarium toxins on plant parasitic nematodes have been reported, Mani and Sethi (1984) working with culture filtrates of *F. solani reported* reductions in hatch and mobility of *M. incognita.* Fattah and Webster (1983) observed inhibited development of *M. javanica* in roots colonized by *F. oxysporum* f. sp. *Lycopersici.*

In vitro assay, the effect of bacterial isolates, Actinomycetes and yeasts that isolated from pomegranate rhizosphere was examined against J2 % of *M. javanica*. The results showed that, the highest mortality percent was observed in treated with *Pantoea agglomerans* (26.52), *Streptomyces halstedii* (25.82), *Xenorhabdus beddingii* (25.59) and *Pichia guilliermondii* (25.24) with nonsignificantly differences. Several reports noted that suppression of *Meloidogyne* sp. by different rhizobacteria like *Pseudomonas fluorescens* (Siddiqui and Mahmood, 1999; Siddiqui *et al.,* 2001; Hashem and Abo-Elyousr, 2011), *Bacillus* sp. (Siddiqui and Mahmood, 1999; Giannakou *et al.,* 2007), *Rhizobium* sp. (Akhtar and Siddiqui, 2008), but no studies have not been performed on the biological control of this pathogen with *P. agglomerans* and *X. beddingii*. *P. agglomerans* (Cook and Baker, 1983). While, Vasebi *et al.* (2015) reported that there a biocontrol agent used against other plant pathogens.

A large number of soil microorganisms are capable of producing siderophores (Misaghi *et al.,* 1988). The high ability of *P. agglomerans* in siderophore production in CAS-agar medium has been confirming that, this group of bacteria has evolved high-affinity iron uptake systems to shuttle iron into the cell.

P. guilliermondii is seemed to promising biocontrol agent. Although we couldn't define the exact mechanism of disease protection by this strain, it could be hypothesized that the reduction of the disease might be attributed to direct effect of metabolites that induce mortality in J2, or that may have also enhanced host defense mechanism in roots that resist invation and consequent infection by pathogen (Hashem *et al.,* 2008; Hashem and Abo-Elyousr, 2011).

Saccharomyces serevisiae is promising plant growth-promoting for different crops as descriped by Karajeh (2013). *S. cerevisiae* was investigated as a biocontrol agent against root-knot nematode by Noweer and Hasabo (2005); Karajeh (2013) and Mokbel and Alharbi (2014). They showed that, the yeast was reduced root gall formation, egg masses and nematode reproduction ability and inhanced plant growth and fruit yield. High content of total phenolic and hydrogen peroxide in roots of *S. cerevisiae*- treated plants gives a clue on the ability of the yeast to induced plant resistance (Karajeh, 2013).

The management of root-knot nematode by *Streptomyces* sp. was mentioned by a lot of investigators. The actinomycetes enhanced the plant growth, imporoved fruit yield and suppressed root-gall development (Jonathan, 2000; Rajeswari and

Ramakrishnan, 2015). Culture filtrates of actinomycetes exhibited variable response against egg hatchability and mortality of root-knot nematode (Helal *et al.,* 2016).

The culture filtrate of the optimized medium of *Streptomyces fradiae* resulted in higher degree of inhibition in egg hatching and J2 mortality of *M. incognita*. The effectiveness of optimized medium against *M. incognita* is related to higher production of secondary metabolites subsequent to maximization of colonization (Rajeswari and Ramakrishnan, 2015).

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الانتشار، الكثافه العدديه، والمكافحة البيولوجية لنيماتودا تعقد الجذور (*Meloidogyne javanica*) التى تصيب بساتين الرمان في محافظة أسيوط، مصر

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الملخص:

تم عمل مسح لتقدير مدى انتشار الاصابة بنيماتودا تعقد الجذور على الرمان في خمس مناطق (البدارى، منفلوط، صدفا، ساحل سليم، والفتح) في محافظة اسيوط. اوضحت النتائج ان ٤٩٠ من بين ٥٠٠ عينه كانت مصابة بتعقد الجذور النيماتودي بنسبة ٩٨% اصابه. وجدت اعلى اصابة في البدارى، منفلوط والفتح (١٠٠%)، بينما الاقل شوهدت فى ساحل سليم (٩٤%) يليها صدفا (٩٦%). في موسم ٢٠١٣، كانت اعلى اصابة بالطور اليرقي الثاني لنيماتودا تعقد الجذور في بساتين رمان ساحل سليم (٣٩٤ طور يرقي /١٠٠ جرام تربة)، ولكن وجدت اقل اعداد لها في صدفا (٨٨,٨ طور يرقي /١٠٠ جرام تربة). في موسم ٢٠١٤، شوهدت اعلى اعداد للطور اليرقي الثاني في مركز الفتح (٢٧٥,٤)، بينما كا ن مركز منفلوط الاقل في الاصابة (١٣٤,٢). تم عزل فطريات، بكتريا، خمائر، اكتينومايسيتات من المجال الجذري للرمان واختبرت ضد الطور اليرقي الثاني لنيماتودا تعقد الجذور فى المعمل (النسبه المئويه للموت). من بين ٢٩ راشح فطري، وجد التاثير الاعلى للراشح الفطري لثلاث عزلات (٢، ٣، ١٠) بمتوسط نسبة موت ٨,٣٣، ١٠,٠١، ٩,٢٢ على التوالي ، بوجود فرق غير معنوى. تم اختيار وتعريف العزلة الفطرية رقم ٣ *Fusarium verticilloids* . ومن بين ١٧ عزلة من البكتريا والخمائر والاكتينومايسيتات، تم ملاحظه اعلى نسبه موت فى حاله العزلات رقم ٨، ٩، ١٠، ١١، ١٢، ١٥، و١٦ بمتوسط نسبة موت ٢٤,٢٢، ٢٣,٦٩، ٢٥,٥٩، ٢٥,٨٢، ٢٦,٥٢، ٢٢,١٣، و٢٥,٢٤ على التوالي، بدون فروق معنوية. وطبقا للصفات الموروفولوجية والفسيولوجية فان العزلات رقم ١٠، ١١، ١٢، ١٦ تم تعريفها على انها *Xenorhabdus beddingi, Streptomyces halstedii, Pantoea agglomerans* و *Pichia guilliermondii*