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The Integration Impact of Fluopyram and Oxamyl with *Purpureocillium lilacinum* on the Management of *Meloidogyne incognita* and Tomato Growth

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ABSTRACT

The using of chemical nematicides against the root-knot nematodes (*Meloidogyne* spp.) infections is the only famous route to most farmers in Egypt. Root-knot nematodes are one of the destructive pests around the world. Moreover, biological agents as microbial pesticides are eco-friendly, effective and alternative tools to synthetic pesticides globally. In the current study, single and integration impacts of the nematicides fluopyram and oxamyl as well as *Purpureocillium lilacinum* against the root-knot nematode, *Meloidogyne incognita* on tomato plants in two separated pot experiments. The recorded reduction parameters of *M. incognita* were J_2/250g soil, galls and egg masses / 5g roots. The total dry weight, root and shoot–lengths of tomato plants were also noticed. The total soluble protein /g fresh weight of tomato leaves was also recorded. The single applied treatments recorded general mean reduction (GMR) in soil population density at range of 70.33 to 85.24%, root galling recorded GMR at range of 57.62 to 77.35% and egg masses recorded GMR at range of 63.34 to 79.67%. However, the integrated treatments (in sequence) elucidated significant reductions in soil populations (from 72.22 to 88.85%), root galling (from 60.07 to 74.96%) and egg masses (from 62.81 to 81.90%). The applied treatments significantly increased the majority of measured plant indices, as well as exhibited fluctuation in the level of total soluble proteins (TSP) in both trials.

Key words: *Meloidogyne* spp., *Purpureocillium lilacinum*, fluopyram, oxamyl and IPM

INTRODUCTION

Tomatoes are one of the popular vegetable crops in Egypt and worldwide (Abd El-Ghany, 2011). According to FAOSTAT (2020) Egypt is one of the biggest tomato producers' globally which produce about 6.8 million metric tons. Infection with plant parasitic nematodes cause significant suppression in tomato yield (Abd-Elgawad, 2014). However, the root-knot nematodes (*Meloidogyne* spp.) are the most catastrophic genus in plant parasitic nematodes (PPNs) on tomato roots (Moens *et al.*, 2009). There are millions of $ dollars ($118 billion) loss annually because of the root-knot nematodes (Atkinson *et al.*, 2012). Therefore, management of PPN is a vital process which must contains multi-measures to keep the soil population

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below the economical threshold level (Khalil *et al*., 2022).

Non-fumigant nematicides that follow carbamates or organophosphates group is one of the common control methods (Rich *et al*., 2004). These non-fumigant nematicides acted against the root-knot nematode by inhibiting acetylcholin-esterase (AChE) at cholinergic synapses in the nematode nervous system (IRAC, 2022). Lately, various investigations showed that the new registered nematicide; fluopyram recorded remarkable efficiency towards root-knot nematodes in certain crops Storelli *et al*., 2020; Massoud *et al*., 2021). Originally fluopyram is a fungicide that produced by Bayer Crop Science (Ji *et al*., 2019).

Bio-diversity of soil is important source to provide the antagonistic microbes. Certain attempts have been made for managing the pest or disease problems with soil microbes. The nematophagous fungus; *Paecilomyces lilacinus* (newly; *Purpureocillium lilacinum*) was discovered in Peru (Oclarit *et al*. 2009). Several reports indicated that *P. lilacinum* was found to parasitize eggs, juveniles and females of root-knot and cyst nematodes (Atkins et al., 2005 and Kiewnick and Sikora, 2006). In this investigation the efficacy of *P. lilacinum*, fluopyram and oxamyl was evaluated on the root-knot nematode, *M. incognita* under pot conditions, and to study the integration effects between *P. lilacinum* and both nematicides.

MATERIALS AND METHODS

The applied treatments during the experiments

1) Velum prime® 40% SC (Fluopyram) applied at the recommended dose of 250 ml/ feddan which equivalent 0.0005 ml /pot.

2) Oxamytod® 24% SL (Oxamyl) applied at the recommended dose of 3L/ feddan which equivalent 0.006 ml /pot.

3) Bio-Nematon® containing 1×109 cfu/g of the fungus *Purpureocillium lilacinum*, applied at the recommended dose of 4 kg/ feddan which equivalent 0.008 g /pot.

4) The integrated treatments were applied in sequence as follow: -

•Sequence 1: ½ recommended dose of Fluopyram +½ recommended dose of *P.lilacinum.*

•Sequence 2: ¾ recommended dose of Fluopyram + ½ recommended dose of *P.lilacinum.*

•Sequence 3: one recommended dose of Fluopyram + ½ recommended dose of *P. lilacinum*.

• Sequence 4: ½ recommended dose of Oxamyl +½ recommended dose of *P. lilacinum.*

•Sequence 5: ¾recommended dose of Oxamyl + ½ recommended dose of *P. lilacinum.*

•Sequence 6: one recommended dose of Oxamyl + ½ recommended dose of *P. lilacinum.*

Source of root-knot nematode (*Meloidogyne incognita*) inocula

The isolated eggs of the root-knot nematode (M.incognita) were originally obtained from the root of cucumber (*Cucumis sativus* cv. Dahab) with sodium hypochlorite method of Hussey and Barker (1973). The eggs suspension was passed through a 200-mesh sieve and collected on a 400-mesh sieve to obtain free eggs before carrying out the experiments. The second stage juveniles (J2s) were obtained from the hatched eggs by Baermann plate technique (Ayoub, 1980). The species of the root knot nematode (*M. incognita*) was identified by using the perineal pattern technique (Taylor and Nelscher, 1974).

Activity of fluopyram and oxamyl on Purpureocillium lilacinum

The effect of fluopyram and oxamyl was investigated on *P. lilacinum* using the radial growth technique method (Zambonelli et al., 1996). Appropriate volumes of the stock solutions of fluopyram and oxamyl products either in distilled water were added to molten nutrient agar (potato dextrose agar Medium, PDA) to obtain range of concentrations for fluopyram (100, 125, 250, 500, 1000 µg

ml-1) and oxamyl product (500,1000, 1250, 2500 and 5000 µg ml-1) immediately before pouring into the Petri dishes (5.5 cm in diameter). Each concentration was tested in triplicate. The discs of mycelial (0.2 cm diameter) of *P. lilacinum*, taken from 8-day-old cultures on PDA plates, were transferred aseptically to the center of Petri dishes. The treatments were incubated at 25ºC in the dark. Colony growth diameter was measured after the fungal growth in the control treatments had completely covered the Petri dishes. Percentage of mycelia growth inhibition was calculated according to Harlapur et al. (2007) as follow: -

Mycelial growth inhibition = [(DC-DT) /DC] × 100

where: DC and DT are average diameters of fungal colony of control and treatment.

Total protein assay

The amount of total protein was calculated using the method outlined by Bradford (1976), with some modifications suggested by Dixon (1985).

Since mg protein/g fresh weight = ((O/K) *100)/0.25,

K (BSA protein) = 0.029 mg/ml

O = the absorption at 595nm, the total protein was calculated as mg protein/gm fresh weight.

The design and procedures of the experiments

Two pot trials were conducted to evaluate the impact of nematicides namely; fluopyram and oxamyl, as well as nematophagous fungus *P. lilacinum* singly and in sequence. Four weeks old tomato seedlings (cv.086) were used as a host plant to the root-knot nematode *M. incognita*. The pot was 17.5 cm diameter which filled with approximately 2kg of autoclaved loamy sand soil (pH= 8.11, OM= 0.9%, sand: silt: clay= 85:9:6%). The transplanted seedlings were infested with the 2nd stage juveniles' suspension of *M. incognita* at the rate of 2000 J_2 / pot by making holes around the root system and each replicate contains one plantlet. Eleven treatments were applied and each treatment was replicated five times. Both experiments were carried out separately. In the termination of the experiments (about eight weeks after inoculation), the plants were uprooted and evaluated for the shoot and root indices (total dry weight, shoot height, root length and total protein). Furthermore, soil population, root galling and egg masses of *M. incognita* were assessed. Isolation of soil population was done by using the sieving and Baermann technique (Ayoub, 1980), while egg masses on roots were stained with Phloxine B stain (0.15 g/l water) for 15 minutes, then roots were washed to remove residual stain (Holbrook et al., 1983).

Statistical analysis

In both experiments, the obtained data have been analysis by using a computer Costat program (2005) version 6.303. The used experimental design was complete randomized (CRD). Statistically significant differences between the means were compared using analysis of variance (ANOVA) with the least significant differences (LSD) and *P*-values at 0.05 probabilities.

RESULTS

The recent registered nematicide fluopyram (Velum prime®) and the commonly used nematicide oxamyl (Oxamytod®) in Egypt, were investigated against the root-knot nematode (*M. incognita*) on tomato plants under plastic house conditions singly or in sequence with the bio-nematicide *Purpureocillium lilacinum* (Bio-Nematon®). The impact of single or sequenced treatments of fluopyram, oxamyl and *P. lilacinum* (PL) was studied on the soil population density in two successive pot trials (**Table 1)**. In sequence treatments fluopyram and oxamyl in recommended dose, in addition to half (½) and three quarters (¾) of recommended dose were also applied. The obtained results (**Table 1)** exhibited that application of fluopyram, oxamyl and *P. lilacinum* (PL) singly decreased the soil population density with general mean reduction (GMR) of 85.24, 78.57 and 70.33%, successively. In sequenced treatments of fluopyram and PL at different rates the soil population density was decreased with GMR from 75.45 to 88.85%. While, the sequence between oxamyl and PL in different rates recorded GMR from 72.22 to 84.09%. The tomato root galling was minimized significantly with fluopyram, oxamyl and PL by GMR of 77.35, 67.67 and 57.62 %, respectively (**Table 1**). However, the sequenced treatments of fluopyram and PL in various rates decreased galls with GMR of 62.90 to 74.96%. Whilst, the sequence between oxamyl and PL in various rates recorded GMR in root galls from 60.07 to 68.70%. The egg masses/5g roots were also decreased by 81.17, 68.07 and 62.55% with fluopyram, oxamyl and PL, successively. The sequenced treatment of fluopyram and PL achieved GMR in egg masses ranged from 67.04 to 81.90%, while the sequence between oxamyl and PL recorded GMR ranged from 62.81 to 70.37% (Table 1).

Table (1) The effect of fluopyram, oxamyl and *Purpureocillium lilacinum* alone and in sequence against *Meloidogyne incognita* on tomato plants under plastic house conditions during two successive pot trials.

Treatments	J_{2s}/ 250g soil					Galls / 5 g roots					Egg masses/ 5 g roots				
	1st trial		2nd trial		GMR (%)	1st trial		2nd trial		GMR (%)	1st trial		2nd trial		GMR (%)
	Mean	R%	Mean	R%		Mean	R%	Mean	R%		Mean	R%	Mean	R%	
Fluopyram	209.60f	88.25	436.80d	82.22	85.24	30.00e	77.94	71.20e	76.75	77.35	54.60ef	81.17	90.40f	78.17	79.67
Oxamyl	416.40d	76.66	479.60d	80.47	78.57	44.40cd	67.35	98.00cd	67.99	67.67	70.40d	75.72	120.80de	70.84	73.28
P. lilacinum	572.60b	67.90	669.20b	72.75	70.33	65.80b	51.62	111.40bcd	63.62	57.62	108.60b	62.55	148.60bc	64.12	63.34
Sequence 1	433.60d	75.70	609.40bc	75.19	75.45	48.60cd	64.26	117.80bc	61.53	62.90	92.60c	68.07	140.80cd	66.01	67.04
Sequence 2	309.60e	82.65	466.60d	81.00	81.83	37.00de	72.79	101.80bcd	66.75	69.77	46.00f	84.14	122.20de	70.50	77.32
Sequence 3	226.00f	87.33	236.60e	90.37	88.85	28.40e	79.12	89.40de	70.80	74.96	28.40g	90.21	109.40de	73.59	81.9
Sequence 4	493.80c	72.32	684.80b	72.12	72.22	54.80bc	59.71	121.20b	60.42	60.07	99.20bc	65.79	166.40b	59.83	62.81
Sequence 5	458.40cd	74.30	579.20c	76.42	75.36	50.80c	62.65	110.40bcd	63.95	63.30	86.6c	70.14	163.60bc	60.50	65.32
Sequence 6	336.40e	81.14	318.60e	87.03	84.09	38.40de	71.76	105.20bcd	65.64	68.70	67.80de	76.62	148.60bc	64.12	70.37
Inoculated plants	1784.00a	0.00	2456.20a	0.00	0.00	136.00a	0.00	306.20a	0.00	0.00	290.00a	0.00	414.20a	0.00	0.00

Means in each column followed with the same letter are not significantly different at 5 % level; R %: Reduction percent; GMR%: General mean reduction percent of two trials; Sequence 1: ½ recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 2: ¾ recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 3: recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 4: ½ recommended dose of Oxamyl +½ recommended dose of *P. lilacinum*; Sequence 5: ¾ recommended dose of Oxamyl +½ recommended dose of *P. lilacinum* and Sequence 6: Recommended dose of Oxamyl +½ recommended dose of *P. Lilacinum.*

The impact of fluopyram, oxamyl and *P. lilacinum* alone or in sequence on the tomato plant indices e.g. the total dry weight (TDW), root length and shoot height was illustrated in Table (2) and Fig. (1). The total dry weight exhibited that the single treatments such as fluopyram, oxamyl and PL were recorded the GMI of 115.36, 70.45 and 34.36%, respectively. However, the sequenced treatments showed that fluopyram and PL recorded GMI at range of 65.59 to 94.01%, while oxamyl and PL recorded GMI at range of 46.16 to 87.25%. The untreated uninoculated plants (healthy plants) recorded GMI of 101.25%. The obtained results showed that all applied treatments recorded increasing in root length of tomato plants. The single treatments such as fluopyram, PL and oxamyl exhibited GMI of 63.98, 44.55 and 44.18%, respectively. In sequenced treatment of fluopyram + PL and oxamyl + PL recorded GMI ranges were from 54.98 to 68.25% and from 38.81 to 75.70%, consecutively. The treatment of fluopyram only recorded GMI in shoot height estimated by 45.48%, followed by PL and oxamyl with GMI of 33.11 and 15.27%, respectively. The sequenced treatments as fluopyram and PL or oxamyl and PL were recorded GMI at range of 40.56 to 50.26% and from 29.01 to 52.97%, respectively. Moreover, the healthy plants were increased by 30.63% over inoculated plants (Fig. 1). Regarding the total soluble protein (TSP), levels were fluctuated during both trials (Table 2 and Fig. 1).

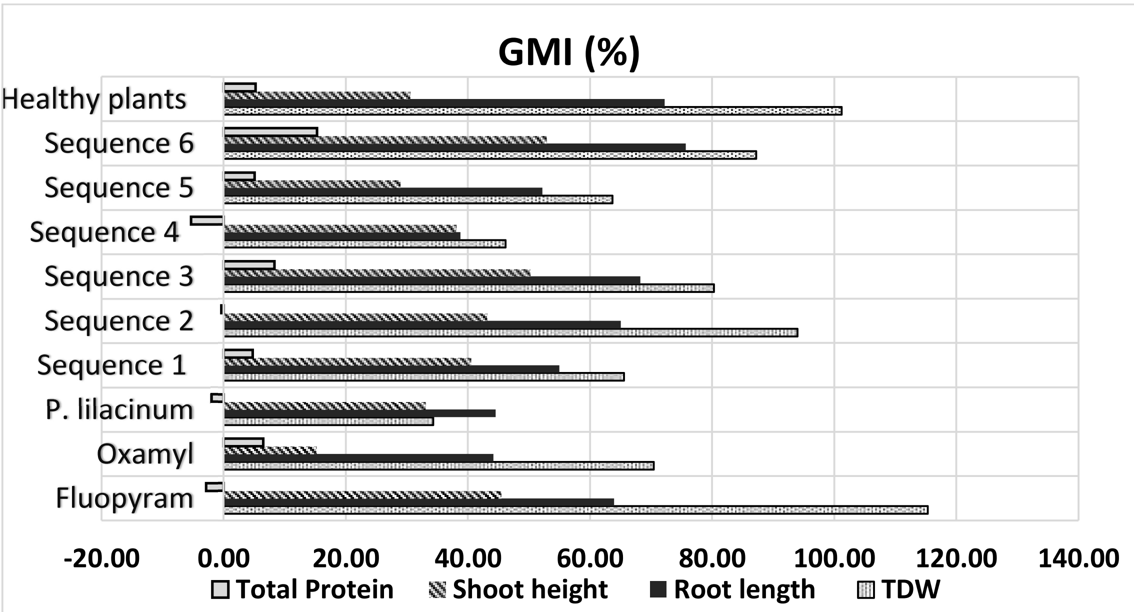


Fig. (1): The general mean increase percent (GMI %) of tested treatments singly or in sequence on tomato growth under plastic house conditions during two successive pot trials. Sequence 1: ½ recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 2: ¾ recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 3: recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 4: ½ recommended dose of Oxamyl +½ recommended dose of *P. lilacinum*; Sequence 5: ¾ recommended dose of Oxamyl +½ recommended dose of *P. lilacinum* and Sequence 6: Recommended dose of Oxamyl +½ recommended dose of *P. lilacinum.*

Table (2) The efficacy of fluopyram, oxamyl and *Purpureocillium lilacinum* alone and in sequence on tomato growth under plastic house conditions during two successive pot trials.

Treatments	TDW (g)				Root length (cm)				Shoot height (cm)				Total Pro. (mg protein/ g F.W.)			
	1st trial		2nd trial		1st trial		2nd trial		1st trial		2nd trial		1st trial		2nd trial	
	Mean	I%	Mean	I%	Mean	I%	Mean	I%	Mean	I%	Mean	I%	Mean	I%	Mean	I%
Fluopyram	20.50b	86.73	15.93a	143.98	19.00bc	52.00	13.90abc	75.95	48.80bcd	32.97	50.40ab	57.99	291.02d	-3.99	282.90c	-1.81
Oxamyl	12.61ef	14.84	14.76abc	126.06	16.90c	35.20	12.10bcd	53.16	44.80d	22.07	34.60e	8.46	320.29bcd	5.51	311.23abc	7.45
P. lilacinum	12.79ef	16.46	9.94f	52.25	18.10bc	44.80	11.40cd	44.30	45.70cd	24.52	45.20abc	41.69	360.08a	15.95	240.20d	-19.91
Sequence 1	13.76de	25.32	13.44abcd	105.85	17.70bc	41.60	13.30abc	68.35	49.90bcd	35.97	46.30ab	45.14	314.43cd	3.75	305.81abc	5.81
Sequence 2	16.68c	51.91	15.42ab	136.10	18.80bc	50.40	14.20ab	79.75	50.80bc	38.42	47.20ab	47.96	302.28cd	-0.12	286.33bc	-0.59
Sequence 3	16.79c	52.90	13.57abcd	107.75	20.70ab	65.60	13.50abc	70.89	53.00ab	44.41	49.80ab	56.11	319.64bcd	5.32	324.88ab	11.34
Sequence 4	14.35de	30.70	10.55ef	61.62	19.10bc	52.80	9.86de	24.81	57.00a	55.31	38.60cde	21.00	337.28abc	10.27	238.11d	-20.97
Sequence 5	15.53cd	41.41	12.14cdef	85.97	19.70bc	57.60	11.60cd	46.84	44.30d	20.71	43.80bcd	37.30	328.43abcd	7.85	295.00bc	2.36
Sequence 6	19.15b	74.40	13.07bcde	100.09	19.40bc	55.20	15.50a	96.20	52.80ab	43.87	51.70a	62.07	354.12ab	14.54	343.01a	16.03
Healthy plants	24.02a	118.78	12.00def	83.71	22.80a	82.40	12.80bc	62.03	53.20ab	44.96	37.10de	16.30	322.18abcd	6.06	301.23bc	4.38
Inoculated plants	10.98f	-----	6.53g	-----	12.50d	-----	7.90e	-----	36.70 e	-----	31.90e	-----	302.64cd		288.03bc	0.00

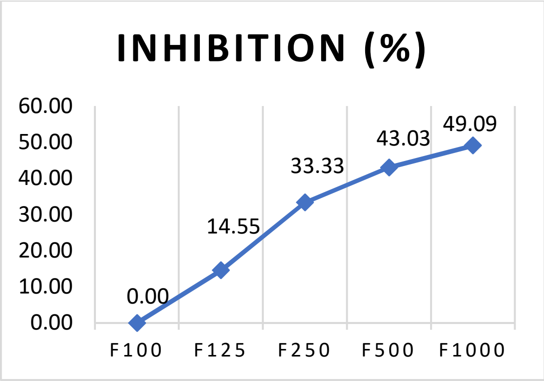
Means in each column followed with the same letter are not significantly different at 5 % level; I %: increase percent; GMI%: General mean increase percent of two trials; Sequence 1: ½ recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 2: ¾ recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 3: recommended dose of Fluopyram +½ recommended dose of *P. lilacinum*; Sequence 4: ½ recommended dose of Oxamyl +½ recommended dose of *P. lilacinum*; Sequence 5: ¾ recommended dose of Oxamyl +½ recommended dose of *P. lilacinum* and Sequence 6: Recommended dose of Oxamyl +½ recommended dose of *P. lilacinum*

The obtained results revealed that fluopyram, PL, sequence 2 and 4 minimized the TSP by 2.90, 1.98,0.36 an 5.35%, respectively. Application of oxamyl, sequence 1, 3, 5 and 6 recorded GMI estimated by 6.48, 4.78, 8.33, 5.11 and 15.28%, respectively. Also, healthy plants recorded increase in TSP estimated by 5.22% over inoculated plants.

Data presented in Table 3 showed the effect of non-fumigant nematicides on *P. lilacinum* with radial growth technique method under laboratory conditions. Results exhibited that *P. lilacinum* was more sensitive to fluopyram than oxamyl with inhibition effect (EC_{50}) estimated by 858.82 and 31453.09 µg/ml, respectively. However, fluopyram elucidated inhibitions in the growth of *P. lilacinum* ranged from 0.00 to 49.09% with the concentrations from 100 to 1000 µg/ml, whilst oxamyl recorded inhibitions in the growth of *P. lilacinum* at range from 6.06 to 27.27% with concentrations from 500 to 5000 µg/ml (Fig. 2). These results indicated that using oxamyl with *P. lilacinum* in IPM programs is safer than using fluopyram.

Table (3): EC_{50}, fiducial limits and slop of fluopyram and oxamyl on the growth of *Purpureocillium lilacinum* with radial growth technique method under laboratory conditions.

Treatment	EC_{50} (µg/ml)	Fiducial Limits (Lower-Upper)	Slope ±SE
Fluopyram	858.82	617.78-1534.88	1.07± 0.20
Oxamyl	31453.09	11522.93-653202.48	0.81± 0.20



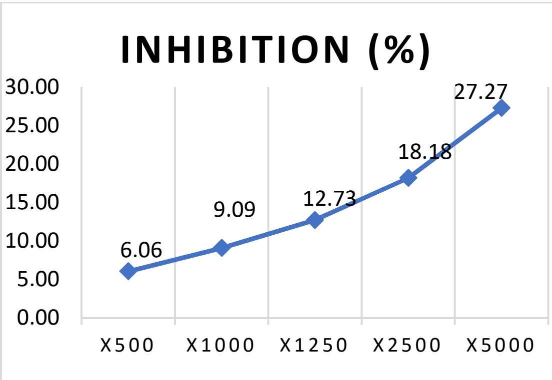


Fig. (2): Inhibition (%) of fluopyram (F) and oxamyl (X) on the growth of *Purpureocillium lilacinum* with radial growth technique method under laboratory conditions.

DISCUSSION

The results of current study are in agreement with those obtained by El-Ashry *et al*. (2021) who found that using *P. lilacinum* alone was effective to control the root-knot nematode (*M. incognita*) and suppressed galls (73.85%), egg masses (71.37%) and soil population (58.97%) on tomato plants significantly under greenhouse conditions. Meanwhile, the combination between *P. lilacinum* + abamectin increased the reduction in galls (86.31%) and egg masses (66.17%), while soil population recorded 74.43%. These results of integration may imply that combination between abamectin and *P. lilacinum* gave synergism, also it may possibly induce the systemic resistance of plants against *M. incognita* (Sharma *et al*., 2020). The fungus, *P. lilacinum* was observed to produce leucine toxins, chitinases, proteases, and acetic acid to promote the infection (Khan *et al*., 2004). In carnation plants, *Paecilomyces lilacinus* suppressed soil population, galls and egg masses of root-knot nematodes by 40.12, 58.00 and 75.81%, consecutively (Kimenju *et al.*, 2014). It was reported that formulated *P. lilacinus* (25% WP) at 1.5 kg succeeded to diminish the population density of *M. incognita* for two seasons consequently by 58.44 and 85.63% on tomato (Hano and Khan, 2016). Narasimhamurthy *et al*. (2017) reported that *P. lilacinus* was the least effective treatment against rice root-knot nematode; *Meloidogyne graminicola* in compared with *Pseudomonas fluorescens, Pochonia chlamydosporia, Trichoderma harazianum* and *Bacillus subtilis*. The fungus *P. lilacinus* recorded the least reductions in galls, soil population and egg masses by 25.65, 49.68 and 36.53%, consecutively.

Oxamyl is a systemic nematicide that belongs to carbamate group. Also, oxamyl is very effective against different genera of plant parasitic nematodes such as; *Meloidogyne incognita* (Ibrahim *et al*., 2018) and *Tylenchulus semipenetrans* (Sweelam *et al*., 2021). However, oxamyl alone or along with mycorrhizae were decreased the larvae of citrus nematode, *T. semipenetrans* infected citrus seedlings under greenhouse conditions by 74.90 and 74.65 % (Sweelam *et al*., 2021).

Based on our results fluopyram was the most effective treatment against the nematode parameters (galls, egg masses and soil populations) and this may be attributed to its systemic effect in tomato roots (Giannakou and Kamaras, 2021). Fluopyram belongs to succinate dehydrogenase inhibitors according to FRAC at group7 (Rieck and Coqueron, 2012). However, fluopyram showed high efficacy against the soil population of root-knot nematodes (*Meloidogyne* spp.) on guava trees for two successive seasons at range of 71.75 to 77.85%, in compared with fosthiazate which recorded reduction at range of 76.70 to 82.95%. Also, in the same study, the biological agent; *P. lilacinus* decreased J_2 in soil at range of 68.75 to 92.92% (Massoud *et al*., 2021). Also, it was reported that fluopyram can decrease the soil population density at planting or protecting the plantlets against the initial penetration of *M. incognita* (Dahlin *et al.*, 2019).

On the other hand, in the current study the effect of fluopyram on *P. lilacinum* under *in vitro* conditions may be attributed to that fluopyram is originally considered a fungicide which is effect on complex II respiration inhibitors and cause a fast and severe depletion in cellular energy (Khalil and Selim, 2021 and Broeksma *et al*., 2014).

Author Contributions:

Conceptualization, MSK, RES; data curation, MSK, RES; formal analysis, MSK; Investigations, MSK, RES; Methodology, MSK, RES; writing original drafts, and writing and editing MSK,

RES; All authors have read and agreed to the purplish version of the manuscript.

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Data Availability Statements:

The data presented in this study are available on request from the corresponding author.

Conflicts of interest:

The author declares no conflict of interest.

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