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Enhancing Application Efficiency of *Pseudomonas* SPP. and *Serratia marcescens* Isolates against *Meloidogyne incognita* in Tomato Plants.

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ABSTRACT

To preserve environmental resources and ensure their sustainability, the search for natural and safe alternatives for controlling pests that attack crops is an urgent necessity. So, the feasibility evaluation of applying plant growth-promoting bacteria (PGPB) isolates as self-reproducing bioagent in controlling root-knot nematodes, *Meloidogyne incognita* is needed, and compare their effect with traditional control methods in the laboratory and greenhouse. Obtained data revealed that egg masses hatching was inhibited by 64.51% when treated with *Pseudomonas putida/fluorescens* isolates, while, decreased to 39.34% with *Serratia marcescens* isolates treatment. While the larvicidal effect of *Pseudomonas* isolates was 99.34% and 88.36 in *S. marcescens* isolates treatments. The tested PGPB showed mediated ovicidal and larvicide lower than oxamyl and higher than composted cattle manure extract (CCME) with surpassing the larvicidal effect.

In the greenhouse experiment, all tomato growth parameters measurement data increased especially in treatments: the combination of PGPB isolates + CCM followed by *P. putida/fluorescens* + *S. marcescens* isolates then oxamyl treatment and the lowest increases were the CCM treatment. The inoculation of the tested PGPB isolates + CCM and oxamyl application reduced highly galling reduction percentage and reproduction (IJs/100 g soil) of *M. incognita* as compared with CCM with a percent reduction of 50.52% (86.07), 58.71% (77.78), and 24.99% (46.26), respectively. Therefore, the results confirm the feasibility and effectiveness of mixing PGPB isolates with manure or its derivatives, better than the individual application of PGPB or manure. The inoculation of the tested PGPB isolates + CCM and oxamyl application reduced highly galling reduction percentage and reproduction (IJs/100 g soil) of *M. incognita* as compared with CCM with a percent reduction of 50.52% (86.07), 58.71% (77.78), and 24.99% (46.26), respectively. Therefore, the results confirm the feasibility and effectiveness of mixing PGPB isolates with manure or its derivatives, better than the individual application of PGPB or animal manure.

INTRODUCTION

Soil-borne diseases are a chronic problem exhausted farmers due to increasing their cumulative population in the soil causing more control difficulty. Among these soil-borne diseases, plant-parasitic nematodes e.g. root-knot nematode (RKN) pathogen *Meloidogyne incognita* are the most important and widespread nematodes. Because of

their economic importance on the wide host range of invasive plants and quickly adapted in different agricultural environments especially in tropical conditions, they considered among the most dangerous pathogens (Luc *et al.,* 2005).

Many common applicable control methods were used for the reduction of RKN population in infested soil included soil fumigation and nematicides application (Wright, 1981). Fumigants and nematicides soil application causes adverse effects on environmental, wildlife risks, and disturb soil biodiversity (Ibekwe *et al.*, 2001). Moreover, the temporary control effect, which disappears after the pesticide removal, followed by an outbreak in the nematode population after the decrease. The pest outbreaks resulting from chemical control came from the reduction or elimination of biological control agents inhabited in soil. All these reasons motivate researchers to continuously search for effective, safe, and natural alternatives (Oka *et al.,* 2000).

Many factors influence RKN reproduction in soil includes abiotic and biotic factors involved biological interaction between two different biological organisms negatively effect on RKN population and infection (Abd-Elgawad and Kabeil, 2012; Davies et al., 1991; Kerry, 2000).

The ecosystem involved different kinds of biological interactions either affect directly or indirectly, negatively or positively on populations as classified by (Odum *et al.,* 1960). The optimal biological control includes predators, parasitoids, pathogens, and competitors (Flint and Dreistadt, 1998) caused direct damage to target pests as parasitism, predation, and competition.

PGPB plays a crucial role by interacting with plants improving healthy and inducing plant resistance mechanisms subsequently the ability to defend against various diseases and pests (Pouteau *et al.*, 1994). The use of PGPB is a promising alternative method to improve plant efficiency in the utilization of chemical fertilizers, enabling a reduction of fertilizer application on crops (Diaz *et al.*, 2019). Beside, prescience PGPB in plant tissues promotes plant growth by secretion of hormones and enzymes or facilitating the nutrient uptake (Aquino *et al.,* 2019). Also, enhancing the antioxidant response of crops (Tiepo *et al.,* 2020). The direct effect of some microbial bioagent by secretions of egg hatching inhibitors and decline or directly killing infective juveniles (Siddiqui and Mahmood, 1999).

There is increasing interest to use friendly tactics for managing nematodes, by creating an appropriate environment for the growth and reproduction of beneficial microbial communities effect on nematode, plant, or directly rhizosphere occupation.

Among promising biocontrol agents, PGPB (*Pseudomonas* spp. and *Serratia marcescens*) showed potency in controlling *Meloidogyne incognita* (Akhtar and Siddiqui, 2009; Khanna *et al.,* 2019; Ovcharenko *et al.*, 2010) in vegetable crops (Mohamed *et al.*, 2009). So, the study tried to assess the successful ability of Egyptian strains of indigenous rhizobacteria in the suppression of RKN, *Meloidogyne incognita* as well as, elucidate of the comparative effect of applying PGPB species separately and its mixture or inoculated with composted cattle manure (CCM) extract *in vitro*, also, composted cattle manure comparing with standard chemical nematicides on tomato plants under greenhouse conditions to provide optimum tactics to enhance the PGPB presence in the soil after application.

MATERIALS AND METHODS

Root-knot Nematode, *Meloidogyne incognita* Inoculum Source:

The identified pure inoculum of *M. incognita*, previously isolated by (El-Ashry *et al.,* 2019) was used. The culture was maintained in the greenhouse on the tomato, *Solanum lycopersicum* L. susceptible cultivar Super Strain B.

Rhizobacteria Inocula Source:

The tested rhizobacteria (*Serratia* sp. and *Pseudomonas* spp.) were was previously isolated from collected soil samples infested with root-knot nematodes from different areas in El-Sharqia Governorate, Egypt. The obtained bacteria isolates were identified by using16S rDNA gene sequencing (Hegazy et al., 2019). The tested isolates used in the study were *Pseudomonas putida* (PP29 and PP22), *P. fluorescens* (PF131), and *Serratia marcescens* (A10, A15, and A20).

Laboratory Experiments:

Effect of Rhizobacteria Isolates on *M. incognita* Eggs Hatching:

A homogenate 5 egg masses in size were added to 10 ml of each prepared rhizobacteria isolate suspension at a concentration of 10^8 cfu /ml in Petri dishes (5 cm diameter). The control treatment similarly was prepared in 10 ml distilled water only. Each treatment was replicated five times. All treatments were incubated at 24 ±2˚C. Numbers of newly hatched juveniles were counted periodically using a research microscope (100 X magnification) after 1, 2, 3, and 4 days of treatment. The hatching inhibition percentage was calculated in comparison with the control treatment, according to the following equation:

$$\text{Egg hatching inhibition (\%)} = \frac{\text{No. of juveniles in control -No. of juveniles in treatment}}{\text{No. of juveniles in control}} \times 100$$

Effect of Rhizobacteria Isolates on the Survival of *M. incognita* Juveniles:

In vitro, J2 experiments were conducted to test the toxicity of tested rhizobacteria. 0.1 ml containing about 100 J2 were pipetted into Petri dishes (9 cm diameter) filled with 10 ml of rhizobacteria isolates adjusted to 10^8 cfu/ml. The control treatment consisted of the 100 IJs maintained in 10 ml distilled water alone. Each treatment was replicated five times and the dishes were kept at (24±2°C) as optimum temperature for IJs survival (Dunphy and Webster, 1986). All dishes were sealed tightly with parafilm to avoid vaporization of the solution.

Periodically examination was conducted by pipetting 0.5 ml treatment solution into a Hawksley counting slide and examined by the aid of a research microscope at 100X. The nematode juvenile showing inactive straight posture or inactive (S) posture or did not show any movement after prodding were considered dead and any other types of movement were scored as alive (Ishibashi and Takii, 1993; Nardo and Grewal, 2003). The juvenile's mortality recorded after 1, 2, 3, and 4 days, and the mortality percentage calculated by the following equation:

$$\text{Mortality(\%)} = \frac{\text{No. of dead juveniles}}{\text{Total No. of exposed juveniles}} \times 100$$

Comparison effectiveness between Combinations of the Tested Rhizobacterial Isolates Application and Conventional Control:

The comparison treatments included the following applications:

A) Ten milliliters volume mixture (1:1) of tested rhizobacteria (*Pseudomonas* spp. and *Serratia marcescens*) isolates were applied.

B) Ten milliliters of composted cattle manure extract (CCME) was used. The solution of composted cattle manure was prepared by adding 0.25 Kg of composted cattle manure/ L distilled water to a beaker then incubated for 12 h at room temperature. Then obtained liquid suspension was filtered and sieved through a 200-mesh sieve nested upon a 400-mesh sieve to obtain the extract.

C) Vydate 24 % SL (oxamyl) (2 L/feddan) as standard nematicides recommended for controlling root-knot nematode was used. Besides, control treatment free of any control

agent. All these treatments were implemented on egg masses and the second juvenile stage of *M. incognita* in two separate laboratory experiments implemented at the above-mentioned technique.

Greenhouse Experiment:

Tomato (*Solanum lycopersicum* L.) seedlings VFNT cultivar were transplanted in 20 cm diameter plastic pots filled with 1700 g mixture of sterilized sandy soil (73.5 % sand, 12.5 % clay, 8.1 % silt, 100 g peat moss, and 3 mg urea fertilizer per kg of soil). After three weeks of sowing, seedlings were thinned to three plants per pot.

Every plant seedling was inoculated with 1000 newly hatched infective juveniles (IJs) of *M. incognita*. The second-stage juveniles were added by pipetting 2 ml of the inoculum suspension into four holes around the root system. The treatments were done according to the following scheme: each rhizobacterium applied alone, rhizobacteria in combination (*Serratia* isolates + *Pseudomonas* isolates), composted cattle manure, and the combination of all tested rhizobacterial isolates loaded on composed cattle manure besides, control healthy and infected with nematodes. The application rate of rhizobacteria was 10 ml /plant of 10^8 cfu/ml concentration and incorporated with the upper 5cm of soil around each plant using special spouted. While the composted cattle manure (CCM) applied to seedlings at the rate of 5 g/plant incorporated with the upper 3cm of soil around each plant. Control treatments included inoculation of *M. incognita* IJs alone as well as healthy plants without nematode inoculum. Each treatment was replicated five times.

The treatments were arranged in the greenhouse in a randomized complete block design and incubated at 28 ± 3°C. Forty -five days after inoculation, plants were removed carefully from pots, and data on plant growth included root weight (g), root length (cm), shoot weight (g), and the number of root galls /plant roots were recorded. While, the recorded parameters related to nematode development were root weight (g), root length (cm), shoot weight (g), number of root galls/plant roots, gall diameter, number of egg masses/plant roots, number of IJs/100 g, and RF (Final population /Initial population). The root-knot gall index (RGI) was assessed using (Taylor and Sasser, 1978) scale of 0 = No galling; 1 = 1:2 galls; 2 = 3: 10 galls; 3 = 11: 30 galls; 4 = 31:100 galls and 5 = more than 100 galls. Gall diameter was also measured at its greatest diameter (El-Deeb et al., 2018). Also, samples of 100 g soil were processed for nematode extraction using a combination of sieving and Baermann trays technique (Hooper, 1990). The parameters changing the percentage of increase or decrease imputed to "negative or positive" control values and the current equations were used:

$$\text{Reduction (\%)} = ((\text{Control-Treated})/\text{Control}) \times 100$$

$$\text{Increase (\%)} = ((\text{Treated-Control})/\text{Control}) \times 100$$

Statistical Analysis:

The completely randomized design was applied for laboratory experiments, while experimental units were arranged in a randomized complete block design in the greenhouse experiment. Data were subjected to statistical analysis using MSTAT version 4, where, analysis of variance and means were compared using Duncan's multiple range test at $P \leq 0.05$ probability.

RESULTS AND DISCUSSION

The pesticides used in agriculture production follow two types based on the target organism for pesticide mechanism. The first type of pesticides used against the pest causing mortality or development/ behavior disruptions. Most chemical pesticides follow the first type which has the affinity to conjugate with a vital molecular target causing stop a crucial

physiological process. The second type including very few pesticides besides microbial bioagents interacts with the host plant whether biological interactions or as plant resistance elicitors. Elicitors are substances or organisms that induce releasing the potential defense potential of a healthy host plant. Few of the microbial bioagents can parasitize on the disease, but many of them have multiple mechanisms such as secreting toxins, antibiotics, and decomposing enzymes. Among the microbial pesticides, PGPB is a multifunctional bacterium. As, PGPB enhance phytoremediation by the production of siderophores (Tahir *et al.,* 2013), phytohormones, and chelators in addition to their ability to biodegrade contaminants and facilitate their removal (Chun *et al.,* 2020) or *S. marcescens* ABHI001 in the bioremediation of organic pollutants, such as p-cresol(Singh *et al.,* 2017). Also, PGPB used as a biofertilizer can dissolve phosphate highly and making available for plants (Akhtar *et al.,* 2012; Mohamed *et al.,* 2018). Besides, PGPB can be mixed with different agrochemicals e.g. mineral fertilizers and pesticides without reducing its efficiency (Esfahani *et al.,* 2016; Mohamed et al., 2018; Saedi et al., 2017). PGPB showed potency against a wide range of pests e.g. nematode and fungi and practical in the disease complex cases (Ying *et al.,* 2015). Among the different PGPB, *P. putida*, *P. fluorescens*, and *Serratia marcescens* get more attention practically in root-knot nematode control (Amin, 2014; Mohamed *et al.,* 2009).

The Ovicidal and Larvicidal Effect of *Serratia marcescens* and the Tested *Pseudomonas* spp. Isolates on the Egg Masses and the Second Infective Juveniles of RKN, *Meloidogyne incognita:*

Emerged juveniles of root-knot nematode *M. incognita* hatched from egg masses flooded in the suspensions of *Pseudomonas* spp. and *Serratia marcescens* isolates after different incubation periods shown in Table (1). The direct contact between the tested PGPB isolates for 2 days led to 84.45 and 82.84 % hatching reduction in *P. putida* (PP22 and PP29) respectively with significant difference comparing other treatments. While *P. fluorescens* (PF131) caused significant hatching reduction (38.33 %). Different isolates of *Serratia marcescens* (A10, A15, and A20) bring about the lowest hatching inhibition percentage range (18.49: 32.05 %). The successive days of incubation, hatching inhibition percentage data showed the same trend as the aforementioned, with a slight difference in the inhibitory efficacy of the *P. fluorescens* (PF131), to be close to the effect of the tested *S. marcescens* isolates, with no significant difference between them. After 10 days incubation period, the efficiency of the tested PGPB isolates order significantly based on nematode egg masses hatching inhibition. The tested isolates PP22, PP29, PF131, (A10 and A15), and A20 recorded 75.48, 69.04, 49.00, (47.96 and 39.50), and 30.56% hatching inhibition.

Inhibition Effect on Egg Masses of *M. incognita*:

Emerged juveniles of RKN, *M. incognita* hatched from egg masses immerged in the suspensions of *Pseudomonas* spp. and *Serratia marcescens* isolates after different incubation periods were demonstrated in Table (1). The nematicidal property of the rhizosphere bacteria on egg hatching (Percentage inhibition in egg hatching) of *M. incognita* under laboratory conditions was studied after 2, 4, 6, 8, and 10 days. Laboratory bioassay by using *M. incognita* egg masses varied according to rhizobacteria isolates. All tested PGPB isolates showed a superior inhibition ($P \leq 0.05$) after two-day incubation with nematode egg mass ranged from 82.84 % reduction with *Pseudomonas* spp. isolates while, *S. marcescens* showed a fluctuated potency recorded 32.05 and 21.98 % inhibition percentage with *S. marcescens* (A10) and *S. marcescens* (A20), respectively, whereas *S. marcescens* (A15) scored the least reduced hatching with 18.49%.

On the other hand, when the period of the tested bacteria was increased to four days, the percentage of emerged juveniles was decreased obviously and all treatments

showed increasing in hatching inhibition and *P. putida* (PP22) recorded the highest reduction percentage (86.60). After the 6th day of exposure, all bacteria isolates showed a reduction in egg hatching inhibition continued to the end of the experiment. Treatments of *Pseudomonas* spp. showed the high significant effectiveness especially *P. putida* (PP22) which surpassed all other tested bacterial isolates recorded 87.85 % hatching reduction as shown in Table 1. The same trend was observed after the 8th day of exposure. It was true with tested the tested PGPB isolates at 10th day of exposure, a significant difference ($P \leq 0.05$) was observed and *P. putida* (PP22) exposed the highest inhibition percent (75.48) with no significant difference with *P. putida* (PP29) whereas, *S. marcescens* showed the least efficacy against egg masses hatching of RKN, *M. incognita.*

Table 1. Effect of tested *Pseudomonas* spp. and *Serratia marcescens* isolates on egg masses hatching of root-knot nematodes, *Meloidogyne incognita* after various times of exposure

Bacterial isolates	Isolate code	Emerged juveniles after different exposure periods (d)				
		2	4	6	8	10
Pseudomonas spp.	*P. putida* (PP29)	12.80^{d} (82.84)	46.80^{d} (84.16)	88.40^{c} (86.51)	142.80^{c} (82.80)	309.60^{d} (69.04)
	P. putida (PP22)	11.60^{d} (84.45)	39.60^{d} (86.60)	79.60^{c} (87.85)	107.60^{c} (87.04)	245.20^{d} (75.48)
	P. fluorescens (PF131)	46.00^{c} (38.33)	109.80^{c} (62.85)	250.60^{b} (61.77)	372.80^{b} (55.10)	510.00^{c} (49.00)
Serratia marcescens	*S. marcescens* (A10)	57.40^{b} (32.05)	229.20^{b} (22.46)	305.40^{b} (53.41)	436.80^{b} (47.39)	520.40^{bc} (47.96)
	S. marcescens (A15)	60.80^{b} (18.49)	231.60^{b} (21.65)	295.60^{b} (54.91)	464.40^{b} (44.07)	605.00^{bc} (39.50)
	S. marcescens (A20)	58.20^{b} (21.98)	224.60^{b} (24.01)	287.60^{b} (56.13)	420.40^{b} (49.37)	694.40^{b} (30.56)
Control		74.60^{a}	295.60^{a}	655.60^{a}	830.40^{a}	1000^{a}

The number between parentheses refers to the reduction percentage; Different letters in the same column represent significant differences ($P < 0.05$) among different bacterial isolates.

The mortality percentages resulted from exposure *M. incognita* second juveniles to the tested PGPB different isolates elucidated in Table (2). Among the tested PGPB isolates PF131 significantly surpassed until the experimental endpoint recorded 52.86% mortality and a short latent period appeared after two days of treatment followed by PP22 and PP29 caused significant mortality 43.60 and 41.01 %, respectively. While A10 showed intermediated mortality 47.07% finally, A15 and A20 resulted in mortality 29.29 and 26.06 %. After 4 days of incubation, all *Pseudomonas* spp. isolates and A10 isolate showed significant difference differed from the rest of the *Serratia marcescens* isolates. After 7 days of incubation, the pseudomonas isolates to outperform, especially PP22 and PP29 isolates while PF131 intermediate between them followed by *S. marcescens (*A15 and A20). The 10th day exposure ascertained the superiority of *Pseudomonas* spp. isolates especially PP22 and PF131 while *Serratia marcescens* isolates ranked second for efficiency with a significant difference.

Table 2. The second juvenile mortality percentages of root-knot nematode, *Meloidogyne incognita* after incubation with the tested isolates of *Serratia marcescens* and *Pseudomonas* spp. at different time intervals.

Bacteria	Isolates	Exposure periods (d)			
		2	4	7	10
***Pseudomonas* spp.**	***P. putida* (PP29)**	41.01^{b}	72.84^{a}	83.13^{b}	98.92^{ab}
	***P. putida* (PP22)**	43.60^{b}	79.25^{a}	90.10^{a}	99.28^{a}
	***P. fluorescens* (PF131)**	52.86^{a}	83.54^{a}	87.32^{ab}	99.82^{a}
Serratia marcescens	***S. marcescens* (A10)**	47.07^{ab}	73.66^{a}	83.13^{b}	95.69^{b}
	***S. marcescens* (A15)**	29.29^{c}	43.21^{b}	56.46^{c}	84.05^{c}
	***S. marcescens* (A20)**	26.06^{c}	44.44^{b}	59.58^{c}	85.34^{c}

Different letters in the same column represent significant differences ($P < 0.05$) among different bacterial isolates.

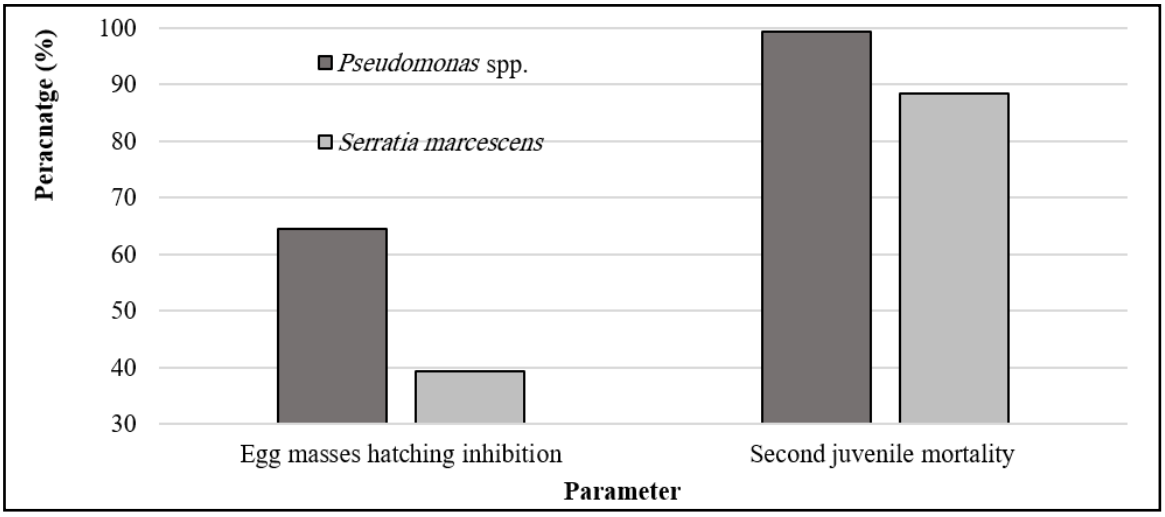

Fig. 1. Percentage hatching inhibition and mortality in *M. incognita* resulted from the tested *Serratia marcescens* isolates and Pseudomonas / fluorescens isolates.

Results indicated that *Pseudomonas* spp. and *S. marcescens* isolates have significant potential as a biocontrol agent against egg masses and J2 of RKN, *M. incognita in vitro* experiments (Fig.1). Inoculation of egg masses with PGPB was significantly reduced newly J2 hatched and increased J2 mortality. As well as *Pseudomonas* spp. isolates were more effective than *S. marcescens* isolates against eggs (ovicidal effect) and J2 mortality (larvicidal effect) of *M. incognita* and can be regarded as a potential biocontrol agent.

Reduction in egg masses hatching and the IJ_2 mortality in the present study might be attributed to the presence of nematode egg is surrounded by a gelatinous matrix to preserve the water content necessary for egg development. Change in water control and content or insufficiency required for embryonic development leads to egg hatching failure. The PGPB active ingredient is extracellular secretions considering no biological interaction between PGPB and any stage of RKN. The secreted bioactive molecules produce by PGPB included siderophores, phytohormones, and chelators (Chun *et al.,* 2020; Mandal *et al.,* 2017). Besides, the degradable enzymes e.g. chitinases, gelatinase, and protease (Guo et al., 2020; Khatamidoost *et al.,* 2015; Navarro-González *et al.,* 2019; Safni *et al.,* 2018; Zaghloul *et al.,* 2015). These enzymes enable PGPB causing egg hatching failure due to liquidizing gelatin matrix surrounded egg masses and causing damage to infective juvenile nematode by degrading cuticle which chitin involved in composition causing mortality or water imbalance or bleeding. On the other hand, secondary metabolites may include a toxic

molecule whether simple or complex molecules. Toxic simple molecules include ammonia, hydrogen cyanide (Khan *et al.*, 2016; Siddiqui *et al.*, 2006; Zavaleta-Mejia and Van Gundy, 1989). Besides complex molecules e.g. prodigiosin is red pigment secreted as a secondary metabolite, produced by *Serratia marcescens*. This pigment is a natural alkaloid that has three pyrrole rings in chemical structures. Prodigiosin has been described as a stronger antioxidant activity (Lapenda *et al.*, 2020; Mandal *et al.*, 2017; Rahul *et al.*, 2014). These secondary metabolites may be volatile or nonvolatile (Khatamidoost *et al.*, 2015; Zavaleta-Mejia, 1985; Zavaleta-Mejia and Van Gundy, 1989; Zhai *et al.*, 2018) with direct nematicidal, fumigant, and repellent activities on egg or juveniles with multiple modes of RKN control (Zhai *et al.*, 2018). So, the cultural filtrate of PGPB approved the nematicidal effect (Rangeshwaran *et al.*, 2012). The bacterial extraction treatment of *Pseudomonas fluorescens* strains UTPF5 kills almost 100% of the larvae hatching after 24 h and a complete ban on egg hatch (Bagheri *et al.*, 2014). Although bacterial cell suspension showed more efficient mortality on nematode *M. incognita* compared with their cultural filtrates (Zaghloul *et al.*, 2015) for the presence of renewable sources for secretion toxic secondary metabolites and enzymes. Therefore, the nematotoxic effect of PGPB was positively correlated with bacterial concentrations, exposure periods, and chitinases production (Channppa et al., 2008; Kassab *et al.*, 2017). Consequently, it becomes clear that the difference between isolates of the same bacterial species is due to the qualitative and quantitative difference between the different isolates in the secreted extracellular metabolites that define the activity range (breadth or narrow) of bacteria on pests. To ensure the maximum effectiveness of the bacteria, it is recommended to mix them upon application.

The Comparative Effect between PGPB, CCME, and Oxamyl on Hatching and Juvenile Mortality of RNK, *M. incognita*:

For studying the comparative effectiveness of the different control methods, whether the chemical control method, represented by the (Vydate "oxamyl'' 24% SL) as standard nematicides or cultural control method. The cultural control method was represented by the extract of composted cattle manure and inoculation with a combination of PGPB isolates and calculating the hatching reduction percentage based on control treatment contains only egg masses, as shown in Table (3). By looking at the different treatments over the intervals period, data clearly showed that the oxamyl nematicide surpasses other treatments in hindering egg hatching of nematodes. The juveniles who emerged from oxamyl treatment showed highly consistent inhibition (90: 95%) until the experimental endpoint. It ranked second in terms of effectiveness occupied by the tested PGPB isolates. Although, low hatching inhibition percentage (44.86%) on the second day of the incubation increased gradually with the long incubation period (4^{th} and 8^{th} day; 66.76 and 65.16%), then decreased inconsiderably recorded 56.29% on the tenth day after treatment with a significant difference. While the extract of CCME treatment showed a limited hatching inhibition effect during the initial incubation periods. Then, its efficiency increased reaching the maximum during the 6^{th} and 8^{th} day of treatment followed by decreasing again during the 10^{th} day of treatment to record 44.52% less PGPB.

The comparative effect between the different control methods included chemical control method, represented by the (Vydate "oxamyl'' 24% SL) or cultural control method, represented by CCME and inoculation with the tested PGPB isolates combination and calculating mortality percentage of the second juveniles as shown in Table (4). Oxamyl treatment after 2-day exposure recorded 60.81% mortality with a significant difference, also, oxamyl killed all exposed juveniles after only 4 days with a short latent period. While the PGPB isolates ranked second after oxamyl with a significant difference caused 39.98 % mortality after 2 days, then dead juveniles raised gradually until the 10^{th} days recorded

mortality 93.85%. while the cattle manure extract caused the lowest juvenile mortality (18.59%) after 2 days. Then raised gradually to caused 75.43 % mortality in the experimental endpoint.

The efficacy of oxamyl as ovicidal and larvicidal nematicide was evident after treating egg masses or J2 of RKN, *M. incognita.* While the tested PGPB isolates exhibited potency as a larvicidal more than ovicidal effect, similarly with CCME but with lower potency nematicide, as shown in Fig. (2).

Table 3.The comparative effect of rhizobacteria mixture (*Pseudomonas* spp. and *Serratia marcescens*) with extracts of drenched CCM and oxamyl nematicide on egg masses hatching of RKN, *Meloidogyne incognita* after different time intervals.

Treatments	Egg hatching and emerged juveniles after different time intervals				
	2	4	6	8	10
Rhizobacteria	41.13^{b} (44.86)	146.93^{c} (50.29)	217.86^{b} (66.76)	324.13^{b} (65.16)	480.76^{c} (56.29)
Cattle manure extract	68.80^{a} (7.77)	224.60^{b} (24.08)	287.60^{b} (56.13)	393.60^{b} (57.69)	610.2^{b} (44.52)
Oxamyl	6.80^{c} (90.88)	13.80^{d} (95.33)	29.40^{c} (95.51)	42.00^{c} (95.48)	45.00^{d} (95.90)
Control	74.60^{a}	295.60^{a}	655.60^{a}	930.40^{a}	1100^{a}

The number between parentheses refers to the reduction percentage; The same letter (s) in each row indicates no significant difference (P ≤ 0.05) between treatments according to Duncan's multiple range test.

Table 4. The comparative effect of rhizobacteria mixture (*Serratia marcescens* and *Pseudomonas* spp.) isolates mixture with extract of drenching CCM and oxamyl nematicide on the second juvenile mortality of root-knot nematodes, *Meloidogyne incognita* after different time intervals.

Treatments	J2 mortality after different time intervals (d)			
	2	4	7	10
Rhizobacteria	39.98^{b}	66.15^{b}	76.61^{b}	93.85^{b}
Cattle manure extract	18.59^{c}	44.24^{c}	58.33^{b}	75.43^{b}
Oxamyl	60.81^{a}	100^{a}	100^{a}	100^{b}

The same letter (s) in each row indicate no significant difference (P ≤ 0.05) between treatments according to Duncan's multiple range test.

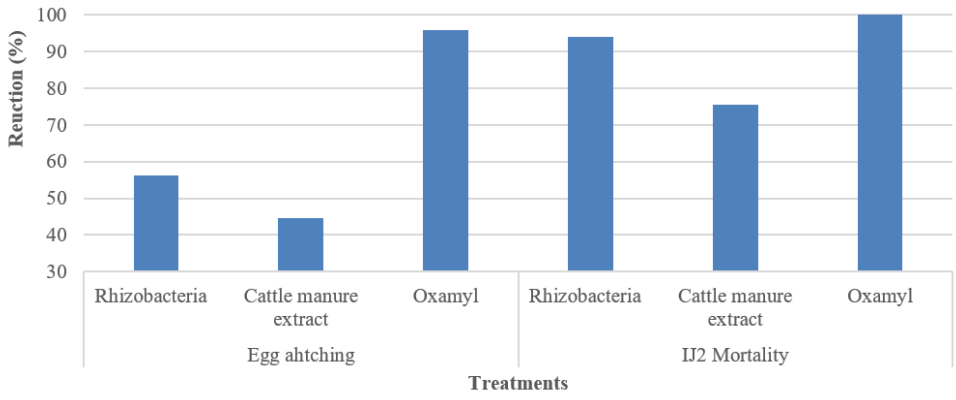

Fig. 2 Effect of tested PGPB isolates, CCME, and oxamyl on egg masses hatchability and IJ2 mortality of *Meloidogyne incognita* after 10 days incubation.

The changing reality of the actual application always depends on the comparison between the actual application (composted cattle manure), the available (oxamyl nematicide), and the desired (PGPB application). Previous studies confirmed the feasibility of the combination of different PGPB (species/isolates/strains) better than that of either of them alone (Ara and Ehteshamul-Haque, 2016; El-Nagdi *et al.,* 2019; Siddiqui and Akhtar, 2008) because of the synergistic effect between combined bacteria. Treatment with *Serratia marcescens* alone resulted in the least effective low potency (Ameen and Youssef, 2006; Youssef and Ameen, 2008).

When comparing the nematicides applications with PGPB, research work showed initial incomparable potency (Khan et al., 2016; Raddy et al., 2013) vanished soon as the active ingredient dissipated in the environment to return nematode population built-up in infested soil. As for application, PGPB ensures the relatively less potency, with the continuous protection as a result of colonization of the rhizosphere and built-up population gradually with some precautions. Although, some strains (*P. fluorescens* strains CHAO) proved to be more effective than fenamiphos nematicide in nematode egg production on olive seedlings roots. (Khalighi and Khodakaramian, 2012). This is maybe due to the presence of mutations that increase the production of secondary metabolites. The limited potency of compost may be due to the toxic levels of ammonium, (Lazarovits *et al.,* 2001; López-Pérez *et al.,* 2005) beside some metabolites include enzymes and minerals water-soluble resulted from fermentation during composting manure although the main role of manure by alterations in soil structure, the stimulation of antagonistic organisms, and improved plant tolerance.

Effect of PGPB, CCM, and Oxamyl on Tomato Plants Growth Parameters and Reproduction Parameters of *M. incognita*:

The measured plant growth parameters of infect tomato plants with RNK, *M. incognita,* and received different treatments under study are illustrated in Table (5). By scrutinizing data, all recorded plant growth parameters were raised without exception. Whereas oxamyl treatment surpassed in fresh root weight and root length recorded increasing 20.22 and 23.67%, respectively. While the combination of *Pseudomonas* spp. isolates + *S. marcescens* isolates + CCM surpassed in fresh shoot weight, the number of leaves/ plants, and stem diameter causing increasing 25.77, 76.92, and 68.00%, respectively. Therefore, the combination of *Pseudomonas* spp. isolates + *S. marcescens* isolates showed prepotency with most growth parameters better than applied separately. The least effective treatment with the most botanical measurements was CCM treatment recorded the lowest inconsiderable increase. Therefore, the results confirm the feasibility and effectiveness of mixing PGPB isolates with manure or its derivatives, better than the individual application of PGPB or manure (Table 5).

There were significant differences ($P \leq 0.05$) between different PGPB treatments, CCM, and oxamyl in infected root plant parameters (gall formation, root gall index (GI), galls size (more than 4 mm)) and soil parameter besides reproductive factors (RF) of root-knot nematodes (Table 6). Current results revealed that all PGPB species or its mixture, as well as its mixture with CCM, reduced tomato root galling (as shown by the number of galls and root gall index, GI) and *M. incognita* reproduction (as directed by the number of egg masses on roots and final number of IJs in soil or reproduction factor, RF).

Under greenhouse conditions, pots treated with a mixture of *Pseudomonas* spp. and *S. marcescens* plus CCM gained the highest level in percent reduction of gall numbers (50.52) after oxamyl treatment (58.71). Among the tested rhizobacteria, a mixture of rhizobacteria (32.44) surpassed *Pseudomonas* spp. (19.91) and *S. marcescens* (21.89) in percent reduction of galls followed by composted cattle manure (24.99) while cattle manure plus a mixture of rhizobacteria (50.52) showed the maximum nematicidal effect.

Pots treated with oxamyl equal to pots treated with a mixture of rhizobacteria plus CCM in GI (3.3 for each) while positive control gained the high root gall index (4.0) followed by various treatments of rhizobacteria and composted cattle manure. Regarding the efficiency of the tested rhizobacteria and composted cattle manure in comparison with oxamyl in the reduction of *M. incognita* egg masses, results clearly showed that oxamyl effect (70.68%) followed by the mixture of rhizobacteria plus CCM (66.78 %) achieved the highest significantly effect in minifying numbers of egg masses compared to other tested materials. Whereas *Pseudomonas* spp. achieved the lowest significant effect (14.20%) compared to untreated tomato plants.

Table 5. Plant growth parameters of infected tomato plants with *Meloidogyne incognita* after treatment with rhizobacteria and various mixtures in comparison with composted cattle manure and oxamyl nematicide in the greenhouse tests.

Treatment	Fresh root weight	Fresh shoot weight	Number of leaves/ plants	Root length (cm)	Stem diameter (mm)	Plant height (cm)
Healthy plants	12.06^{a}	14.77^{a}	14.0^{a}	16.34^{a}	8.60^{a}	24.40^{a}
M. incognita	9.74^{e}	11.91^{f}	7.80^{f}	11.91^{d}	5.00^{e}	16.80^{f}
***M. incognita* +P**	10.60^{c} (8.82)	14.31^{c} (20.15)	11.80^{c} (51.28)	12.77^{d} (7.22)	7.40^{bc} (48.00)	22.80^{b} (35.71)
***M. incognita* +S**	10.14 ^{de} (4.10)	14.03^{cd} (17.80)	11.60^{d} (48.71)	12.39^{d} (4.03)	6.80^{c} (36.00)	20.00^{d} (19.04)
***M. incognita* +S + P**	11.06^{d} (13.55)	14.56^{d} (22.25)	13.40 ^{e} (71.79)	13.65^{c} (14.60)	7.80^{d} (56.00)	18.60^{e} (10.71)
***M. incognita* + O**	11.71^{ab} (20.22)	12.35^{ef} (3.69)	12.20^{b} (56.41)	14.73^{ab} (23.67)	6.80^{b} (36.00)	21.20^{c} (26.19)
***M. incognita* + CCM**	10.02^{de} (2.28)	12.83^{e} (7.72)	11.00^{c} (41.02)	12.16^{d} (2.09)	6.30^{d} (26.00)	16.90^{f} (0.59)
***M. incognita* + S + P + CCM**	11.77^{b} (20.84)	14.98^{b} (25.77)	13.80^{b} (76.92)	14.60^{ab} (19.96)	8.40^{a} (68.00)	22.60^{b} (34.52)

Pseudomonas spp. isolates (P), *S. marcescens* isolates (S), Vydate "oxamyl'' 24% SL (O), composted cattle manure (CCM); Number between parentheses refer to the increasing percentage; Means in each column followed by the same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range test.

Egg masses Index (EI) for treated materials were 5.0, 4.6, and 4.0 with positive control plants, *Pseudomonas* spp. and *S. marcescens*, respectively (Table 6). The least number in galls ≥ 4 mm was recorded from oxamyl applied at the recommended dose/pot (17.00),followed by pots treated with mixture rhizobacteria plus CCM (19.00) and pots treated with S+P (23.00), while the greatest number (34.80) in galls ≥ 4 mm was obtained with positive control. There were significant differences ($P \leq 0.05$) in J2 population density in pot soils between various treatments of rhizobacteria and CCM in comparison with chemical nematicide, oxamyl. Treatment with a mixture of S + P + CCM recorded the least number of J2 of *M. incognita* populations (9.33/100 cc (g) soil) with a percent reduction 86.07 while, pots treated with the recommended dose of oxamyl produced 17.33 in J2 of *M. incognita* populations with percent reduction 77.78 followed by a mixture of S. + P. (32.00) with percent reduction of 52.23) as compared to 78.0 in the control.

The reproduction factor (RF) of *M. incognita* decreased significantly in pots treated with S + P + CCM followed by pots treated with the recommended dose of oxamyl, while treatments of *Pseudomonas* spp. and *S. marcescens* caused the least decrease as compared with positive control treatment with 0.139, 0.259, 0.769, 0.739 and 1.170 in the abovementioned treatments. Generally, the mixture of treated bacteria and composted cattle manure surpassed rhizobacteria species alone in the reduction of gall formation, egg masses, and reproduction of *M. incognita*.

Table 6 Root-knot nematodes parameters related to pathogenesis in tomato roots and population in the soil after treatment with rhizobacteria and various mixtures in comparison with CCM and oxamyl nematicide in the greenhouse tests.

Treatment	Root parameters					Soil parameters and reproduction factor	
	Number of galls (%)	Root Gall Index	Number of egg-masses (%)	Egg mass Index	No. gall ≥ 4 mm	IJs/100g soil (%)	RF (P_F/P_I)
Healthy plants	0.00^{d}	0	0.00^{c}	0	0.00^{d}	0.00^{g}	0.0
M. incognita	67.00^{a}	4.0	108.00^{a}	5.0	34.80^{a}	78.0^{a}	1.170
***M. incognita* +P**	53.66^{b} (19.91)	4.0	92.66^{ab} (14.20)	4.0	27.40^{b}	51.33^{c} (34.19)	0.769
***M. incognita* +S**	52.33^{b} (21.89)	4.0	83.66 ^{ab} (45.06)	4.6	27.20^{b}	49.33^{c} (36.75)	0.739
***M. incognita* +S + P**	42.33^{bc} (32.44)	4.0	59.33^{c} (39.66)	4.0	23.00^{b}	32.00^{d} (52.23)	0.480
***M. incognita* + O**	27.66^{d} (58.71)	3.3	31.66^{d} (70.68)	4.0	17.00^{c}	17.33^{e} (77.78)	0.259
***M. incognita* + CCM**	47.00^{b} (24.99)	4.0	51.67^{c} (47.45)	4.0	26.20^{b}	36.00^{d} (46.26)	0.390
***M. incognita* + S + P + CCM**	31.00^{d} (50.52)	3.3	32.66^{d} (66.78)	4.0	19.00^{c}	9.33^{ef} (86.07)	0.139

Pseudomonas spp. isolates (P), *S. marcescens* isolates (S), Vydate "oxamyl'' 24% SL (O), Cattle manure (CCM); Number between parentheses refer to the reduction percentage; Means in each column followed by the same letter(s) are not significantly different at 5% level of probability according to Duncan's multiple range test.

In all the previous studies that examined the relationship between application PGPB and plants showed a consensus on increasing plant growth parameters and yield. Perhaps this increase is due to several reasons, including the availability elements necessary for plant growth clutched on soil particles by siderophores and chelators secrete by PGPB in the rhizosphere (Chun *et al.*, 2020; Khatamidoost *et al.*, 2015). Besides the ability to dissolve phosphorous as well as the secretion of plant hormone (indole acetic acid) that lead to the superiority of inoculated plants (Khan et al., 2016; Mohamed *et al.*, 2018). Raising plant resistance after inoculation with PGPB causing root colonization.

PGPB habitat rhizosphere is an unreal threat because of entering bacteria to plant through root wounds resulted from continuous root growth. Plant take a quick response toward the xenobiotic enhancing whether structural or bio/chemical defenses to stop the progress of the bacteria, so the bacteria are considered elicitors causing induced systemic resistance (ISR) (Abd-Elgawad and Kabeil, 2012; Kella et al., 2017). Elicitor-induced reactive oxygen species and related scavenging enzymes for stimulating plant defense reactions in a moderately resistant tomato (Nikoo *et al.*, 2014). As well as, increased catalase, chitinase, peroxidase, peroxidase, phenylalanine ammonia-lyase, and polyphenol oxidase significantly in inoculated plants PGPB, besides increasing phenol content (Anita and Samiyappan, 2012; Devapriyanga *et al.*, 2011; Norabadi *et al.*, 2014). Increasing enzymes related to plant defense were not specific to host plant or PGPB strain or isolates.

Plant obtains nutritional needs and is subjected to fake pathogenesis which induces plant defenses and results in resistant plants. The healthy plant improves the plant growth parameters (Akhtar *et al.*, 2012; Dutta *et al.*, 2020; Haque *et al.*, 2018; Jahanbazian *et al.*, 2015; Ketabchi *et al.*, 2016). Vigor plant growth correlated positively with restriction nematode infection with varying degrees of resistance from one plant to another.

During the formulation of bioagents, the colony-forming units loaded on filler organic substance e.g. floor and molasses (Táborsky, 1992) enable bioagent growth after field application. This nutrition source is temporary so, mixing PGPB with alternative

nutritional sources such as urea. Application of *Serratia* sp. in combination with urea fertilizer had the greatest effect, as compared to other treatments (Ketabchi *et al.,* 2016; Saedi *et al.,* 2017) enhancing the presence of bacteria (Saedi *et al.,* 2017). Also, organic wastes, manure, plant litters, and sawdust (Siddiqui and Akhtar, 2008). The best delivery mechanism through enrichment manure with PGPB for managing nematode disease complex and reaping maximum yield under field condition (Rao *et al.,* 2017). Because of the nematode disease suppression ability of the bacterial isolates were related to its root colonizing ability (Kalaiarasan *et al.,* 2006; Santhi and Sivakumar, 1995). Besides, root colonized with PGPB combined organic wastes increased the root colonization caused by the AM fungus (Akhtar and Panwar, 2013; Siddiqui and Akhtar, 2008). Organic waste inoculated with PGPB guarantees a constant source for carbon and nitrogen required for the growth and multiplication bacteria.

Therefore, it could be concluded that the effectiveness of PGPB depends on the species, strains, and isolates. To ensure its efficiency after application, mixtures of bacteria (species and/or isolates) must be used. The mechanism for applying bacteria must ensure the presence of a permanent source of nutrition, such as organic waste or compost inoculated with bacteria and distributed in-furrow then transplanted in this inoculated organic matter as a protectant treatment.

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ARABIC SUMMARY

تعزيز كفاءة تطبيق عزلات بكتيريا *Pseudomonas* spp. و*Serratia marcescens* ضد نيماتودا تعقد الجذور *Meloidogyne incognita*

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من أجل الحفاظ على المصادر الطبيعية وضمان استدامتها، أصبح البحث عن البدائل الطبيعة والآمنة للسيطرة على الآفات التي تهاجم المحاصيل أمرا ملحا، ولهذا تهدف الدراسة لتقييم جدوى استخدام عزلات من البكتيريا المشجعة لنمو جذور النبات (PGPB) كعوامل مكافحة حيوية لمكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* ومقارنة تأثيرها مع طرق المكافحة التقليدية معمليا وتحت ظروف الصوبة.

قدرت نسبة تثبيط فقس كتل البيض في المعاملات التي استخدم فيها عزلات بكتيريا *Pseudomonas fluorescens /putida* 64.51 % ومع عزلات بكتيريا *Serratia marcescens* بـــ 39.34 %. بينما أحدث تأثيرها كمبيد يرقات نسبة موت مقداره 99.34 % مع عزلات بكتيريا *Pseudomonas* و88.36 % مع بكتيريا *S. marcescens*. ولقد أظهرت عزلات PGPB المختبرة تأثيرها كمبيدات بيض ويرقات أقل من مبيد الأوكساميل وأعلى تأثيراً من المستخلص المائي لمخلفات الأبقار والذي تفوق تأثيره كمبيد فعال على الطور اليرقي.

أظهرت نباتات الطماطم في تجربة الصوبة زيادة في كل المقاييس الخاصة بالنمو وبصفه خاصة في المعاملة التي جمعت بين عزلاتPGPB مخلفات الأبقار المتحللة يليها المعاملة التي جمعت عزلات *P. putida/fluorescens* و*S. marcescens* يليها معاملات الأوكساميل، بينما نتج عن المعاملات المطبق فيها مخلفات الماشية المتحللة أقل زيادة. ولقد نتج عن المعاملات التي جمعت PGPB ومخلفات الماشية والأوكساميل أعلى نسب خفض سواء في معدل تكون العقد أو التكاثر (عدد الأطوار اليرقية\100 جرام تربة) نيماتودا تعقد الجذور مقارنة مع مستخلص مخلفات الأبقار المتحللة وبنسب مئوية مقدارها (50.52 & 86.07) و (58.71 & 77.78) و (24.99 & 46.26) على التوالي. ولهذا فإن النتائج المتحصل عليها تؤكد علي جدوى وفاعلية خلط عزلات PGPB مع المخلفات الحيوانية أو مشتقاتها بصورة أفضل من استخدام كل من البكتيريا أو المخلفات الحيوانية منفردة.