



EVALUATION THE ACTIVITY OF *HIRSUTELLA* SP. FUNGUS AND THE NEMATICIDE VEROX TO CONTROL ROOT KNOTS NEMATODE *MELOIDOGYNE* SPP. ON FIG SEEDLINGS

Ali Kadhim Jabir Al-Awabid¹, Saad Tareq Abdulmalek Yass²

Researcher, Plant Protection Department, College of Agricultural Engineering Sciences, University of Baghdad, Baghdad, Iraq.
ali.kadim1204a@coagri.uobaghdad.edu.iq

Assistant Professor, College of Agricultural Engineering, University of Baghdad, Baghdad, Iraq. Saad.t@coagri.uobaghdad.edu.iq

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ABSTRACT

This study was conducted in the plastic house of the College of Agricultural Engineering Sciences at the University of Baghdad to evaluate the activity of *Hirsutella* sp. fungus and Nematode Verox against *Meloidogyne* spp. causative agent of root knots in Fig seedlings. Treatment of *Meloidogyne* spp. eggs with *Hirsutella* sp. at 20ml/L and with Verox at 2g/L caused significant inhibition in eggs hatching after 3,5 and 7 d .The number of non- hatching eggs were 96.70, 54.00and 29.30 eggs respectively in petri plates treated with *Hirsutella* sp., 106.70, 60.00and 46.00 eggs respectively in petri plates treated with Verox compared with 116.70, 111.00and96.00 eggs respectively in control. The treatment caused significant inhibition of juvenile-2 at the same periods .The number of juvenile-2 were 123.30, 114.00and 109.30 petri plates treated with *Hirsutella* sp at 20ml/L110.00, 63.00 and 26.70 in seedlings treated with Verox at 2g/L compared with 136.70, 132.00and120.00 in control after 3,5 and 7 d respectively. High significant reduction in root knots index and disease severity were observed in seedlings treated with *Hirsutella* sp. and Verox, 2.00 and 1.33% compared with 3.33 and 3.32% in control respectively .Significant increase in Phenylalanine Ammonia- Lyase and Chitinase activity induced in Fig seedling treated with *Hirsutella* sp. and Verox. PAL activity attained to 17.43and19.23 and 18.85 mg cinnamic acid/h/g fresh weight in seedlings treated with *Hirsutella* sp.16.76, 18.56and 18.05 mg cinnamic acid/ h /g fresh weight in seedlings treated with Verox compared with 15.30, 17.13and 15.74 mg cinnamic acid /h/g fresh weight in control after 3,5and 7 d respectively. The treatment induced significant increase in nitrogen and potassium in the leaves, Nitrogen and potassium percentage were attained to 7.619% and 2.120% in seedling treated with *Hirsutella* sp. 3.558%and 2.080% in seedlings treated with Verox compared with 2.858% and 1.820% in control respectively. Significant increase in leaf area and in seedlings height were observed in treated seedlings, 157.467 cm²and2.667 cm in seedling treated with *Hirsutella* sp, 134.687 cm²and 3.837 cm in seedlings treated with Verox compared with 105.883 cm²and 0.662 cm in control respectively. The treatment of Fig seedlings with *Hirsutella* sp and Verox induced significant increase in leaf chlorophyll content, foliage fresh weight, root fresh weight, foliage dry weight that attained to 37.17, 44.67, 60.63, 16.90 and 17.2ag respectively in Seedlings treated with *Hirsutella* sp, 35.83 , 44.33 , 52.50, 14.63 and 19.20g respectively in seedling treated with Verox, compared with 30.90, 28.57, 39.00 ,11.63 and 12.07g in control respectively.

Keywords: *Hirsutella* sp., Nematicide Verox, *Meloidogyne* spp, induce resistance, Fig Tree.



تقييم فعالية الفطر *Hirsutella sp* ومبيد النيماتودا Verox لمقاومة النيماتودا تعقد الجذر *Meloidogyne spp* على شتلات التين

على كاظم جابر العوايد¹، سعد طارق عبد الملك ياس²

¹الباحث، قسم وقاية النبات، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق. ali.kadim1204a@coagri.uobaghdad.edu.iq

²استاذ مساعد دكتور، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق. Saad.t@coagri.uobaghdad.edu.iq

الخلاصة

أجريت هذه الدراسة في البيت البلاستيكي لكلية علوم الهندسة الزراعية في جامعة بغداد لتقييم نشاط *Hirsutella sp*. الفطر والديدان الخيطية ضد *Meloidogyne* العامل المسبب لعقد الجذور في شتلات التين. معاملة بيض *Meloidogyne spp* مع *Hirsutella sp* عند 20 مل/ لتر ومع المبيد Verox عند 2 غم/ لتر تسبب في تثبيط كبير في فقس البيض بعد 3، 5، 7 أيام. بلغ عدد البيض غير الفقس 96.70، 54.00، 29.30 بيضة على التوالي في أطباق بتري المعاملة بـ *Hirsutella sp*، 106.70، 60.00، 46.00 بيضة على التوالي في أطباق بتري المعاملة بـ المبيد Verox مقارنة بـ 116.70، 111.00، 96.00 بيضة على التوالي في معاملة المقارنة. تسبب المعاملة في تثبيط كبير ليافاعات الطور الثاني في نفس الفترات. كان عدد يافعات الطور الثاني 123.30، 114.00، 109.30 طبق بتري تمت معالجتها بـ *Hirsutella sp* عند 20 مل / لتر 110.00، 63.00، 26.70 في الشتلات المعاملة بالمبيد Verox عند 2 غم / لتر مقارنة بـ 136.70، 132.00، 120.00 في السيطرة بعد 3.5. 7 أيام على التوالي. لوحظ انخفاض كبير في دليل عقد الجذور وشدة الإصابة في الشتلات المعاملة بـ *Hirsutella sp* و Verox 2.00 و 1.33 مقارنة بـ 3.33 و 3.32٪ في السيطرة على التوالي. زيادة معنوية في نشاط Phenylalanine Ammonia – Lyase و Chitinase المحرض في شتلة التين المعاملة بـ *Hirsutella sp* و Verox. بلغ نشاط PAL 17.43، 19.23، 18.85 مليغرام من حمض سيناميك/ ساعة/ غم وزن طازج في الشتلات المعاملة بـ *Hirsutella sp* 16.76، 18.56، 18.05 مليغرام حمض سيناميك/ ساعة/ غم وزن طازج في الشتلات المعاملة بالمبيد Verox مقارنة بـ 15.30، 17.13، 15.74 مليغرام حمض سيناميك/ ساعة/ غم وزن طازج تحت السيطرة بعد 3، 5، 7 أيام على التوالي. أدت المعاملة إلى زيادة معنوية في النيتروجين والبوتاسيوم في الأوراق، وبلغت النسبة المئوية للنيتروجين والبوتاسيوم 7.619٪ و 2.120٪ في الشتلات المعاملة بـ *Hirsutella sp* و 3.558٪ و 2.080٪ في الشتلات المعاملة بالمبيد Verox مقارنة بـ 2.858٪ و 1.820٪ في الشتلات. السيطرة على التوالي. لوحظت زيادة معنوية في مساحة الورقة وارتفاع الشتلات المعاملة، 157.467 سم²، 2.667 سم في الشتلات المعاملة بـ *Hirsutella sp* 134.687 سم²، 3.837 سم في الشتلات المعاملة بالفيروكس مقارنة بـ 105.883 سم²، 0.662 سم على التوالي. أدت معاملة شتلات التين مع *Hirsutella sp* و Verox إلى زيادة معنوية في محتوى كلوروفيل الأوراق، الوزن الرطب للأوراق، الوزن الرطب للجذر، الوزن الجاف للأوراق بلغ 37.17 غم، 44.67 غم، 60.63 غم، 16.90 غم، 17.2 غم على التوالي في الشتلات المعاملة. مع *Hirsutella sp* 35.83 غم، 44.33 غم، 52.50 غم 14.63 غم، 19.20 غم على التوالي في الشتلات المعاملة بالفيروكس، مقارنة بـ 30.90، 28.57 غم، 39.00 غم، 11.63 غم، 12.07 غم على التوالي.

الكلمات المفتاحية: *Hirsutella sp*، مبيد النيماتودا Verox، *Meloidogyne spp*، المقاومة المستحثة، شجرة التين

INTRODUCTION

The Fig *Ficus carica* L. family Moraceae is one of the most important fruiting trees in world and Iraq. The genus *Ficus* was reported to include up to 800 species (Van, 2013).

It has been reported that Fig trees are subjected to infection with many pathogens. Of these pathogens root knots Nematode is the most important that affect quality and quantity of Fig production (Martinuz, 2012). In addition to high losses caused by root knots Nematode, it caused breakdown of plant resistance to other pathogens and formed disease complex with other pathogen making its control more difficult (Qiao et al., 2013).

Among 80 species of *Meloidogyne spp*. identified in the world, *M. huapla*, *M. arenaria*, *M. Incognita*, *M. javenica* represent 95% and distributed in all agricultural areas in the world (Abu Gharbia, 2010). The second larval stage, juvenile-2 (J2), penetrate the root toward the vessels causing hyperplasia and hypertrophy that leading to form galls (Bellafiore & Briggs, 2010).

The control of root knots Nematode was restricted for long time on highly toxic Nematicides. Due to enormous problems to ecosystem and human caused by Nematicide the



research was oriented to search for natural means, safe and effective, to central root knots Nematode (**khun-in et al., 2015**)

Many natural enemies, fungi, bacteria, Virus and nematophagous, found to be effective against Nematode. It was reported that were ported to include up to species, include up to 70 species, attack insects and nematoda (**Sung et al., 2007**). *Hirsutella* sp. produce conidia that adhere with Nematode, penetrate reproduce to Nematode Leading to kill it (**Sun et al., 2015**).

Verox Nematicide composed of Rhizobacteria and amino acids. Plant growth promoting rhizobacteria showed high activity as biocide (**Diby & weichad, 2014**). It was found that the genus *Bacillus* sp. caused decrease. in Nematode *Heterodera glycines* population in soybean in laboratory and field (**Xiang et al., 2017**). PGPR parasitize roots and rhizosphere producing secondary metabolites that affect Nematode movement, eggs hatching and J-2 penetration (**Siddiqui & Mahmood, 1999**). PGPR Showed high activity against *Meloidogyne incognita* Chitwood in laboratory and field studies on different hosts (**Xiang et al., 2017; Mhatre et al., 2019**).

The study was conducted to evaluate the efficiency of the fungus *Hirsutella* sp. and Verox Nematicide against *Meloidogyne* spp. causative agent of root knots on Fig seedlings

MATERIALS AND METHODS

Meloidogyne inoculum

The Nematode inoculum was prepared as described by (**Hussey & Barker, 1973**). Samples from Fig seedlings root infected with *Meloidogyne* spp. showing root knots were collected. The infected roots were cut to small pieces, 2 cm length, and dipped in 1% sodium hypochlorite solution to 4 min in flask with agitation. The hypochlorite solution with the root pieces was transferred to 300, 150 and 25µm sieves respectively. The pieces were subjected to light flow water to eliminate NaOCl. The eggs were collected from 25µm sieve by light flow water in flask, 125 mL, observed under microscope at 40× and the eggs with juvenile- 2 were counted in 1 mL of the total volume. The eggs suspension was poured in dark petri plates, 20 cm diam, with small volume of distilled water and maintained at 25°C for 1-3 d to obtain juvenile-2.

Evaluation the effect of *Hirsutella* sp. fungus and Verox Nematicide on *Meloidogyne* spp. eggs hatching and Juvenile-2 viability.

One ml of *Meloidogyne* spp. inoculum containing 250± 5 eggs was placed in each of petri plates. *Hirsutella* sp. was added into the plates at 5, 10 and 20 ml/L. One mL of Verox Nematicide at 1, 1.5 and 2 g/L was added to other plates containing the inoculum. The plates were incubated at 25± 5°C and the number of eggs and dead juvenile-2 were counted after 3, 5 and 7 d of treatment (**AL-Ubaidy, 1985**). Distilled water was added to other plates containing the inoculum for control, in three replicates for each concentration. The plates were distributed in Complete Randomized Design.

Effect of *Hirsutella* sp. and Verox Nematicide on root knots index and disease severity on Fig seedlings infected with *Meloidogyne* spp.

Healthy Fig seedlings, black variety, at one year age with 45-50 cm Length were used in this experiment. The seedling roots were dipped in *Hirsutella* sp. at 20ml/L and others in Verox Nematicide at 2g/L for 15 min and transplanted. 20 mL of *Meloidogyne* spp. inoculum containing 2500±50 eggs and juvenile-2 were distributed in pits around the root (4 pits) after 3 d. The treatments were distributed in Complete Randomized. Design (CRD) with 3 replicates. Fig seedlings inoculated with *Meloidogyne* spp. and others non- inoculated were transplanted as control. The Fig seedlings were pulled out after 60 d of inoculation and the roots were



tested. Plants were carefully uprooted and roots were washed under tap water to remove adhering soil. To determine foliage and roots fresh and dry weight, foliage and roots were separately weight and dried at 70 °C for 48h or until weight is fixed. Root knots index was determined as described by (Smart & Dube, 1987) with index of 5 degrees, where, 0 = healthy root, 1= knots on 1-25% of root, 2 = knots on 26-50% of root, 3= knots on 51-75 of root, 4 = knots on 76-100% of root. The disease severity was estimated by (Mckinney, 1923) equation.

$$\text{Disease severity (\%)} = \frac{\sum \text{number of plants} \times \text{infection degree}}{\text{Total number of plants} \times \text{higher degree}} \times 100$$

Determination of phenyl alanine ammonia – Lyase (PAL) and Chitinase activity.

Leaves of Fig Seedlings were collected after 3, 6 and 4 d of treatment. The leaves were rinsed with tap water then with sterilized distilled water and cut to small pieces by Sterilized scissor. PAL activity was estimated as described as described by (AL-Jarah, 2011) and Chitinase activity as described by (Nawani & kapadnis, 2005) as modulated by (Lee *et al.*, 2009).

Effect of treatment with *Hirsutella* sp. and Verox on Seedling height and area.

Seedlings height were measured from soil surface to higher part of growing point before treatment and at the end of experiment. Leaf area was determined by taking 3 leaves from each experiment unit by Al-Zaidi method as described by (Sadik *et al.*, 2011).

Estimation of nitrogen and potassium in treated Fig seedlings.

Nitrogen percentage was determined in the leaves after 3, 6 and 9 d of treatment. The leaves were dried in oven at 50°C and ground. The leaves powder was added to a mixture of sulfuric and perchloric (1:2) for digestion. The nitrogen in the solution was determined by kjeldhal system (Haynes, 1980). The potassium was determined by flame photometer as described by (Haynes, 1980). The data was statistical analysis using Gen State 12 and compared by LSD 0.05.

Estimation of Chlorophyll leaf content in treated Fig seedlings.

Chlorophyll content was determined after 45 d of inoculations by Mater chlorophyll content (AL-Zahawi, 2007).

RESULTS AND DISCUSSION

Effect of *Hirsutella* sp. fungus and Verox Nematicide on eggs hatching and juvenile-2 viability of *Meloidogyne* spp.

The treatment of *Meloidogyne* spp. eggs with *Hirsutella* sp. at concentrations 5, 10 and 20 ml/L and with Verox at 1, 1.5 and 2 g/L caused significant inhibition in eggs hatching compared with control after 3, 5, 7 d (Table -1). It was found that the concentrations 20 ml/L of *Hirsutella* sp. and 2g/L of Verox were the more effective. The number of viable non-hatching eggs treated with *Hirsutella* sp. at 20 ml/L were 96.70, 54.00 and 29.30 at 38.68, 21.6 and 11.72% respectively compared with 116.70, 111.00 and 96.00 at 46.68, 44.4 and 38.4%, respectively in control. The number of viable eggs treated with Verox at 2g/L were 106.70, 60.00 and 40.00 eggs at 42.68, 24.0 and 16.00% after 3, 5 and 7 d of treatment respectively Compared. with 116.70, 111.00 and 96.00 at 46.68, 44.4 and 38.4% respectively in control.



Table(1) Effect of the fungus *Hirsutella* sp. and the Nematicide Verox on *Meloidogyne* spp. Eggs.

Treatments		viable eggs	percentage (%)	viable eggs	percentage (%)	viable eggs	percentage (%)	means
The agent	concentration	after 3 d	after 3 d	after 5 d	after 5 d	after 7 d	after 7 d	
<i>Hirsutella</i> sp.	5ml/L	100.00	40	81.00	32.4	53.30	21.32	78.10
	10 ml/L	100.00	40	63.00	25.2	37.30	14.92	66.77
	20 ml/L	96.70	38.68	54.00	21.6	29.30	11.72	60.00
Verox	1 g/L	113.30	45.32	72.00	28.8	45.30	18.12	76.87
	1.5 g/L	110.00	44	69.00	27.6	42.70	17.08	73.90
	2 g/L	106.70	42.68	60.00	24	40.00	16	68.90
control		116.70	46.68	111.00	44.4	96.00	38.4	107.90
means		106.2	42.48	72.9	29.14	49.1	19.65	
		transaction		the d		overlap		
LSD (5 %)		12.57**		8.23**		21.78**		

Results of this study indicated that the fungus *Hirsutella* sp. at 20ml/L and the Nematicide Verox at 2g/L were of high activity to inhibit eggs hatching of *Meloidogyne* spp. parasitized the eggs producing secondary metabolites toxic to eggs. It was reported that *Hirsutella* sp. caus reduction in eggs hatching of *Meloidogyne incognita* to Laboratory conditions (Hallmann *et al.*, 2019; Abokora,2021). Verox at 2g/L showed high activity against eggs of *Meloidogyne* compared with control. Verox is composed of plant growth promoting rhizobacteria (PGPR), I produce secondary metabolites (enzymes and toxins) including Chitinase that decompose chitin in eggs and toxin affecting eggs hatching (Siddiqui & Mahmood, 1999; Chauhan *et al.*, 2015).

The treatment of juvenile - 2 of *Meloidogyne* spp. with *Hirsutella* sp. fungus at 5, 10and20 ml/L and with Verox at, 1,1.5and 2 g/L induced significant inhibition of juvenile-2 compared with control after 3, 5and7 d. The results (Table-2) Indicated that *Hirsutella* sp. at 20 ml/L and Verox of 2g/L were more efficient, where the number of viable juveniles treated with *Hirsutella* sp., 123.30, 114.00 and109.30 at 49.32, 45.60and43.72% after 3, 5and 7 d respectively. The viable juvenile - 2 treated with Verox of 2g/L were, 110.00, 63.00and 26.70 at 44, 25.2and 10.68% after 3,5and7 d respectively compared with 136.70, 132.00and120.00 at 54.68, 52.8and48.0% respectively in control.

Table (2): Effect of *Hirsutella* sp. and Verox Nematicide on viability of root knots Nematode *Meloidogyne* spp. juvenile-2.

Treatments		viable juvenile-2	percentage (%)	viable juvenile-2	percentage (%)	viable juvenile-2	percentage (%)	means
The agent	concentration	after 3 d	after 3 d	after 5 d	after 5 d	after 7 d	after 7 d	
<i>Hirsutella</i> sp.	5ml/L	133.30	53.32	129.00	51.6	117.30	46.92	126.53
	10 ml/L	126.70	50.68	118.00	47.2	112.00	44.8	118.90
	20 ml/L	123.30	49.32	114.00	45.6	109.30	43.72	115.53
Verox	1 g/L	133.30	53.32	123.00	49.2	112.00	44.8	122.77
	1.5 g/L	113.30	45.32	72.00	28.8	32.00	12.8	72.43
	2 g/L	110.00	44	63.00	25.2	26.70	10.68	66.57
control		136.70	54.68	132.00	52.8	120.00	48	129.57
means		125.2	35.96	107.3	42.91	89.9	50.09	
		transaction		the d		overlap		
LSD(5%)		8.25**		5.40**		14.30**		



Results of this study indicated that the fungus *Hirsutella* sp. at 20ml/L and the Nematicide Verox at 2g/L were of high activity to juvenile 2 viability of *Meloidogyne* spp. parasitized juvenile-2 producing secondary metabolites toxic to juvenile-2. *Hirsutella rhossiliensis* reported to be internal parasite produce stick substances to attract juvenile-2, induced reduction in juvenile-2 viability up to 95% (Hallmann *et al.*, 2019; Wang *et al.*, 2007). Verox at 2g/L showed high activity against Juvenile of *Meloidogyne* compared with control. Verox is composed of plant growth promoting rhizobacteria (PGPR), I produce secondary metabolites (enzymes and toxins) including Chitinase that decompose chitin in eggs and toxin affecting juvenile-2 viability (Siddiqui & Mahmood, 1999; Chauhan *et al.*, 2015).

Effect of treatment Fig seedlings infected with *Meloidogyne* spp on disease index and severity.

Results (Table-3) showed that treatment of Fig seedling with *Hirsutella* sp. fungus and Verox and inoculating with *Meloidogyne* spp. induced high significant reduction in both disease index and disease severity. The disease index and severity were found to be 2.00 and 1.33 m seedlings treated with *Hirsutella* sp. and Verox respectively compart with 3.33 and 83.5 in contest respectively.

Table (3): Effect of *Hirsutella* sp. and Verox in discase index and severity treatment on Fig seedling inoculating with *Meloidogyne* spp.

Treatments	discase index	discase severity
<i>Hirsutella</i> sp.+Nematode	2.00	50.00
Verox +Nematode	1.33	33.33
Nematode	3.33	83.33
Healthy seedlings (Control)	0.00	0.00
LSD(5%)	0.7643**	

High reduction in root knots index and disease severity were observed upon treatment big seedlings with *Hirsutella* sp. and Verox. This may come from reduction. the number of juvenile-2 penetrated the roof Lending To reduction in knot formation on the root. It has been reported that *Hirsutella* sp. was active in reduction Juvenile-2 of *Meloidogyne incognita*, number of knots and Nematode population (Abokora, 2021), The treatment with PGPR caused reduction in root knots number and disease severity (Liu *et al.*, 2022).

Effect of treatment Fig seedlings with *Hirsutella* sp. and Verox on phenylalanine Ammonia-Lyase (PAL) and Chitinase enzymes activity.

Results (Table-4) showed that treatment Fig seedling with *Hirsutella* sp. fungus and vortex caused significant increase in PAL activity compared with control. PAL activity attained to 17.43, 19.23and 18.85 mg cinnamic acid/ h/g fresh weight in seedling treated with *Hirsutella* sp., 16.76, 18.56and18.05 mg cinnamic acid /h/g fresh weight in seedling treated with Verox compared with 15.33, 17.13and15.74 mg cinnamic acid/h/ g fresh weight in control after 3, 6and 9 d of treatment respectively. The significant differences in PAL activity were found more obvious at 3 d of treatment. No significant difference in PAL activity between treatments offer bod of d of treatments has been observed.



Table (4): Effect of treatment Fig seedlings with *Hirsutella* sp. and Verox on phenylalanine Ammonia-Lyase (PAL) and Chitinase enzymes activity.

Treatments	PAL activity mg cinnamic acid/ h/g fresh weight			means	Chitinase activity units/mL			means
	3 d	6 d	9 d		3 d	6 d	9 d	
<i>Hirsutella</i> sp.+Nematode	17.43	19.23	18.85	18.50	7.20	11.30	29.36	15.96
Verox +Nematode	16.76	18.56	18.05	17.79	7.16	11.28	29.27	15.91
Nematode	15.33	17.13	15.74	16.07	7.18	11.20	29.36	15.91
Healthy seedlings (Control)	9.46	9.59	9.14	9.40	6.30	11.30	29.27	15.62
lsd(5%)	0.15				0.098			

The effect of *Hirsutella* sp. and Verox on *Meloidogyne* spp. may be indirectly through induction systemic resistance in big seedlings against *Meloidogyne* spp. as proved by increase in PAL and Chitinase activity. It was reported that Nematophagous fungus *Arthrobotrys oligospora* induced increase, enzymes activity related with resistance including PAL associated with reduction in Nematode number in tomato compared. with plant, inoculated with *M. incognita* (Mostafanezhad *et al.*, 2014).

Effect of treatment Fig seedlings with *Hirsutella* sp. and Verox on Loves content of Nitrogen and potassium.

Results (Table-5) indicated to significant increase in nitrogen and potassium percentages in the leaves of seedlings treated with *Hirsutella* sp. and Verox compared with control. (Treated with *Meloidogyne* spp.). Nitrogen and potassium percentage were attained to 3.617% and 2.120% in seedlings treated. with *Hirsutella* sp., 3.558% and 2.080% in seedlings treated with Verox compared with 2.858% and 1.820% respectively in control.

Table (5): Effect of treatment Fig seedlings with *Hirsutella* sp. and Verox on Loves content of Nitrogen and potassium.

Treatments	(%)Nitrogen	(%)Potassium
<i>Hirsutella</i> sp.+Nematode	3.617	2.120
Verox +Nematode	3.558	2.080
Nematode	2.858	1.820
Healthy seedlings (Control)	3.150	1.960
LSD(5%)	0.567	0.329

The decrease in the root knot index and the severity of the disease may lead to an improvement in the efficiency of the roots in absorbing elements from the soil, including Nitrogen and Potassium, It has been reported that *Hirsutella* sp. Active in Juvenile-2 reduction of *Meloidogyne incognita*, ganglia number and Nematode number, treatment with PGPR reduced root knot number and disease severity (Abokora, 2021; Liu *et al.*, 2022).

Effect of treatment Fig seedlings with *Hirsutella* sp. and Verox on Leaf area, seedling height and some growth Parameters.

Treatment Fig seedlings with *Hirsutella* sp. and induced significant increase in leaf area compared with control, 157.467 cm² and 134.687 cm² respectively compared with 105.883 cm² in control (Table-6). Significant increases in seedlings height treated with the two agents were observed, 2.667 cm and 3.833 cm respectively compared with 0.667 cm in seedlings inoculated with *Meloidogyne* spp. (control).



The treatment with *Hirsutella* sp. and Verox induced significant increase in growth parameters, Chlorophyll content, fresh and dry weights of foliage and root systems (Table-6). Leaf chlorophyll content, foliage fresh weight, root fresh weight, foliage dry weight, root dry weight found, 37.17, 44.67, 60.63 and 16.90 g, 17.20g respectively in seedling treated with *Hirsutella* sp., 35.83, 44.33, 52.50, 14.63 and 19.20g respectively in seedling treated with Verox compared with 30.90, 28.57, 39.00, 11.63 and 12.08 g respectively in control.

Table (6): Effect of treatment Fig seedlings with *Hirsutella* sp. and Verox on Leaf area, seedling height and some growth Parameters.

Treatments	Leaf area cm ²	increase in seedling height after 60d cm	Chlorophyll leaf content Spad	foliage fresh weight g	root fresh weight g	foliage dry weight g	root dry weight g
<i>Hirsutella</i> sp.+Nematode	157.467	2.667	37.17	44.67	60.63	16.90	17.20
Verox +Nematode	134.687	3.833	35.83	44.33	52.50	14.63	19.20
Nematode	105.883	0.667	30.90	28.57	39.00	11.63	12.07
Healthy seedlings(Control)	156.350	2.833	41.17	40.17	42.90	15.00	17.73
LSD(5%)	6.344	0.5055	2.61	2.246	2.371	1.828	2.130

Panpatte et al. (2021) reported the efficacy of plant growth promoting rhizobacterial (PGPR) effective control against *M. incognita* Nematodes by soil immersion of a fortified PGPR consortium to enhance plant growth parameters and reduce Nematode infestation in cucumber in a potted experiment as well as in the field. It has been reported that *Hirsutella* sp. It was active in reducing Juvenile-2 of *Meloidogyne incognita*, number of ganglia and number of Nematodes (**Abokora, 2021**) This may improve plant growth

CONCLUSION

The restriction of *Meloidogyne* spp. development was found associated with promotion of thy seedlings. This may come directly from the inhibition of juvenile-2 penetration into the cost and indirectly from the secondary metabolites producing by *Hirsutella* sp. and PGPR that provide the plants with nutrient elements and make other more available.

REFERENCES

1. Abokora, M. (2021). Application of Nematophagous Fungi and Salicylic Acid as Biological Control Agents against Root-Knot Nematode, *Meloidogyne incognita* Infected Gladiolus: A Valuable Export Plant. *Egyptian Journal of Agronomatology*, 20(2), 53-63.
2. Abu Gharbia, W. I. (2010). *Plant Nematodes in Arab Countries*. University of Jordan-Dar Ell Wael Publishing, Jordan
3. AL-Jarah, N.S. (2011). *Effect of Effective Microorganisms (EM1) and Magnetic field in protecting cucumber plants from infection By the Causal Agents of rots and damping off*. Ph.D. Theses, College of Agriculture. University of Baghdad, Iraq
4. Al-Ubaidy, H. F.W (1985). *Use of Extracts of Some Plants in Controlling the Root-knot Nematode Meloidogyne javanica on Tomato*. MSc Theses, College of Agriculture. University of Baghdad, Iraq.



5. Al-Zahawi, S.M. A. (2007). *Effect of Organic Fertilizers and Soil Covering on Potato Growth and Yield. Solnum tuberosam L.* MSc Theses, College of Agriculture. University of Baghdad, Iraq
6. Al-Zaidi, A. K. N. (2016). *Effect of Adding Wheat Peat and Spraying With its Extract on Growth and Yield of Red Cabbage.* MSc Theses, College of Agriculture. University of Baghdad, Iraq
7. Bellafiore, S. & Briggs, S. P. (2010). Nematode effectors and plant responses to infection. *Current Opinion in Plant Biology*, 13(4), 442-448.
8. Chauhan, H., Bagyaraj, D. J., Selvakumar, G. & Sundaram, S. P. (2015). Novel Plant growth promoting rhizobacteria-Prospects and potential. *Applied Soil Ecology*, 95, 38-53.
9. Diby, P. & Harshad, L. (2014). Plant-growth-promoting rhizobacteria to improve crop growth in saline soils a review. *Agronomy for Sustainable Development*, 34(4), pp.737-752.
10. Dube, B. & Smart, G.C. (1987). Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. *Journal of Nematology*, 19(2), 222-227.
11. Gusiati, Z. M., Bułkowska, K. & Pokój, T. (2014). Tannic acid as a cost-effective substitute for saponin in soil remediation. *Environmental Biotechnology*, 10, 66-72
12. Hallmann, J., Gutberlet, V., Jakobs-Schönwandt, D., Vorlop, K. D., Müller, J. & Patel, A. V. (2019). Effect of additives on the efficacy of microencapsulated *Hirsutiella rhossiliensis* controlling *Heterodera schachtii* on sugar beets. *Biological Control*, 128, 40-47.
13. Haynes, R. J. (1980). A comparison of two modified Kjeldahl digestion techniques for multi-element plant analysis with conventional wet and dry ashing methods. *Communications in Soil Science and Plant Analysis*, 11(5), 459-467.
14. Hussey, R.S. & Barker, K.R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter*. 57, 1025-1028.
15. Khun-in, A., Sukhakul, S., Chamswarn, C., Tangkijchote, P. & Sasnarukkit, A. (2015). Culture filtrate of *Pleurotus ostreatus* isolate Poa3 effect on egg mass hatching and juvenile 2 of *Meloidogyne incognita* and its potential for biological control. *Journal of International Society for Southeast Asian Agricultural Sciences*, 21(1), 46-54.
16. Lee, Y. G., Chung, K. C., Wi, S. G., Lee, J. C. & Bae, H. J. (2009). Purification and properties of a Chitinase from *Penicillium* sp. LYG 0704. *Protein expression and purification*, 65(2), 244-250.
17. Liu, A., Wang, W., Zheng, X., Chen, X., Fu, W., Wang, G. & Guan, C. (2022). Improvement of the Cd and Zn phytoremediation efficiency of rice (*Oryza sativa*) through the inoculation of a metal-resistant PGPR strain. *Chemosphere*, 302, 134900.
18. Martinuz Guerrero, A. P. (2012). *Interrelationships Between Mutualistic Endophytic Microorganisms, The Root-knot Nematode Meloidogyne incognita and The Sap-Sucking Insect Aphis gossypii on Tomato, Squash and Arabidopsis* Ph.D. Theses, University of Bonn, Germany.
19. McKinney, R. H. (1923). Influence on soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativus*. *Journal of Agricultural Research*, 26, 195-218.
20. Mhatre, P. H., Karthik, C., Kadirvelu, K., Divya, K. L., Venkatasalam, E. P., Srinivasan, S., Ramkumar, G., Saranya, C. & Shanmuganathan, R. (2019). Plant growth



- promoting rhizobacteria: A potential alternative tool for nematodes bio-control. *Biocatalysis and agricultural biotechnology*, 17, 119-128.
21. Mostafanezhad, H., Sahebani, N. & Nourinejhad Zarghani, S. (2014). Control of root-knot Nematode (*Meloidogyne javanica*) with combination of *Arthrobotrys oligospora* and salicylic acid and study of some plant defense responses. *Biocontrol Science and Technology*, 24(2), 203-215.
 22. Nawani, N.N. & Kapadnis, B.P. (2005). Optimization of Chitinase production using statistics based experimental designs. *Process Biochemistry*, 40(2), 651-660.
 23. Panpatte, D. G., Shelat, H. N., Jhala, Y. K. & Vyas, R. V. (2021). Fortified bacterial consortium—A novel approach to control root knot Nematode in cucumber (*Cucumis sativum*). *Biological Control*, 155, 104528.
 24. Qiao, K., Zhang, H., Duan, H., Wang, H., Xia, X., Wang, D. & Wang, K. (2013). Managing *Meloidogyne incognita* with calcium phosphide as an alternative to methyl bromide in tomato crops. *Scientia Horticulturae*, 150, 54-58.
 25. Sadik, S. K., Al-Taweel, A. A., Dhyeab, N. S. & Khalaf, M. Z. (2011). New computer program for estimating leaf area of several vegetable crops. *American-Eurasian Journal of Sustainable Agriculture*, 5(2): 304-309.
 26. Siddiqui, Z. A. & Mahmood, I. (1999). Role of bacteria in the management of plant parasitic Nematodes: a review. *Bioresource technology*, 69(2), 167-179.
 27. Sun, J., Park, S. Y., Kang, S., Liu, X., Qiu, J. & Xiang, M. (2015). Development of a transformation system for *Hirsutella spp.* and visualization of the mode of Nematode infection by GFP-labeled *H. minnesotensis*. *Scientific Reports*, 5(1), 1-12.
 28. Sung, G. H., Hywel-Jones, N. L., Sung, J. M., Luangsa-Ard, J. J., Shrestha, B. & Spatafora, J. W. (2007). Phylogenetic classification of Cordyceps and the clavicipitaceous fungi. *Studies in Mycology*, 57, 5-59.
 29. Xiang, N., Lawrence, K. S., Kloepper, J. W., Donald, P. A., Mcinroy, J. A. & Lawrence, G. W. (2017). Biological control of *Meloidogyne incognita* by spore-forming Plant growth-promoting rhizobacteria on cotton. *Plant Disease*, 101(5), 774-784.