

**MANAGEMENT OF ROOT-KNOT NEMATODE, *MELOIDOGYNE INCOGNITA* AND IMPROVE DEFENSIVE ABILITY OF CUCUMBER PLANT USING *SERRATIA MARCESCENS*, SALICYLIC ACID AND FOSTHIAZATE (MELOTHERIN®) THROUGH ADAPTIVE PROCESSES AND ANTIOXIDANT CAPACITY**

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**ABSTRACT:** Plant parasitic nematodes are dangerous pests that infect many agricultural crops including cucumber. A greenhouse experiment was conducted in the experimental farm of Botany and Microbiology Department, Faculty of Science, Al-Azhar University, to evaluate the efficacy of Salicylic acid and Fosthiazate (melotherin®) as abiotic elicitors and *Serratia marcescens* as biotic elicitor against root-knot nematode, *Meloidogyne incognita* infecting cucumber plants. The highest mortality percentages of *M. incognita* 2<sup>nd</sup> juveniles *in vitro* 96 hours after applying tested materials were recorded with Fosthiazate (melotherin®) followed by salicylic acid and *Serratia marcescens* by 74.2%, 27.87% and 13.19 % respectively. Under greenhouse conditions, similar results were obtained, where Fosthiazate (melotherin®) was the most potent treatment in reducing nematode galls, egg masses, females, and developmental stages in infected cucumber roots by 86.15%, 93.89%, 67.81% and 73.22% respectively as well as decreased 2<sup>nd</sup> juveniles in the soil by 79.48%, followed by Salicylic acid and *Serratia marcescens* respectively. Regarding to plant morphological and biochemical parameters, application of *Serratia marcescens* followed by salicylic acid showed significant improvements in cucumber vegetative growth and yield as well as photosynthetic pigments and antioxidant enzymes. It could be suggested that application of Fosthiazate (melotherin®), *Serratia marcescens* and Salicylic acid could be commercially used as safe, effective and cheap nematicides against root-knot nematodes.

**Key words:** Biological control, abiotic elicitors, biotic elicitor, antioxidant enzymes

## INTRODUCTION

Cucumber is one of the most important moist vegetables that frequently consumed either fresh or pickled especially in the summer, where it contains a large amount of water and a low percentage of calories (Swamy, 2017). Cucumber has many benefits, including body moisturizing, acidity preventing, blood sugar lowering, and blood vessels health maintaining. Cucumber is also used in manufacture of many cosmetics (Hossain, *et al.*, 2020, Ugwu and Suru 2021).

Plant parasitic nematodes are living organisms found in soil and water, which have the ability to attack most agricultural crops causing serious losses in their quantity and quality, and in some cases, it may destroy the

entire crop (Attia, *et al.*, 2021, Pulavarty, *et al.*, 2021). Nematodes also may facilitate spread of other diseases and raise the cost of agricultural operations and resistance (Reddy 2013).

Cucumber is susceptible to several harmful nematodes that live in the soil especially sandy and light soils (Valenzuela, *et al.*, 1995). One of the most dangerous nematodes that infect cucumbers in greenhouses is the root-knot nematode, *Meloidogyne* sp. which infect roots leading to formation of tumors and knots, hence, size of infected root dramatically increases (Asaturova, *et al.*, 2022). The infected roots may branch above the tumor area, causing plant dwarfing, leaf yellowish, leaf drooping, and in case of severe infections the seedlings may be totally die (Ali and Zewain, 2019, Ismail, 2022).

Chemical control can severely reduce nematode population densities in the soil within a short period, providing a protection for sensitive young seedlings (Barker and Olthof 1976, Ghorbani, *et al.*, 2008). On the other hand, chemical nematicides have many limitations due to their harmful impacts on environment, human and animal health, furthermore they cost high prices (Czaja, *et al.*, 2015, Tudi, *et al.*, 2021). Therefore, application of some biotic and abiotic alternatives represents one of the most important approaches in controlling nematode infections, because they are safe on environment and human health as well as cost low prices (Abd-Elgawad, 2020, 2021). The use of microorganisms as anti-plant pathogens has proven to be highly effective in many studies (Sharaf, *et al.*, 2016, Attia, *et al.*, 2022, Attia, *et al.*, 2022, Tariq, *et al.*, 2022). Applied studies proved the efficiency of using biotic and abiotic inducers in reducing the severity of nematode infection and improving plant immunity (Waller, 1999, Sharaf, *et al.*, 2016). There is a lot of research conducted, especially in recent years, indicating the potential role of some bacteria in nematode control (Nielsen, 2012). *Serratia marcescens* have a wide range of suppressive activities on different types of nematodes (Mohamed, *et al.*, 2009, Mokbel and Alharbi 2014, Kella, *et al.*, 2017), including free-living, predatory nematodes as well as nematodes parasitic on animals and plants (Li, *et al.*, 2015).

The study carried out by (Sharaf, *et al.*, 2016) applied a strain of *Serratia marcescens* against the root-knot nematode *Meloidogyne incognita* infecting tomato plants showed 86% and 88% reduction in root masses and egg masses, respectively. Also, a remarkable improvement was observed in the vegetative and physiological growth characteristics after treating infected plants.

Salicylic acid (SA) is the first natural plant derivative used in the manufacture of aspirin and plays a signaling molecule in inducing systemic acquired resistance to many parasitic infections (Tripathi, *et al.*, 2019, Bernardino, *et al.*, 2020). Salicylic acid is an expressive material for acquired systemic resistance and is responsible for a series of plant physiological processes and

is produced during the phenomenon of systemic resistance in large quantities (Kiraly, *et al.*, 1991, Blainski, *et al.*, 2018).

Fosthiazate is a phosphonic ester, organic phosphonate and organothiophosphate insecticide (Shalaby, *et al.*, 2021). It has a role as an EC 3.1.1.7 (acetylcholinesterase) inhibitor, an agrochemical and a nematicide. Fosthiazate is a member of the organophosphate pesticides or nematicides and used to control nematode species on vegetables (Li, *et al.*, 2020).

The aim of the present study was to evaluate the efficiency of the biostimulant *Serratia marcescens* and salicylic acid as well as Fosthiazate (melotherin®) in eliminating root-knot nematodes that destroy economic crops, as well as to prove the effectiveness of the treatments on the recovery of infected plants and the improvement of systemic resistance.

## MATERIALS AND METHODS

### 1. Plant materials

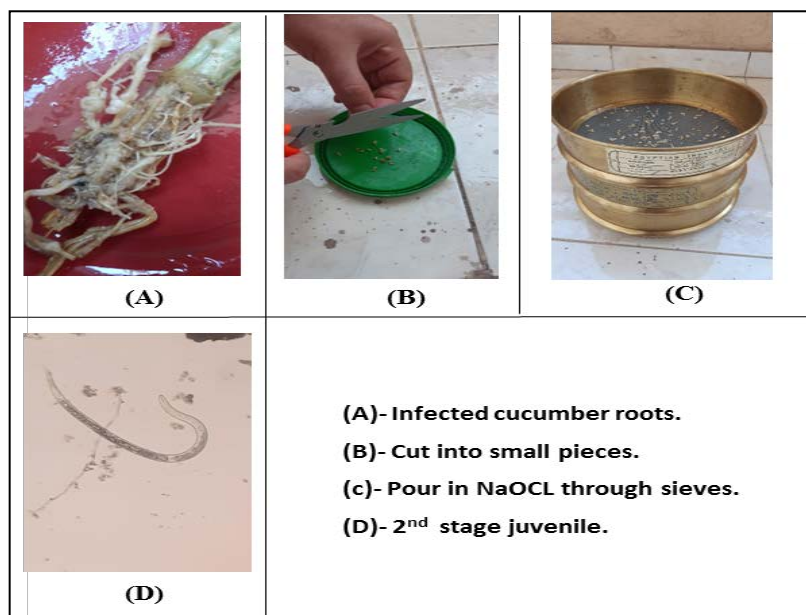
For the current work, four weeks age cucumber seedlings were obtained from Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt.

### 2- Root-knot nematodes

Nematode specimens used in this research was *Meloidogyne incognita* was reared in a greenhouse on cucumber plants. Eggs of *M. incognita* were extracted from roots in 0.5 % sodium hypochlorite (Nitao, *et al.*, 1999) and caught on a 25 µm sieve. Second stage juveniles (J2) were hatched from these eggs on Baermann funnels and only (J2) less than 2 days old were used for experimentation (Fig. 1).

### 3- Green house experiment

Twenty-five seedlings of cucumber plants were planted in plastic bags, and were divided into five groups, each group containing five seedlings. After seven days of cultivation, four groups were inoculated with nematodes, three of them were treated with the elicitors (*Serratia marcescens*, Fosthiazate (melotherin®) and Salicylic acid) and the fourth group was left without any treatment.



**Fig. (1): Extraction of 2<sup>nd</sup> stage juveniles of *M. incognita* nematode.**

The fifth group, was left without nematode injection, as well as without any treatment as a check group. Finally, the groups were assigned as follows:

- 1- *Serratia marcescens* 4 ml /L.
- 2- Fosthiazate (melotherin®) 0.5 ml /L.
- 3- Salicylic acid 4 ml/L.
- 4 - Nematodes only control.
- 5 – Check.

Each treatment was replicated five times and were arranged in a complete randomized block design. Pots were kept in the greenhouse at 25 ± 5°C receiving water and ordinary nutrient solution as required. plants were harvested 60 days after nematode inoculation.

#### 4- Source and application methods of inducers

*Serratia marcescens* was kindly obtained from biofertilizer production unit, Soil, Water and Environment Research Institute (Agriculture Research Center ARC Giza Egypt). The bacterial concentration of the obtained suspension was justified at 10<sup>9</sup> (CFU/ml). Fosthiazate (melotherin®) 90 % EC (O-Ethyl S-(1-methylpropyl) (2-oxo-3 thiazolidinyl)

phosphonothioate) obtained by AL-SALAM International for Development & Agriculture Investment, Egypt from AGROBEST GROUP-Turkey, and finally salicylic acid 100 mM (sigma company).

#### 5- Determination of nematode and plant parameters

##### 5- 1- Nematode parameters

Plants were carefully uprooted, washed, then weights and lengths of shoots and roots for each treatment were determined. Root knot nematode galls and egg masses were counted per one-gram roots stained in phloxine B. Reduction percentages of root knot nematode galls and egg mass numbers were counted in comparison with nematode only. Also, roots were stained in lactic acid fuchsin (Ghareeb, *et al.*, 2019). The second juvenile stage (J2s) extracted from 250 gm soil using Baermann funnel technique. Galls, egg mass, developmental stages were counted under stereoscope, while J2s were counted using counting slide using light microscope. Reduction percentages of all nematode parameters in all treatments were compared with plants infected with nematode only.

## 5-2- Plant parameters

### Plant growth and yield characters

Heights of shoots, root length (cm) and yield parameters for plants of each treatment and control were determined.

### Physiological responses

Determination of photosynthetic pigments from leaves was carried out according (Pareek, *et al.*, 2017) the technique of Catalase activity was assayed according to (Aebi, 1984, Attia, *et al.*, 2021). Peroxidase activity was assayed according to (Bergmeyer, 1974). The activity of polyphenol oxidase enzyme was determined according to the method adopted by (Matta and Dimond, 1963).

## 6- Statistical analyses

Experimental data were subjected to one-way analysis of variance (ANOVA) and the differences between means were separated using the (L.S.D) at 5% level of probability using Co-state software.

## Results

### Nematode parameters

#### Mortality% of the second stage juvenile *in vitro*

The obtained results in Table (1) and Fig. (2) appeared that, all tested elicitors increased the nematode juvenile mortality. These increases were constant with increasing period of application. Melotherin® was the most effective treatment in raising nematode juvenile mortality followed by salicylic acid and *S.marcescens* respectively as follows:

Melotherin® recorded (55.18%, 56.52%, 66.66% and 74.2%) in nematode mortality after 24, 48, 72 and 96 hours, respectively. While salicylic acid recorded (18.55%, 23.28%, 25.52% and 27.87%) in nematode mortality after 24, 48, 72 and 96 hours, respectively.

Finally, *S.marcescens* recorded (8.51%, 10.35%, 13.7% and 15.26%) in nematode mortality after 24, 48, 72 and 96 hours, respectively.

### Nematode galls, egg masses, females, developmental stages, and 2<sup>nd</sup> juvenile reduction *in vivo*

Results in Table (2) and Fig. (3) appeared that all elicitors greatly reduced the number of nematode galls, females, egg masses and development stages in infected cucumber roots as well as suppressed the nematode juveniles in the soil especially melotherin® and salicylic acid. Use of melotherin® recorded (86.15%, 93.89%, 67.81%, 73.22% and 79.48%) reduction in numbers of galls, egg masses, females, development stages, and 2<sup>nd</sup> juveniles, respectively. While, application of salicylic acid recorded (44.72%, 44.02%, 43.12%, 51.48% and 34.56%) reduction in numbers of galls, egg masses, females, development stages, and 2<sup>nd</sup> juveniles, respectively. Whereas, *S.marcescens* recorded (29.96%, 27.99%, 22.5%, 37.75% and 29.11%) reduction in numbers of galls, egg masses, females, development stages, and 2<sup>nd</sup> juveniles, respectively.

### Plant parameters

#### Plant growth and yield parameters

Data achieved in Table (3) and Fig. (4) indicated that, growth and yield parameters in nematode-infected cucumber plants were significantly decreased than that of non-infected healthy ones. Regarding to the effect of tested elicitors, infected cucumber plants treated with *S.marcescens* recorded the most potent improvement in growth and yield parameters recording (111 cm, 32.37 cm, 22.86 and 12) for shoot height, root length, leaf numbers and fruit numbers, respectively. Salicylic acid achieved the second order in enhancing growth and yield parameters recording (100 cm, 30 cm, 20 and 10) for shoot height, root length, leaf numbers and fruit numbers, respectively. Whereas, melotherin® recorded the least effective treatment in growth and yield parameters recording (74.68 cm, 26 cm, 19.68 and 9.56) for shoot height, root length, leaf numbers and fruit numbers, respectively.

**Table (1): Effect of tested elicitors and period of application on *Meloidogyne incognita* juveniles mortality *in vitro*.**

Treatment	Incubation periods															
	24 h				48 h				72 h				96 h			
	Dead juveniles	Alive juveniles	Mortality (%)	Mortality (%)	Dead juveniles	Alive juveniles	Mortality (%)	Mortality (%)	Dead juveniles	Alive juveniles	Mortality (%)	Mortality (%)	Dead juveniles	Alive juveniles	Mortality (%)	
<i>S. marcescens</i>	1.66	17.66	8.51±1.48 c	8.51±1.48 c	2.0	17.33	10.35±0.17 c	10.35±0.17 c	2.66	16.66	13.7±0.68 c	13.7±0.68 c	3.0	16.66	15.26±0.74 c	
Melotherin®	10.66	8.66	55.18±0.18 a	55.18±0.18 a	11.66	9.0	56.52±1.01 a	56.52±1.01 a	12.66	6.33	66.66±0.75 a	66.66±0.75 a	15.33	5.33	74.2±0.77 a	
Salicylic acid	3.33	14.66	18.55±1.91 b	18.55±1.91 b	4.66	15.3	23.28±1.16 b	23.28±1.16 b	5.0	14.66	25.52±0.77 b	25.52±0.77 b	5.66	14.66	27.87±0.74 b	
Control	1.33	18.33	6.75±1.62 c	6.75±1.62 c	1.66	17.33	8.69±1.57 c	8.69±1.57 c	2.33	17.0	12.01±0.05 c	12.01±0.05 c	2.66	17.33	13.19±0.66 c	
LSD (5%)			4.76	4.76			3.61	3.61							5.37	

Means in each column followed with the same letter are not significantly different at 5 % level.

**Table (2): Impact of tested elicitors on reducing nematode parameters *in vivo*.**

Treatment	Nematode parameters <i>in vivo</i>																			
	Galls in /1 g root				Egg masses / 1 g root				Females/1 g root				Developmental stages /1 g root				2 <sup>nd</sup> stage juveniles /250 g soil			
	Average no.	Reduction %	RGI	RGI	Average no.	Reduction %	EI	EI	Average no.	Reduction %	Average no.	Reduction %	Average no.	Reduction %	Average no.	Reduction %	Average no.	Reduction %		
<i>S. marcescens</i>	102.83 b	29.96	5	5	47.16 b	27.99	4	4	82.63 b	22.5	4	37.75	90.66 b	37.75	563.33 b	29.11	563.33 b	29.11		
Melotherin®	20.32 d	86.15	3	3	4 d	93.89	2	2	34.36 d	67.81	2	73.22	39 d	73.22	163 c	79.48	163 c	79.48		
Salicylic acid	81.16 c	44.72	4	4	36.61 c	44.02	4	4	60.56 c	43.12	4	51.48	70.66 c	51.48	520 b	34.56	520 b	34.56		
Control	146.83 a	-	5	5	65.5 a	-	4	4	106.36 a	-	4	-	145.66 a	-	794.66 a	-	794.66 a	-		
LSD (5%)	15.16				5.42				10.99				14.88					64.82		

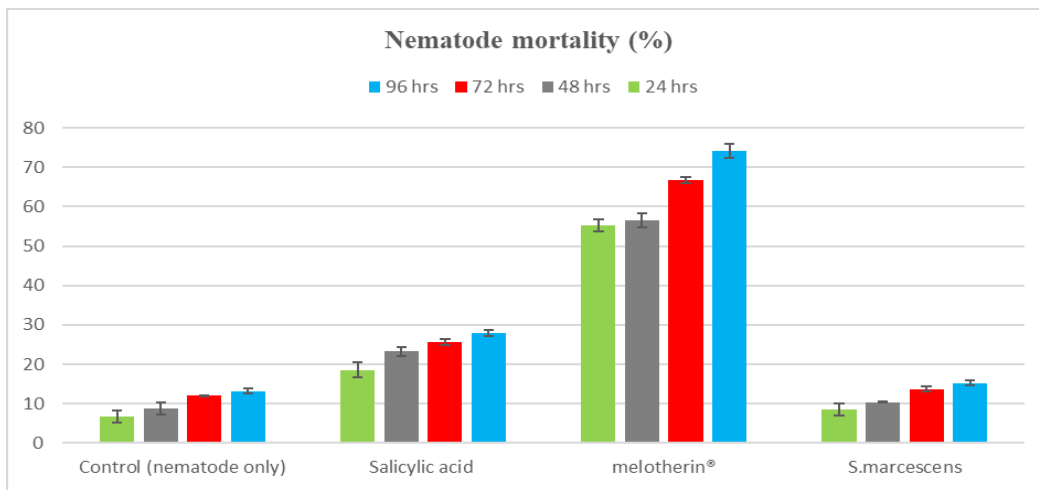
Means in each column followed with the same letter are not significantly different at 5 % level.

\*Root gall index (RGI) or egg-mass index (EI) was determined according to the scale of Taylor & Sasser (1978) as follows: 0= no galls or egg mass, 1= 1-2 galls or egg mass, 2= 3-10 galls or egg mass, 3= 11- 30 galls or egg mass, 4= 31-100 galls or egg mass and 5= more than 100 galls or egg mass.

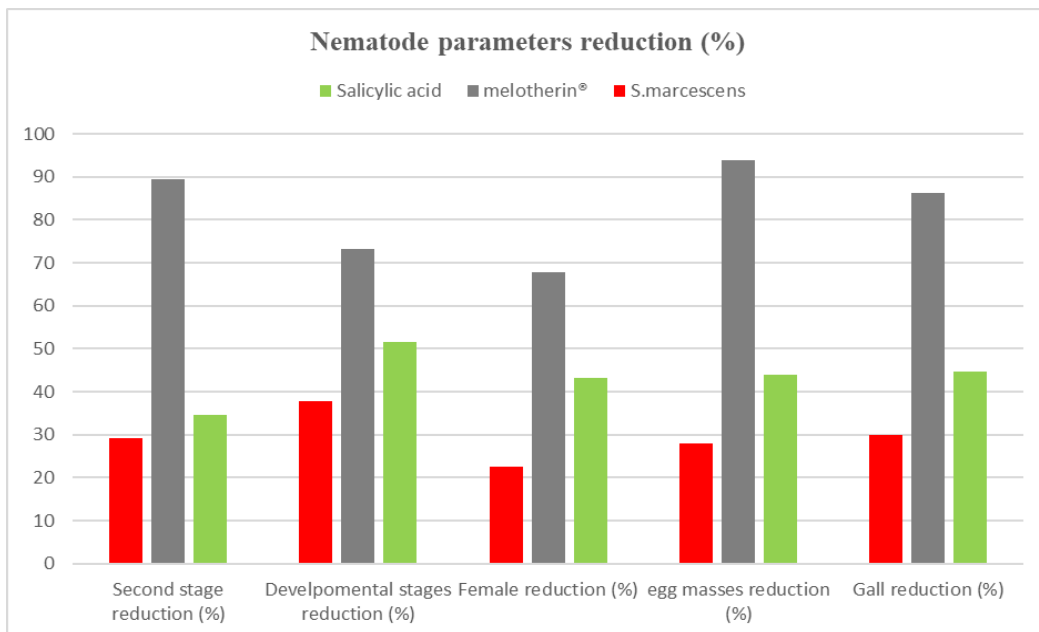
### Physiological responses

Data generated in Tables (4, 5) and Figs. (5, 6) show that, nematode infection caused significant decreases in photosynthetic pigments including (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids). Regarding to the effect of tested elicitors, it was found that, all elicitors alleviated photosynthetic pigments due to nematode infection but did not reach the values of nematode free (control). Whereas, *S.marcescens* recorded the highest alleviating values of photosynthetic pigments, followed by

Salicylic acid and melotherin®, respectively. Concerning to antioxidant enzymes (peroxidase, catalase, polyphenol oxidase and superoxidedismutase) nematode infection caused significant increases in their activities comparing to control ones. Regarding to the effect of tested elicitors, it was found that, all tested elicitors achieved high values of antioxidant enzymes activity more than that of nematode only. Whereas, *S.marcescens* recorded the highest values of antioxidant enzymes activity, followed by Salicylic acid and melotherin®, respectively.



**Fig (2): Impact of tested elicitors and period of application on second stage mortality of juveniles *in vitro*.**

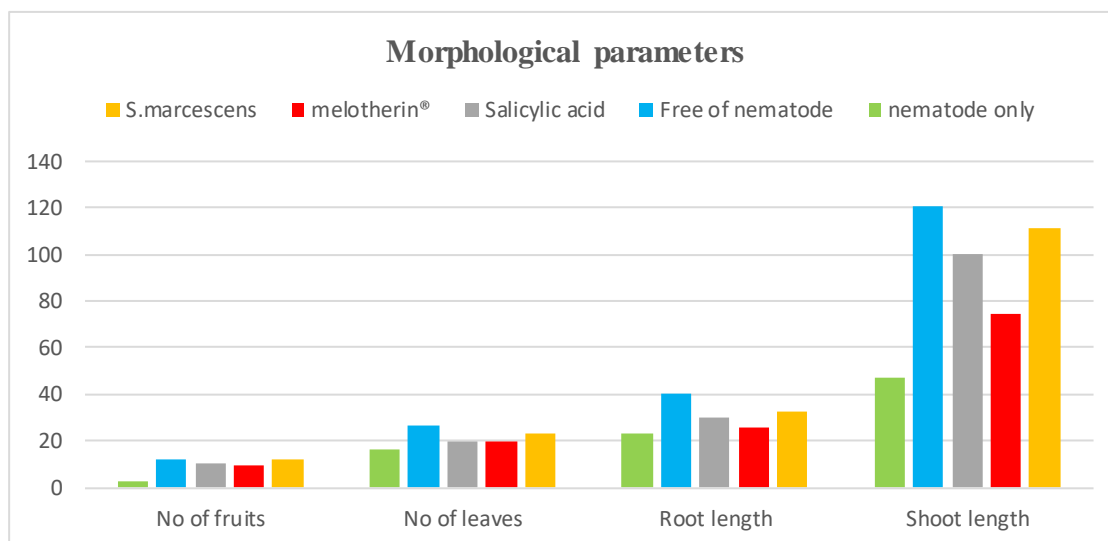


**Fig (3): Impact of tested elicitors on reducing nematode parameters *in vivo*.**

**Table (3): Effect of the tested elicitors on growth and yield parameters of cucumber plants infected with *Meloidogyne incognita*.**

Treatment	Shoot length average (cm)	Root length average (cm)	No. of leaves/plant	No. of fruits/plant
<i>S.marcescens</i>	111.0 b	32.37 b	22.86 b	12.0 a
Melotherin®	74.68 d	26.0 c	19.68 c	9.56 b
Salicylic acid	100.0 b	30.0 b	20.0 b	10.0 a
Nematode only	47.23 e	23.0 d	16.43 d	2.34 c
Free of nematode	120.61 a	40.35 a	26.69 a	12.35 a
LSD (5%)	3.67	2.81	1.75	1.24

Means in each column followed with the same letter are not significantly different at 5 % level.

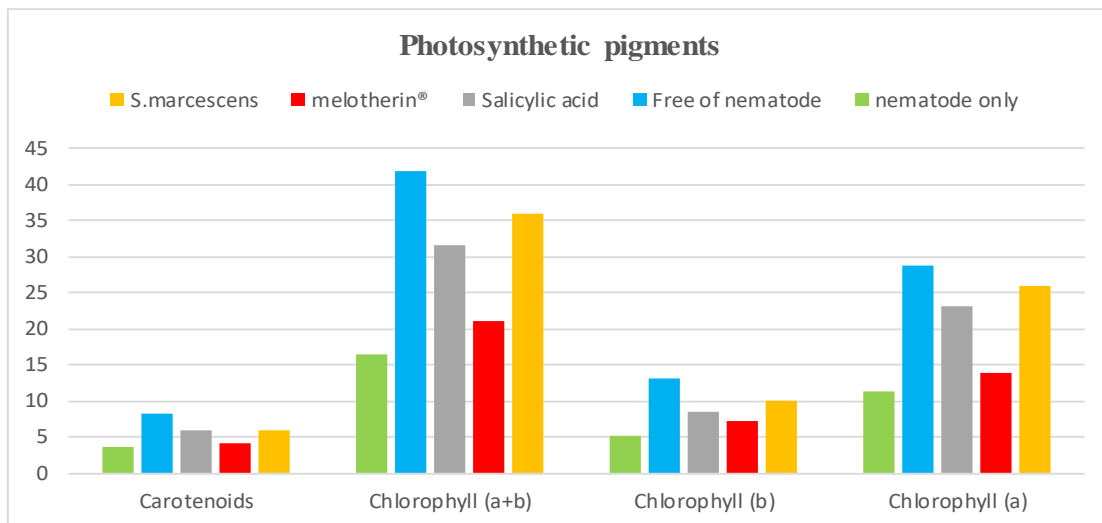


**Fig (4): Effect of the tested elicitors on growth and yield parameters of cucumber plants infected with *Meloidogyne incognita*.**

**Table (4): Effect of the tested elicitors on photosynthetic pigments of cucumber plants infected with *Meloidogyne incognita*.**

Treatment	Chlorophyll (a)	Chlorophyll (b)	Chlorophyll (a+b)	Carotenoids
<i>S.marcescens</i>	26.0 b	10.0 b	36.0 b	6.03 b
Melotherin®	13.9 c	7.16 c	21.06 d	4.16 d
Salicylic acid	23.0 b	8.05 b	31.5 b	6.03 b
Nematode only	11.36 d	5.03 d	16.4 e	3.6 d
Free of nematode	28.66 a	13.06 a	41.73 a	8.1 a
LSD (5%)	1.69	0.98	1.61	0.64

Means in each column followed with the same letter are not significantly different at 5 % level.

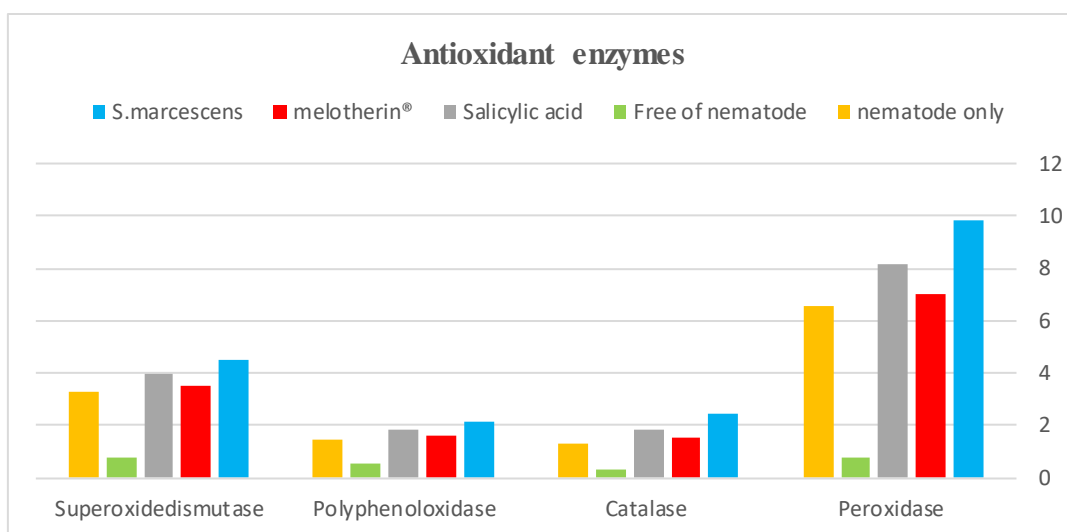


**Fig (5):** Effect of the tested elicitors on photosynthetic pigments of cucumber plants infected with *Meloidogyne incognita*.

**Table (5):** Effect of the tested elicitors on antioxidant enzymes of cucumber plants infected with *Meloidogyne incognita*.

Treatment	Peroxidase	Catalase	Polyphenoloxidase	Superoxidedismutase
<i>S.marcescens</i>	9.86 a	2.43 a	2.13 a	4.5 a
Melotherin®	7.0 c	1.5 c	1.6 c	3.5 c
Salicylic acid	8.13 b	1.83 b	1.86 b	3.96 b
Nematode only	6.56 c	1.27 c	1.43 d	3.25 d
Free of nematode	0.74 d	0.31 d	0.51 e	0.75 e
LSD (5%)	0.68	0.29	0.14	0.22

Means in each column followed with the same letter are not significantly different at 5 % level.



**Fig (6):** Effect of the tested elicitors on antioxidant enzymes of cucumber plants infected with *Meloidogyne incognita*.



## Discussion

The problem of nematode infection disturbs many farmers and attracts the attention of researchers trying to control it. Recently, the trend of scientists in using bio-inducers as nematode pesticides was appeared. In this study, the selection of *S.marcescens* was based previously proven in many previous studies through the ability of bacteria *S.marcescens* to produce HCN, IAA and siderophores. HCN has been established as a biological resistance agent, due to its severe injuriousness to pathogens (Attia, *et al.*, 2020). In this regard, many findings show the significance of *S.marcescens* which produces HCN in resisting pathogens as safe and ecofriendly alternatives (Samada and Tambunan, 2020, Elnahal, *et al.*, 2022). HCN is a compound that participates in many biological processes and has antifungal activity, in addition to its effective role in inducing plant resistance against pathogens (Meena, *et al.*, 2020, Costa, *et al.*, 2022, Dimkić, *et al.*, 2022). HCN plays an effective role in inhibiting the respiration process in the cytochromes of pathogen cells (Schippers, *et al.*, 1990, Ramette, *et al.*, 2003). IAA contributes as the main processes of development and division cells, especially the elongation shoot and root, which leads to the improvement of plants (Bhalerao, *et al.*, 2002, Li, *et al.*, 2018, Sehrawat, *et al.*, 2022). Many studies proved that the organisms that stimulate plant growth are characterized by their ability to produce indole, which explain the role of these organisms in improving plant growth (McSteen, 2010, Jasim, *et al.*, 2014, Singh, 2014, Scagliola, *et al.*, 2016, Kapadia, *et al.*, 2022). Iron plays a direct role in the growth and development of plant cells, some microorganisms synthesize low-molecular-weight iron-chelating molecules called Siderophores (Duca, *et al.*, 2014, Swarnalatha, *et al.*, 2022). Recent studies confirm the need of plants for phosphorous for improving the plant resistance against plant biotic stresses (Enebe and Babalola 2018, Dowarah, *et al.*, 2021, Abd Alhakim, *et al.*, 2022). The obtained results in the current study, appeared that, all tested treatments increased nematode juvenile mortality. These increases were constant with

increasing period of application. While melotherin® was the most effective treatment in raising nematode juvenile mortality, recorded (55.18%, 56.52%, 66.66% and 74.2%) in nematode mortality after 24, 48, 72 and 96 hours, respectively. While salicylic acid recorded (18.55%, 23.28%, 25.52% and 27.87%) in nematode mortality after 24, 48, 72 and 96 hours, respectively. Finally *S.marcescens* recorded (8.51%, 10.35%, 13.7% and 15.26%) in nematode mortality after 24, 48, 72 and 96 hours, respectively. The current results are consistent with many studies (Woods, *et al.*, 1999, Huang, *et al.*, 2016, Wu, *et al.*, 2019) that have proven the effectiveness of the pesticide, as it is described as a systemic phosphorous nematicide that affects the central nervous system of nematodes, with the distinction of eliminating all stages of nematodes. Nematodes represent a danger to the plant by affecting growth, as the infection leads to a delay in vegetative growth and the transfer of nutrients from the soil through the root system to the rest of the plant parts. In this study, the treatments were proven to be effective in recovering and improving vegetative growth characteristics. Data achieved in the current study indicated that, growth and yield parameters in nematode-infected cucumber plants were significantly decreased than that of non-infected healthy ones. These results agree with (Vega, *et al.*, 2006, Sharaf, *et al.*, 2016). Regarding to the effect of tested elicitors, it found that infected cucumber plants treated with *S.marcescens* gave the most potent improvement in growth and yield parameters recording (111 cm, 32.37 cm, 22.86 and 12) for shoot height, root length, leaf number and fruit number, respectively. Salicylic acid achieved the second order in enhancing growth and yield parameters recording (100 cm, 30 cm, 20 and 10) for shoot height, root length, leaf number and fruit number, respectively. Whereas, melotherin® recorded the least effective treatment regarding growth and yield parameters recording (74.68 cm, 26 cm, 19.68 and 9.56) for shoot height, root length, leaf number and fruit number, respectively. As shown in the results of the current study, the enormous ability of salicylic acid to improve growth characteristics, and the

results are supported by many studies (Molinari and Baser, 2010, Javid, *et al.*, 2011, Martínez-Medina, *et al.*, 2017). Salicylic acid provides plant protection against various types of stress, and it also plays an important role in regulating ion uptake, hormonal balance and stomata movement (Mukherjee, *et al.*, 2012, Tripathi, *et al.*, 2019).

The obtained data in the present study, showed that, nematode infection caused significant decreases in photosynthetic pigments including (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) and these results are consistent with many previous studies (Korayem, *et al.*, 2012, Bali, *et al.*, 2018, Labudda, *et al.*, 2018). This lack of photosynthetic pigments may be due to the nematode caused oxidative bursts in the cells and the failure of the plant to carry out the photosynthetic process (Ahmed, *et al.*, 2009). Regarding to the effect of tested elicitors in the present study, it was found that, *S.marcescens* recorded the highest alleviating values of photosynthetic pigments followed by Salicylic acid and melotherin®, respectively. This means the plant's response and its overcoming of nematode stress, which confirms the efficiency of biological treatment (*S.marcescens*) or Salicylic acid and melotherin® in eliminating the nematode threat to the plant. The strongest evidence of resistance to injury and coping with stress is the high activity of antioxidant enzymes (El-Batal, *et al.*, 2019, Attia, *et al.*, 2021). Regarding the effect of tested elicitors, it was found that, all tested elicitors achieved high values of antioxidant enzymes activity more than that of nematode only. Whereas, *S.marcescens* recorded the highest values of antioxidant enzymes activity, followed by Salicylic acid and melotherin®, respectively. High activity of antioxidant enzymes reduces free radicals and improves plant immunity (Shah, *et al.*, 2001, Xie, *et al.*, 2008).

## Conclusion

The present study aimed to evaluate Salicylic acid and Fosthiazate (melotherin®) as abiotic elicitors and *Serratia marcescens* as biotic

elicitor against root-knot nematode, *Meloidogyne incognita*. The *in vitro* results confirmed the ability of Fosthiazate (melotherin®) to reduce nematode numbers, as it was shown that the mortality of *M.incognita* 2<sup>nd</sup> juvenile was increased generally with increasing application period. Moreover, *in vivo* results confirmed that application of Fosthiazate (melotherin®), *Serratia marcescens*, and SA on nematode infested cucumber plants significantly decreased number of galls, egg masses and females. Finally, the use of Salicylic acid and Fosthiazate (melotherin®) as abiotic elicitors and *Serratia marcescens* as biotic elicitor are promising in the field of safe and clean agriculture.

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## التحكم فى نيماتودا تعقد الجذر (*Meloidogyne incognita*) وتحسين القدرة الدفاعية لنبات الخيار باستخدام *Serratia marcescens* وحمض الساليسيليك والفوسيازيت (**melotherin®**) من خلال العمليات التكيفية والقدرة المضادة للأكسدة

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### الملخص العربى

تعتبر النيماتودا الممرضة للنبات من أخطر الآفات التي تصيب المحاصيل الزراعية بما في ذلك محصول الخيار. أجريت تجربة علمية بصوبة قسم النبات والميكروبيولوجي بكلية العلوم جامعة الأزهر لتقييم كفاءة كل من حمض السلسليك والمبيد النيماتودي التجاري فوسيازيت (**melotherin®**) **Fosthiazate** كمستحضات غير حيوية وأيضاً البكتيريا *Serratia marcescens* كمستحث حيوي في مكافحة نيماتودا تعقد الجذور *Meloidogyne incognita* اثناء إصابتها لنبات الخيار تحت ظروف الصوبة البلاستيكية.

أشارت النتائج إلى أن تطبيق المبيد النيماتودي التجاري فوسيازيت (**melotherin®**) على يرقات الطور الثاني لنيماتودا تعقد الجذور في المعمل لمدة 96 ساعة أدى إلى أعلى نسبة وفيات لهذه اليرقات ، تلاه حمض السلسليك ثم بكتيريا *Serratia marcescens* بنسبة موت وصلت إلى 74.2%، 27.87%، 13.19% على التوالي.

أما في ظروف الصوبة البلاستيكية فقد تم تحقيق نتائج مماثلة، حيث كان المبيد النيماتودي هو المعاملة الأكثر فاعلية في تقليل أعداد العقد الجذرية وكذلك كتل البيض والإناث والأطوار اليرقية التطورية لنيماتودا تعقد الجذور في جذور نبات الخيار المصابة بنسبة بلغت 86.15%، 93.89%، 67.81%، 73.22% على التوالي. كذلك كان المبيد النيماتودي الأكثر فاعلية في تقليل أعداد الأطوار اليرقية الثانية لنيماتودا تعقد الجذور في التربة بنسبة بلغت 79.48%. تلي المبيد النيماتودي في الفاعلية كل من حمض السلسليك ثم بكتيريا *Serratia marcescens* على التوالي.

أما فيما يتعلق بالقياسات المورفولوجية والكيميائية لنبات الخيار فقد اظهرت النتائج ان استخدام البكتيريا *Serratia marcescens* أدى إلى تحسين الصفات المورفولوجية كطول الساق والجذر وعدد الأوراق والثمار وكذلك صبغات البناء الضوئي والإنزيمات المضادة للأكسدة تحسينا معنوياً. ثم جاء في المرتبة الثانية حمض السلسليك ثم جاء أخيراً المبيد النيماتودي فوسيازيت (**melotherin®**).

ومما سبق يمكن اقتراح استخدام المبيد النيماتودي التجاري فوسيازيت (**melotherin®**) وحمض السلسليك وبكتيريا *Serratia marcescens* كمبيدات آمنة وفعالة ورخيصة الثمن ضد نيماتودا تعقد الجذور *Meloidogyne incognita*.