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BIOLOGICAL CONTROL OF ROOT-KNOT NEMATODE IN TOMATO PLANTS THROUGH YEAST ISOLATES

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A B S T R A C T

In Egypt, root knot nematodes (RKN) were shown to be significant plant parasites with a variety of host plants. Under laboratory and greenhouse circumstances, the current study examines the antagonistic effects of four distinct yeast isolates on *Meloidogyne* spp. egg hatching and larval mortality. In comparison to the control, the results showed that *Pichia guilliermondii* ATCC 9058 had the highest egg hatching inhibition and larval mortality, which were 93 and 100%, respectively, after 168 hours of exposure. *Pichia guilliermondii* ATCC 9058 at 4% of soil weight, one-week pre nematode inoculation achieved the highest reduction in nematode parameters including number of galls, egg masses, females, and number of juveniles (J2s)/250g soil, by 89.6, 90, 88.7 and 90%, respectively., followed by S. cerevisiae at 4% of soil weight, one-week pre nematode inoculation application, with 84.4, 88.3, 88.4 and 85.3%, respectively. Used yeast isolates could therefore be a useful biocontrol tool for plant parasitic nematodes.

Keywords: Biological Control, Root-Knot Nematodes, *Meloidogyne* spp., yeast.

INTRODUCTION

The tomato (*Lycopersicon esculentum* L.) is the vegetable that people eat the most frequently. Given the great variety of agro-ecologies throughout the nation, it has a very high potential for growth, provided that some production bottlenecks are removed. Tomatoes may be produced in a variety of climates in open fields or greenhouses, and they are the most widely consumed vegetable in the world due to their use as a basic component in a wide range of raw and cooked or processed dishes. The best source of vitamins A, B, C, minerals, and carbs, which provide meals good value and flavor, is said to be the tomato's mature fruits. It is regarded as a significant vegetable crop and one of the foundations of the production of agricultural vegetables in Egypt, where it is consumed either fresh or processed. It is currently by far the greatest vegetable crop in Egypt and ranks second globally in terms of importance (after potatoes) (FAO, 2020). With a total planted area of 428182 feddan, it generates around 6751856 million tonnes. This crop is one of the extremely important ones that effectively competes in the international markets, as evidenced by the export quantity of 41.8 thousand tonnes and the export value of 488.6 thousand dollars in 2018 (Elkhishin, 2020).

Different plant pathogens such as: fungi, bacteria, viruses, and phyto-parasitic nematodes affect tomato plantation. According to Abd-El Gawad 2014, estimated annual yield losses in Egypt during 2011-2012 in tomato due to phyto-nematodes by 12% and amounted to 1753.17 million L.E. Among the plant parasitic nematode, root-knot nematodes (RKNs) (*Meloidogyne* spp.), a plant parasitic nematode, were noted as the most problematic species with variable serious loss to tomato yield (Sasser, 1980). *Meloidogyne* species reduced crop productivity globally by roughly 5% yearly (Sasser, 1987). According to Hussain *et al.,* (2011), *Meloidogyne* spp. can result in vegetable losses of up to 80% losses in tropical and subtropical regions. *Meloidogyne* spp. infections on tomato are widespread in Egypt and

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significantly harm crops, particularly in recently reclaimed sandy areas (Mousa,1997; Abd-Elgawad and Aboul-Eid, 2001; Bakr *et al.,* 2011 and 2020; Abdel-Baset,2022). Stunting, root galls, aberrant physiological features, and low yield output are all symptoms of severity-infected plants (Abd-Elgawad, 2021). Different variables, including the number of soil nematodes, host cultivars, soil type, other plant diseases, and local meteorological conditions, may have an impact on losses (Bakr and Ketta, 2018).

Currently, a lot of farmers handle crop diseases with chemical control. The agroecosystem deteriorates as a result of the frequent usage of chemical nematicides. Nematicides, which are used to control plant-parasitic nematodes, are dangerous for humans and animals in addition to being expensive and perhaps harmful to air, water, other species, and applicators (Bakr *et al.,* 2022a). Because chemical nematicides have been taken off the market due to their danger to people, animals, plants, and the environment, most nematologists worldwide have been searching for the development of safe, environmentally friendly alternative approaches for controlling RKNs over the past ten years (Abdel-lateif and Bakr, 2018; Al-Hendy *et al.,*2021) .

The root-knot nematode species *Meloidogyne* spp. have recently been controlled using numerous safety measures. For the control of nematodes, particularly RKNs, microbial agents have been suggested (Jamshidnejad *et al.,* 2013; Bakr *et al.,* 2022b). The use of antagonistic yeasts for the control of several plant diseases has received significant attention (Karajeh, 2013; Youssef and El-Nagdi, 2021; Kowalska *et al.,*2022). In order to encourage plant growth, they may therefore be a good and promising factor (Poorniammal and Prabhu, 2022). According to several studies diverse genus of yeast fungus exhibits a favourable effect against various RKNs in various crops (Mioranza *et al.,* 2020; Osman *et al.,* 2020; El-Nuby, 2021; El-Sagheer *et al.,* 2021; Abokorah and Fathalla, 2022) .

The current study's goal was to assess various yeast isolates' potential against *Meloidogyne* spp. in a lab setting and on tomato under greenhouse settings.

MATERIALS AND METHODS

Stock culture of *Meloidogyne* spp.: Root-knot nematodes *Meloidogyne* spp. were multiplied on tomato plants (*Lycopersicon esculentum* L.) Hybrid Salymia 65010) transplanting and grown in plastic pots (30 cm in diameter) previously filled by sterilized sand-clay mixture soil (2:1, v/v) at greenhouse, Faculty of Agriculture, Menoufia University, Egypt.

Tomato seed source: Tomato Hybrid Salymia 65010 is produced and manufactured by Syngenta Seeds B.V. Company. Exported and distributed in Egypt by Techno green for Agricultural projects Company.

Yeast isolates source: Four yeast isolates were used in this study to evaluate their efficacy against *Meloidogyne* spp. under laboratory and greenhouse conditions on Tomato. The four yeast isolates were obtained from different trusted sources as follow:

Pichia guilliermondii ATCC 9058 and *Candida albicans* ATCC 10231 were obtained from Microbiological Resources Center, Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Egypt. *Saccharromyces cerevisiae* was obtained from Department of Agricultural Botany, Faculty of Agriculture, University of Menoufia, Egypt. *Saccharromyces* spp. (commercial product) was obtained from a specialized yeast shop in Shebin El-kom, Menoufia, Egypt.

Maintenance of yeast isolates: The used different yeast isolates i.e., *Pichia guilliermondii* ATCC 9058, *Candida albicans* ATCC 10231 and *Saccharromyces cervicaea* were maintained on DifoTM YM Agar medium (Yeast Extract 3g+ Malt Extract 3g+ Peptone 5g+ Dextrose 10g and Agar 20g, dissolved in 1 L of distilled water) and incubated at 30°C for 24 hrs (Azeredo *et al.,* 1998).

Yeast isolates inoculum preparation: Yeast cultures were reared in the specific yeast extract malt extract broth medium (YMB). *Saccharromyces* spp. (commercial) was directly used.

Extraction of *Meloidogyne* spp. Eggs: Three months old heavy galled tomato plant roots infected with *Meloidogyne* spp. were used to collecting *Meloidogyne* spp. eggs. The roots were first removed carefully from the pots and then washed gently with tap water to remove and exclude the adherent soil particles on the roots. Then roots were cut using a knife into small pieces (2cm) and then macerated using an electric blender for two periods of 10 seconds at high speed. To release the highest number of eggs from the roots. The solution with macerated root was then transferred into a flask containing solution of 0.5% sodium hypochlorite (NaOCl) according to Hussey and Barker (1973). Then the solution was well shaken for 3 minutes to help in releasing the eggs from the gelatin matrix. The solution was then sieving through serial sieves to separate eggs from the root tissue. Finally, eggs were collected on 500 mesh sieve (26 µm) after that washed many times using

clear tap water to confirm removing the residual NaOCl. The eggs were then totally transferred to a glass flask containing tap water and then number/ml was calculated using light microscope.

***In vitro* experiments:**

Effect on *Meloidogyne* spp. egg hatching: To evaluate the effect of yeast isolates on egg hatching, 0.9 mL of each yeast isolate and 100 eggs of *Meloidogyne* spp. in 0.1 mL of distilled water were placed together in 5-cm Petri dishes. One hundred eggs of *Meloidogyne* spp. in 0.1 mL of distilled water add to 0.9 mL of distilled water and placed together in 5-cm plastic Petri dishes considered as control. Five replicates were prepared for each separate treatment. Separate group of Petri dishes were incubated for each single period of observation (24, 48, 72, 120 and 168 hrs) under laboratory conditions at 25°**C**±2. Thirty eggs were randomly examined using a light microscope for evaluating the efficiency of the yeast culture filtrates on hatching. Empty and cracked eggs were considered hatched.

Effect on juvenile's mortality: To evaluate the effect of yeast isolates on the mortality of *Meloidogyne* spp. nematode juveniles, 0.9 mL of each yeast isolate liquid culture and 100 juveniles of *Meloidogyne* spp. in 0.1 mL of distilled water were placed together in 5-cm plastic Petri dishes. For control treatment, One hundred juveniles of *Meloidogyne* spp. in 0.1 mL of distilled water plus 0.9 mL of distilled water were placed together in 5-cm plastic Petri dishes. Each single treatment was replicated 5 times. Separate group of Petri dishes were incubated for each single period of observation (24, 48, 72, 120 and 168 hrs) under laboratory conditions at 25°C±2. Thirty juveniles were randomly examined using a light microscope for evaluating the efficiency of the yeast culture filtrates on juvenile's mortality. Inactive juveniles are rigid and elongated with tail and head sometimes slightly wrapped in total. Juveniles were washed with clear tap water to remove any residual culture filtrates then transferred into water for 24 hrs before vitality test is done. juveniles still inactive were considered dead.

Greenhouse experiment: Three-weeks old seedlings of susceptible tomato (*Lycopersicon esculentum* L. Hybrid Salymia 65010) to *Meloidogyne* spp. were transplanted into 15 cm in diameter plastic pots filled with 2.5 Kg of sandy loam soil mixture (2:1, v/v) (one seedling/pot). Three days after transplanting, each seedling was inoculated with about 3000 nematode eggs and larvae by pipetting into 3 holes made around the plant hairy roots. For yeast inoculation, *Pichia guilliermondii* (ATCC 9058), *Candida albicans* (ATCC 10231), *Saccharomyces cerevisiae* and *Saccharomyces* spp. (commercial) were added at different concentrations (1,2 and 4% of soil weight) to form suspensions that were drenched as a soil drench of tomato seedling alone, directly at three different application times as follows, one week prior, at seedlings transplanting and one-week post. Treatments were replicated three times and arranged in a completely randomized block design under greenhouse conditions at approximately 25°C±2. The plants were regularly irrigated and fertilized weekly.

After 60 days from transplanting plants were uprooted and transferred to laboratory. Nematode related parameters i.e., number of galls, egg masses, females, and number of juveniles (J_2s)/250g soil, gall index, nematode final population (Pf) and reproduction factor (Rf) (Goodey, 1957) were recorded. Egg-masses were stained prior to counting by dipping roots in phloxine-B solution (0.15 g/l tap water) for a time of 20 minutes as previously described by Daykin and Hussey (1985). Collecting and counting *Meloidogyne* spp. females were done by cutting the tomato plant root system up to 2 cm pieces then submerging the cutting roots pieces in a beaker containing tap water and keeping for 4 days under room temperature until root tissue became soft. Then roots were washed through 500 and 250 µm sieves to separate the *Meloidogyne* spp. females from the root debris (Mahdy, 2002). The nematode population in soil was enumerated by extraction *Meloidogyne* spp. juveniles (J_2s) using the tray modification of Baermann funnel as described by Barker (1985). Number of J2s/250g soil was enumerated by mixed soil samples carefully and an approximately 250 g of soil taken from each treatment and processed. After extraction, about 1 mL of the extracted nematode suspension was examined using a counting slide under the light microscope.

The reproduction factor (Rf) of root-knot nematode (nematode build-up) was also recorded according to Norton (1978) equation:

$$Rf = \frac{\text{Final population (Pf)}}{\text{Initial population (Pi)}}$$

Initial population (Pi) = 3000 (fixed number) refers to the number of nematode eggs and larvae / pot.

Reduction percentages in some nematode related parameters were calculated using the equation of Henderson and Tilton (1955) and Fleming and Retnakaran (1985):

$$\text{Reduction \%} = \frac{C - T}{C} \times 100$$

$$\text{Efficacy \%} = \frac{T - C}{C} \times 100$$

Whereas: C = Control & T = Treatment

Root gall index values were calculated using the formula by Taylor and Sasser (1978):

0 = No galls or egg mass. 1 = 1-2 galls or egg mass.

2 = 3-10 galls. 3 = 11-30 galls.

4 = 31-100 galls. 5 = More than 100 galls or egg masses.

Vegetative plant growth parameters i.e., shoot and root fresh weights (g), shoot and root lengths (cm) and plant dry weight was recorded. For obtaining fresh and dry weight, the roots were pressed gently between two pads of blotting paper then the fresh weight was recorded using electric balance. Dry weight was recorded by drying the roots in oven under 70°C until the constant weight according to Agaba and Fawole (2016).

Determination of antioxidant enzymes activity: Two antioxidant enzymes activities were determined as follows:

Peroxidase: The Peroxidase activity was measured following the previously described method by Fehrman and Dimond (1967). Sample of Ten grams of tomato plant fresh leaves were totally ground in a cement mortar with 10 ml of phosphate buffer (pH 6), then the extract was centrifuged at 4000 rpm for 15 min. Tow ml of supernatant was diluted to final volume of 10 ml by adding 8 ml distilled water. For the enzyme determination, mixture consisted of 1 ml of extract; 1.5 ml of 0.04 M caticol solution, 1.5 ml H_2O_2 and 1.5 ml phosphate buffer (pH 6) were prepared. Then colorimetrically assayed at 470 nm using spectrophotometer. Control treatment was previously boiled then the same steps. Enzyme activity reflects the increase in optical density after 60 – 120 seconds from add the substrate. The difference value in optical densities between both of t mixture and the control refer to the activity of reaction.

Polyphenol oxidase: Polyphenol oxidase was measured according to previously described method by Broesh (1954). Ten grams of leaves were grounded with 10 ml borate buffer (pH 9) in a mortar. Extracts were centrifuged for 15 min. at 4000 rpm using centrifuge. Then 9 ml distilled water add to 2 ml of supernatant. For preparing the reaction mixture, 2 ml borate buffer (pH 9) 2 ml of 1% caticol, 1 ml of 1% para aminobenzoic acid and 1 ml extract. Then mixture was colorimetrically measured at 575 nm using spectrophotometer. Control was done following the same steps but extracts were previously boiled.

STATISTICAL ANALYSIS

Data were analyzed using LSD test (P =0.05) with (Program: Costat-version: 6.311), analysis of variance was conducted out using the general one-way randomized blocks used for comparison between means.

RESULTS

***In vitro* experiments: Effect of different yeast isolates on *Meloidogyne* spp. egg hatching:** The antagonistic effect of four different yeast isolates i.e., *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae*, *Pichia guilliermondii* ATCC 9058, *Saccharomyces* spp., was tested egg hatching inhibition percentage and inactive juveniles (J_2s) % were evaluated at five different exposure periods (24, 48, 72, 120 and 168h) under laboratory conditions.

Results presented in Table (1), showed that the highest percentage of hatching inhibition at all exposure periods was achieved by *P. guilliermondii* ATCC 9058 with 93% after 168 h of exposure, followed by *S. cerevisiae,* with 83% of hatching inhibition. *Candida albicans* ATCC 10231, recorded moderate percentage of hatching inhibition with (77%). Meanwhile, *Saccharomyces* spp. recorded the least percentage of hatching inhibition by (50%) after 168 h of exposure, compared with control (nematode only+ water).

Table 1. Effect of yeast isolates on the egg-hatch of *Meloidogyne* spp. at different exposure periods.

Treatment	Hatching inhibition % at different exposure periods (h)				
	24	48	72	120	168
Candida albicans	40 ^{c}	47 ^{c}	57 ^{c}	63 ^{c}	77 ^{c}
Saccharomyces cerevisiae	50 ^{b}	53 ^{b}	60 ^{b}	70 ^{b}	83 ^{b}
Pichia guilliermondii	63 ^{a}	70 ^{a}	80 ^{a}	83 ^{a}	93 ^{a}
Saccharomyces spp.	13 ^{d}	23 ^{d}	27 ^{d}	37 ^{d}	50 ^{d}
Control	10 ^{e}	7 ^{e}	3 ^{e}	0 ^{e}	0 ^{e}

Columns followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05).

Effect of different yeast isolates on juvenile's mortality of *Meloidogyne* spp.: Data in Table (2) showed that the highest percentage of juvenile's mortality was achieved by *P. guilliermondii* ATCC 9058, and *S. cerevisiae* with (100%) after 168 h of exposure, followed by *C. albicans* ATCC 10231 with (87%), Meanwhile, *Saccharomyces* spp. recorded (83%) juveniles' mortality after 168 h of exposure comparing with (80%) of the control (nematode only).

Table 2. Effect of yeast isolates on *Meloidogyne* spp. juveniles (J_2s) mortality at different exposure periods.

Treatment	inactive juveniles (J_2s) % at different exposure periods (h)				
	24	48	72	120	168
Candida albicans	40 c	47 c	57 c	80 c	87 b
Saccharomyces cerevisiae	53 b	67 b	77 b	90 b	100 a
Pichia guilliermondii	73 a	87 a	97 a	100 a	100 a
Saccharomyces spp.	30 d	37 d	50 c	73 c	83 bc
Control	0 e	0 e	20 d	53 d	80 c

Columns followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05).

Greenhouse experiment: Effect of yeast isolates on *Meloidogyne* spp. under greenhouse conditions: Data in Table (3) represent the effect of four different yeast isolates i. e., *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae*, *Pichia guilliermondii* ATCC 9058, *Saccharomyces* spp., on *Meloidogyne* spp. under greenhouse conditions at three different application times (one week pre-planting, at planting and one-week post-planting) and three different concentrations (1,2 and 4% of soil weight). Nematode parameters (number of galls, egg masses, females and number of juveniles (J_2s)/250g soil, gall index, nematode final population (Pf) and reproduction factor (Rf)) were evaluated.

Results indicated that the most effective application time in decreasing all nematode parameters by all the four yeast isolates was one week pre-planting, followed by at-planting application, while the least effective application time was one-week post-planting. The most effective concentration in decreasing all nematode parameters was 4% of soil weight, followed by 2% of soil weight, while the least effective concentration was 1% of soil weight. *Pichia guilliermondii* ATCC 9058 at concentration 4% of soil weight, one week pre-planting application, achieved the highest reduction in nematode parameters such as: number of galls, egg masses, females, and number of juveniles (J_2s)/250g soil, with 89.6, 90, 88.7 and 90%, respectively., followed by *S. cerevisiae* (at concentration 4% of soil weight, one week pre-planting application, with 84.4, 88.3, 88.4 and 85.3%, respectively. A moderate reduction in nematode parameters i.e., number of galls, egg masses, females, and number of juveniles (J_2s)/250g soil achieved by *C. albicans* ATCC 10231 at concentration 4% of soil weight, one week pre-planting application, with 59.4, 65.2, 65.2 and 66%, respectively. Meanwhile *Saccharomyces* spp. at concentration 1% of soil weight, one-week post-planting application achieved the least reduction in all nematode parameters with 1, 3.8. 3.7 and 1.7%, respectively, comparing with nematode alone.

Pichia guilliermondii ATCC 9058 at concentration 4% of soil weight, one week pre-planting application, achieved the highest reduction in gall index, nematode final population (Pf) and reproduction factor (Rf), followed by *S. cerevisiae* at concentration 4% of soil weight, one week pre-planting application. A moderate reduction in gall index, nematode final population (Pf) and reproduction factor (Rf), achieved by *C. albicans* ATCC 10231 at concentration 4% of soil weight, one week pre-planting application, Meanwhile *Saccharomyces* spp. at concentration 1% of soil weight, one-week post-planting application was the least effective one, comparing with nematode alone.

Effect of yeast isolates on growth parameters of tomato plants under greenhouse conditions: Results listed in Table (4) represent the effect of four different yeast isolates i. e., *C. albicans* ATCC 10231, *S. cerevisiae*, *P. guilliermondii* ATCC 9058, *Saccharomyces* spp., on plant growth parameters of tomato under greenhouse conditions at three different application times (one week pre-planting, at-planting and one-week post-planting) and three different concentrations 1,2 and 4% of soil weight. Plant growth parameters i.e., shoot and root fresh weights (g), shoot and root lengths (cm) as well as plant dry weight were evaluated.

Data indicated that the most effective application time in increasing all plant growth parameters by all the four yeast isolates was one week pre-planting, followed by at-planting application, while the least effective application time was one-week post-planting. The most effective concentration in increasing all plant growth parameters

was 4% of soil weight followed by 2% of soil weight while the least effective concentration was 1% of soil weight. *P. guilliermondii* ATCC 9058 at concentration 4% of soil weight application, one week pre-planting application, recorded the highest efficacy in plant growth parameters i.e. root and shoot length, fresh shoot and root weight, and plant dry weight with percentages (92.7, 72, 153.8, 153.3 and 237.5%), respectively., followed by *S. cerevisiae* at concentration 4% of soil weight, one week pre-planting application, with 91.9, 75, 153.8, 145.6 and 250%, respectively. A moderate efficacy in plant growth was achieved by *C. albicans* ATCC 10231 at concentration 4% of soil weight, one week pre-planting application, with 59.4, 51, 79, 88.9 and 131.3%, respectively. Meanwhile, *Saccharomyces* spp. at concentration 1% of soil weight, one-week post- planting application achieved the least efficacy in all plant growth parameters with 1.6, 27.6, 7.7, 56.6 and 25%, respectively, comparing with nematode alone.

Table (3): Effect of yeast isolates at different concentrations on nematode parameters of tomato plants infected with root-knot nematodes *Meloidogyne* spp. under greenhouse conditions.

Treatment	Timing	Conc. %	Nematode parameters / root system										
			Number of Galls	Reduction %	Number of Females	Reduction %	Number of EggMass	Reduction %	J2s /250g	Reduction %	[illegible] index (0-5)	PF	RF
Candida albicans	A	1	125.3 ^{hi}	56.6	131.3 ^{i}	59	107.6 ^{i}	59	1775.3 ^{h}	64	5	2139	0.71
		2	122.3 ^{i}	57.6	124.3 ^{ij}	61	102.6 ^{ij}	61	1732.6 ^{h}	64.6	5	2081	0.69
		4	116.9 ^{i}	59.4	111.3 ^{k}	65.2	92.3 ^{k}	65.2	1661.3 ^{h}	66	5	1981	0.66
	B	1	168.9 ^{f}	41.3	176.6 ^{h}	44.8	146.6 ^{g}	44.7	2700.9 ^{f}	44.8	5	3191	1.06
		2	165.6 ^{f}	42.7	172.3 ^{h}	46	121.3 ^{h}	46	2636.3 ^{fg}	46	5	3094	1.03
		4	162.9 ^{f}	43.4	170.9 ^{h}	46.3	122.3 ^{h}	46.2	2604.9 ^{fg}	46.8	5	3060	1.02
	C	1	280.3 ^{bcd}	2.8	302.3 ^{bc}	5.3	250.3 ^{bc}	5.3	3976.6 ^{e}	18.7	5	4808	1.6
		2	276.6 ^{cde}	4.2	296.6 ^{bcde}	7.2	245.3 ^{bcd}	7.2	3919.3 ^{e}	19.9	5	4736	1.58
		4	273.3 ^{de}	5.2	287.3 ^{efg}	10	238.3 ^{def}	10	3859.3 ^{e}	21	5	4693	1.56
Saccaromyces serviceae	A	1	50.3 ^{no}	82.6	47 $^{.3\,nopq}$	85.2	39.3 ^{pqr}	85.2	799.3 ^{lmno}	84	4	935	0.31
		2	47.3 ^{o}	74.3	43.3 ^{opq}	86.5	38.3 ^{qr}	85.6	751.6 ^{mnop}	84.6	4	879	0.29
		4	45.3 ^{op}	84.4	36.9 ^{pq}	88.4	31.3 ^{rs}	88.3	719.9 ^{nop}	85.3	4	832	0.27
	B	1	72.3 ^{k}	75	68.3 ^{m}	78.6	56.6 ^{mn}	78.8	1303.6 ^{i}	73.3	4	1499	0.5
		2	71.3 ^{k}	75.3	65.3 ^{m}	79.6	54.3 ^{mno}	79.5	1273.3 ^{ij}	74	4	1463	0.48
		4	67.3 ^{kl}	76.7	58.3 ^{mn}	81.8	48.3 ^{nop}	81.8	1202.9 ^{ijk}	75.4	4	1375	0.46
	C	1	138.6 ^{g}	51.7	121.3 ^{ijk}	62	100.3 ^{ijk}	62	2714.6 ^{f}	44.5	5	3074	1.02
		2	133.3 ^{gh}	53.8	116.3 ^{jk}	63.6	95.9 ^{jk}	63.6	2597.3 ^{fg}	47	5	2942	0.98
		4	131.3 ^{gh}	54.5	113.3 ^{jk}	64.5	93.3 ^{k}	64.8	2558.3 ^{fg}	50	5	2895	0.96
Pichia guilliermondii	A	1	38.3 ^{pqr}	86.8	48.3 ^{nop}	85	39.9 ^{pqr}	85	607.6 ^{op}	87.6	4	733	0.24
		2	37.3 ^{pq}	87.2	43.3 ^{opq}	86.5	36.6 ^{qr}	86.4	591.3 ^{op}	88	4	707	0.23
		4	30 ^{q}	89.6	35.9 ^{q}	88.7	26.6 ^{s}	90	495.3 ^{p}	90	3	588	0.19
	B	1	59.3 ^{lm}	79.5	69.3 ^{m}	78.3	58.3 ^{m}	78	1051.9 ^{ijkl}	78.5	4	1337	0.45
		2	56.6 ^{mn}	80.6	58.3 ^{mn}	81.8	49.6 ^{mno}	81	999.9 ^{jklm}	79.6	4	1163	0.38
		4	52.3 ^{mno}	81.9	52.3 ^{mn}	83.6	45.3 ^{opq}	83	932.3 ^{klmn}	81	4	1081	0.36
	C	1	91.3 ^{j}	68.4	99.3 ^{l}	69	82.3 ^{l}	69	1775.3 ^{h}	64	4	2047	0.68
		2	88.9 ^{j}	69.1	94.6 ^{l}	70.2	79.3 ^{l}	70	1738.6 ^{h}	64.5	4	2001	0.67
		4	87.3 ^{j}	69.8	89.3 ^{l}	72	74.3 ^{l}	72	1699.3 ^{h}	65.3	4	1949	0.65
Saccaromyces spp.	A	1	278.9 ^{bcd}	3.1	288.3 ^{efg}	9.7	238.3 ^{def}	10	4636.6 ^{abc}	5.2	5	5191	1.73
		2	275.6 ^{cde}	4.5	282.3 ^{fg}	11.5	233.3 ^{ef}	11.6	4457.3 ^{cd}	8.9	5	5247	1.74
		4	272.3 ^{de}	5.6	278.6 ^{g}	12.5	231.3 ^{f}	12.5	4345.3 ^{d}	11	5	5127	1.7
	B	1	274.3 ^{cde}	4.9	288.6 ^{defg}	9.4	238.6 ^{def}	9.5	4814.6 ^{ab}	1.6	5	5616	1.87
		2	270.3 ^{e}	6.3	283.3 ^{fg}	11.2	234.3 ^{ef}	11.4	4553.6 ^{bcd}	6.9	5	5340	1.78
		4	268.9 ^{e}	6.6	280.3 ^{g}	12.2	232.3 ^{f}	12.2	4535.6 ^{bcd}	7.3	5	5316	1.77
	C	1	285.6 ^{ab}	1	307.3 ^{b}	3.7	254.3 ^{b}	3.8	4805.6 ^{ab}	1.7	5	5651	1.88
		2	282.3 ^{abc}	2	300.6 ^{bcd}	6	248.6 ^{bc}	6	4755.3 ^{ab}	2.8	5	5585	1.86
		4	279.3 ^{bcd}	3.1	293.3 ^{cdef}	8	242.3 ^{cde}	8.3	4704.3 ^{abc}	3.8	5	5518	1.83
Nematode alone			288.3 ^{a}	-	319.3 ^{a}	-	264.3 ^{a}	-	4890.6 ^{a}	-	5	5761	1.92
Nontreated (control)			-	-	-	-	-	-	-	-	-	-	-
LSD 0.05			8.141	-	11.240	-	2.659	-	79.120	-	-	-	-

A= one week before seedlings transplanting, B= at the same time of seedlings transplanting, C= one week after seedlings transplanting.

Means in each column followed by the same letter(s) are not significantly different according to LSD test (P≤0.05).

Table (4): Effect of yeast isolates at different concentrations on growth parameters of tomato plants infected with root-knot nematodes under greenhouse conditions.

Treatment	Timing	Conc. %	Plant growth parameters									
			Root length (cm)	% Efficacy	Shoot length. (cm)	% Efficacy	Fresh root weight (g)	% Efficacy	Fresh shoot weight (g)	% Efficacy	Dry weight (g)	% Efficacy
Candida Albicans	A	1	14.9 ^{de}	22	36.9 ^{abc}	27.6	2.6 ^{cdef}	92.3	12.3 ^{defg}	36.7	2.7 ^{nopk}	68.8
		2	14.9 ^{de}	22	37.3 ^{abc}	28.6	1.9 ^{cdef}	46.1	10.9 ^{efg}	22.2	2.8 ^{mnop}	75
		4	20.6 ^{cde}	68.3	43.9 ^{abc}	51	3.3 ^{bc}	79	16.9 ^{bcdefg}	88.9	3.7 ^{ijkl}	131.3
	B	1	14.9 ^{de}	22	43.9 ^{abc}	51.7	1.9 ^{cdef}	53.8	17.3 ^{bcdefg}	91.1	3.4 ^{jklmn}	112.5
		2	18.3 ^{de}	48.7	44.3 ^{abc}	52	3.6 ^{bc}	84.6	15.6 ^{cdefg}	72.2	3.5 ^{jklm}	118.8
		4	18.3 ^{de}	46	50.6 ^{abc}	75	2.9 ^{bcde}	123	18.3 ^{bcdefg}	102.2	3.9 ^{ghijk}	143.8
	C	1	17.3 ^{de}	41.5	40.9 ^{abc}	41	2.6 ^{cdef}	100	17.6 ^{bcdefg}	96.7	3.4 ^{jklmn}	112.5
		2	15.3 ^{de}	24	41.9 ^{abc}	44.8	2.9 ^{bced}	130.8	14.3 ^{cdefg}	56.7	3.5 ^{jklm}	118.8
		4	19.3 ^{cde}	57.7	43.6 ^{abc}	50	3.3 ^{bcd}	146.2	15.9 ^{cdefg}	75.6	4 $_{ghij}$	150
Saccaromyces Serviceae	A	1	14.9 ^{de}	22	44.6 ^{abc}	53.4	1.6 ^{cdef}	30.8	12.3 ^{defg}	37.8	2.5 ^{opk}	56.3
		2	12.9 ^{e}	5.7	38.3 ^{abc}	31	2.3 ^{cdef}	84.6	13.6 ^{cdefg}	50	3 ^{lmnop}	87.5
		4	23.6 ^{cde}	91.9	50.6 ^{abc}	75	3.3 ^{bcd}	153.8	22.3 ^{abcd}	145.6	5.6 ^{bc}	250
	B	1	16.3 ^{de}	32.5	55.6 ^{a}	91.4	2.3 ^{cdef}	69.2	20.9 ^{abcde}	133.3	3.9 ^{ghijk}	143.8
		2	16.9 ^{de}	38.2	48.3 ^{abc}	65.5	3.3 ^{bcd}	146.2	17.6 ^{bcdefg}	95.5	4.1 ^{fghij}	156.3
		4	16.6 ^{de}	35.8	46.9 ^{abc}	61.4	3.3 ^{bcd}	146.2	18.9 ^{bcdefg}	111.1	4.3 ^{fghi}	168.8
	C	1	18.9 ^{de}	54.5	52.9 ^{ab}	49	2.9 ^{bcdef}	115.4	19.3 ^{bcdefg}	112.2	4.1 ^{fghij}	156.3
		2	17.9 ^{de}	46	50.9 ^{abc}	75.9	2.9 ^{bcde}	130.7	24.3 ^{abc}	166.6	5.1 ^{cde}	218.8
		4	14.9 ^{de}	22	43.3 ^{abc}	49	3.3 ^{bc}	161	18.3 ^{bcdefg}	103.3	4.5 ^{efgh}	181.3
Pichia guilliermondii	A	1	15.9 ^{de}	30	46.3 ^{abc}	60	1.6 ^{def}	23	20.9 ^{abcde}	132.2	4.6 ^{efg}	187.5
		2	19.5 ^{de}	58.5	47.3 ^{abc}	63	2.9 ^{bcdef}	115.4	19.3 ^{bcdefg}	114.4	4.8 ^{def}	200
		4	36.3 ^{a}	92.7	49.9 ^{abc}	72	3.3 ^{bcd}	153.8	22.9 ^{abcd}	153.3	5.4 ^{bcd}	237.5
	B	1	27.3 ^{bc}	19.5	49.9 ^{abc}	72	5.3 ^{a}	307.7	26.3^{ab}	191.1	6.9 ^{a}	331.3
		2	27.3 ^{b}	21.9	51.3 ^{abc}	77	2.6 ^{bcdef}	107.7	21.9 ^{abcd}	144.4	5.4 ^{bcd}	237.5
		4	19.3 ^{cde}	56	57.3 ^{a}	97	1.6 ^{def}	23	20.3^{abcdef}	122.2	4.8 ^{def}	200
	C	1	15.3 ^{de}	24	42.3 ^{abc}	44.8	2.9 ^{bcdef}	115.4	14.3^{cdefg}	57.8	3.2 ^{klmno}	100
		2	19.9 ^{cde}	62.6	54.3 ^{ab}	86	4.3 ^{b}	223	23.3 ^{abcd}	157.8	6 ^{b}	275
		4	16.9 ^{de}	38.2	57.3 ^{a}	98	2.3 ^{cdef}	76.9	29.3 ^{a}	222	6.9 ^{a}	331.3
Saccaromyces SPP.	A	1	12.6 ^{e}	1.6	48.9 ^{a}	67.9	1.3 ^{f}	0	13.9 ^{cdefg}	53.3	2.3 ^{pkr}	43.8
		2	15.3 ^{de}	23.6	51.6 ^{abc}	77.6	1.9 ^{cdef}	53.8	21.3 ^{abcd}	137.8	2.9 ^{mnop}	81.3
		4	15.6 ^{de}	26	52.6 ^{ab}	81	2.3 ^{cdef}	61.5	19.3 ^{bcdefg}	114.4	3.8 ^{ghijk}	137.5
	B	1	13.3 ^{e}	8	40.9 ^{abc}	41	1.9 ^{cdef}	38.5	12.6 ^{defg}	40	2.5 ^{opk}	56.3
		2	12.6 ^{e}	3.3	37.3 ^{abc}	27.6	1.3 ^{ef}	7.7	14.9 ^{cdefg}	65.6	2 qr	25
		4	13.9 ^{de}	13.8	45.6 ^{abc}	57.2	2.6 ^{cdef}	92.3	15.3 ^{cdefg}	71.1	2.8 ^{mnop}	75
	C	1	12.6 ^{e}	1.6	34.6 ^{bc}	19	1.3 ^{f}	0	10.3 ^{fg}	11.1	2 qr	25
		2	17.3 ^{de}	40.7	55.3 ^{a}	90.3	2.3 ^{cdef}	69.2	20.3 ^{abcdef}	126.7	4 ^{ghij}	150
		4	13.3 ^{e}	8	42.3 ^{abc}	45.2	2.6 ^{cdef}	100	17.3 ^{bcdefg}	88.9	3.8 ^{higk}	137.5
Nematode alone			12.3 ^{e}	-	29.3 ^{c}	-	1.3 ^{f}	-	9.3 ^{g}	-	1.6 ^{r}	-
Nontreated (control)			15.3 ^{de}	22	34.6 ^{bc}	19	1.6 ^{def}	23	11.3 ^{efg}	22.2	2.6 ^{opk}	162.5
LSD 0.05			1.368	-	1.435	-	0.164	-	1.102	-	0.767	-

Means in each column followed by the same letter(s) are not significantly different according to LSD test (P≤0.05).
A= one week before seedlings transplanting, B= at the same time of seedlings transplanting, C= one week after seedlings transplanting.

Effect of yeast isolates on enzymatic activity of tomato plants: Results in Table (5) represent the enzyme activities of two defense enzyme peroxidase and polyphenol-oxidase in tomato plants. The effect of four yeast isolates on the two enzymes was evaluated at three different application times: one week pre-planting, At-planting and one-week post-planting. Data showed that the most effective application time in increasing the

two enzymes activity by the four yeast isolates was one week pre-planting, followed by at-planting application, while the least effective application timing was one-week post-planting. The highest enzymes activity achieved by *P. guilliermondii* ATCC 9058, followed by *S. cerevisiae*. A moderate increase in peroxidase and polyphenol-oxidase activities was recorded by *C. albicans* ATCC 10231 meanwhile; *Saccharomyces* spp. recorded the least increase in the enzyme activities of the two enzymes, comparing with nematode alone.

Table (5): Effect of yeast isolates on enzymatic activity of tomato plants.

Treatment	Conc. %	Enzyme activities (PPO and POX)					
		Peroxidase (POX) (O.D.g-1 fr.wt. after 2min)			Polyphenol-oxidase (PPO) (O.D.g-1 fr.wt. after 45min)		
		One week pre-planting	At-planting	One week post-planting	One week pre-planting	At-planting	One week post-planting
Candida albicans	**1**	0.77 ^{fg}	0.72 ^{fg}	0.66 ^{fg}	1.2 ^{fg}	1.05 ^{fg}	1 ^{fg}
	2	1 ^{de}	0.94 ^{de}	0.85 ^{de}	1.6 ^{de}	1.45 ^{de}	1.35 ^{de}
	4	1.25 ^{bc}	1.22 ^{bc}	1.05 ^{bc}	2.15 ^{bc}	2 ^{bc}	1.85 ^{bc}
Saccaromyces serviceae	**1**	0.82 ^{efg}	0.7 ^{efg}	0.62 ^{efg}	1.15 ^{fgh}	1.05 ^{fgh}	1.02 ^{fgh}
	2	1.1 ^{cde}	1.02 ^{cde}	0.88 ^{cde}	1.8 ^{cde}	1.64 ^{cde}	1.6 ^{cde}
	4	1.33 ^{ab}	1.28 ^{ab}	1.2 ^{ab}	2.38 ^{ab}	2.15 ^{ab}	2 ^{ab}
Pichia guilliermondii	**1**	0.96 ^{d}	0.89 ^{d}	0.78 ^{d}	1.48 ^{ef}	1.34 ^{ef}	1.28 ^{ef}
	2	1.3 ^{abc}	1.2 ^{abc}	1 ^{abc}	2.07 ^{bcd}	1.88 ^{bcd}	1.68 ^{bcd}
	4	1.4 ^{a}	1.34 ^{a}	1.15 ^{a}	2.52 ^{a}	2.3 ^{a}	2.07 ^{a}
***Saccaromyces* spp.**	**1**	0.65 ^{g}	0.63 ^{g}	0.55 ^{g}	1 ^{gh}	0.95 ^{gh}	0.90 ^{gh}
	2	0.75 ^{f}	0.7 ^{f}	0.65 ^{f}	1.2 ^{fg}	1.1 ^{fg}	1.02 ^{fg}
	4	0.85 ^{ef}	0.82 ^{ef}	0.73 ^{ef}	1.47 ^{efg}	1.32 ^{efg}	1.3 ^{efg}
Nematode alone		0.20 ^{g}	0.24 ^{g}	0.23 ^{g}	0.35 ^{k}	0.33 ^{k}	0.34 ^{k}

Means in each column followed by the same letter(s) are not significantly different according to LSD test (P≤0.05).

DISCUSSION

Obtained results showed that *Pichia guilliermondii* ATCC 9058, achieved the highest egg-hatching inhibition and juveniles (J_2s) mortality, followed by *Saccharomyces cerevisiae*, while the least percentage of egg-hatching inhibition and juveniles (J_2s) mortality were achieved by *Candida albicans* ATCC 10231.

Yeasts acts in different modes of action against phytopathogens, such as: production of lytic enzymes for example: chitinase (Zhang *et al.,* 2011), which consider the responsible about chitin breakage as reported by Gortari and Hours (2008). Also, Chitin is a one of the main nematode-eggshell components, which responsible for stiffening as mentioned by Bird and McClure (1976). Moreover, chitinase production can cause embryonic development disorder which may be a way to inhibit and decrease the hatching of juveniles (Khan *et al.* 2004). Moreover, production of extracellular cell wall-degrading enzymes by *P. guilliermondii* effective in plant pathogens inhibition (Papon *et al.,* 2013). Particularly, different levels in chitinase and b-1,3-glucanase activity have been reported for *Pichia* strains (R13, M8 and US-7) (Wisniewski *et al.,* 1991; Chanchaichaovivat *et al.,* 2008; Zhang *et al.,* 2011). The degradation makes affects shell peeling and fractured or interrupted surface, which lead to partial or total release of the egg content, then the nematode J_2s unviable (Regaieg *et al.,* 2010). These results in agreement with the indicated by Hashem *et al.,* (2008) and El-Qurashi *et al.,* (2019), who reported that the yeast *Pichia gluilliermondii* was one of the treatments, achieved highest mortality of *M. javanica* juveniles *in vitro* after 24 and 48h. Also, Moussa and Zawam (2010) found that treatment with the culture filtrates of *Candida incommunis* and *Wickerhamiella domercqiae* suppressed egg hatching at very high percentage and observed that the yeasts widely attached to *M. inc*ognita eggs and juveniles. Fialho *et al.,* (2012) proved that volatile organic compounds (VOCs) produced by *S. cerevisiae* showed higher mortality of *M. javanica* on tomato.

Data indicated that generally, all treatments had a significant clear effect on nematode parameters these results are in accordance with those obtained by Hashem and Abo-Elyousr (2011), reported that *Pichia guilliermondii* presents a lethal effect on the tomato root-knot nematode *M. incognita*. In a previous study, a commercial product, containing cells of *S. cerevisiae*, significantly affected *M. incognita* J_2s in soil and galling in

squash root (Noweer and Hasabo, 2005). Also, in Egypt, the bio-agent *Saccharomyces uvarum* and *Saccharomyces ludwigii* present a harmful effect to *M. javanica* juveniles and reducing egg masses and galls numbers (Shawky *et al.,* 2006). Similarly, Hashem *et al.,* (2008), indicated that *Candida albicans* Moh Y-5 showed an obvious reduction of *M. incognita* populations compared to the control in grapevines (CV. Flame Seedless) under field conditions. Also, *Candida incommunis* highly decreased galls numbers and egg-masses formation by *M. incognita* and clear promoted the tomato plants growth compared to the control (Moussa and Zawam, 2010). Furthermore, *Saccharomyces cerevisiae* was effective in reducing *M. javanica* infection and reproduction on cucumber roots (Karajeh, 2013). The nematicidal effect of *Pichia guilliermondii* ATCC 9058 may be due to their production of citric acid and riboflavin (RF, vitamin B_2) (Abbas and Sibirny, 2011; West, 2013). Citric acid presents a nematicidal activity against *M. arenaria* (Mokbel *et al.,* 2009), and *M. incognita* in tomato (El-Sherif *et al.,* 2015). The nematicidal potential of citric acid is mostly referred to its acidity which may be fast destroying nematode bodies and eggs cells and tissues. Also, this may be referred to osmoregulation disruption followed by fluid accumulation (Seo and Kim, 2014; Jang *et al.,* 2016). Riboflavin helps in induce antioxidant compounds accumulation in plant cells as reported by Mori and Sakurai (1995) and Taheri and Tarighi(2010) which, can enhance plants resistance against pathogen (Deng *et al.,* 2014; Nie and Xu, 2016). The mechanism of yeast as a bio-control agents may be involve parasitism induced resistance, competition for site and/or make chemical and physical soil properties unsuitable for phyto-pathogens as mentioned by Noweer and Hasabo, (2005) and Karajeh(2013). The toxic effect of yeasts against *M. javanica* might be referred to the yeast ability to use carbohydrates and produce CO_2 and ethyl alcohol which are toxic to *M. javanica* (Mostafa, 2004; Noweer and Hasabo, 2005).

Data indicated that all treatments showed efficacy in enhancing shoot and root fresh weights (g), shoot and root lengths (cm) compared with nematode alone treatment and non-treated control. These results are also in agreement with Hamza *et al.* (2013) who reported that yeast extracts have increased carnation vegetative parameters i.e., root and shoot length, root and shoot weight and plant dry weight. The yeast *S. cerevisiae* was able to promote growth and increase tomato plants fruit yield. Moreover, an improvement of different crops plant growth and yield was noticed by *S. cerevisiae* such as: Egyptian henbane (Youssef and Soliman, 1997), squash (Noweer and Hasabo, 2005), sugar beet (Shalaby and El-Nady, 2008), cucumber (Karajeh, 2013). Also, *Candida ethanolica* and *Pichia guillermondii,* increased tomato plant growth parameters and fruit yield (Arwiyanto, 2014). The increase in the plant's biomass may be due to released and provide different metabolites during yeast fermentation such as vitamins and amino acids (Abou-Zaid, 1984). Not only but also improve photosynthesis as a result to activation of magnesium dechelatase enzymes (Somer, 1987; Mahmoud, 2001). Moreover, Yeast richness by carbohydrates, proteins, lipids, nucleic acid, and various different minerals considered a natural plant growth stimulator as reported by Wareing and Phillips (1970). It contains considerable amounts of mineral elements, proteins, vitamins, carbohydrates, enzymes, cytokinins and indol acetic acid (Moor, 1979; Somer, 1987; Mahmoud, 2001). Which enhances division and cell enlargement as previously discussed (Ferguson, *et al.,* 1987; Nagodawithana, 1991; Nassar *et al.,* 2005). Yeast produces many of naturally active components such as enzymes, phyto-hormones and auxin groups including indole-3-acetic acid and IAA) which promotes plant health then enhance their yield production (Nassar *et al.,* 2005; Moller *et al.,* 2016). Citric acid produced by yeasts decreases soil pH which enhances macronutrient elements uptake and plays an important role in photosynthesis and cellular respiration. Moreover, citric acid is used as chelate agent to some nutrients and make them available for plants (Hasegawa, 2012). Also, Riboflavin (vitamin B_2) plays as coenzyme in many physiological processes inside plants (Gastaldi *et al.,* 1999), promotes plant growth development and antioxidant regulates and enhances plant drought tolerance (Deng *et al.,* 2014). The present study revealed an increasing in the activities of two enzymes, peroxidase (PO) and polyphenol oxidase (PPO) in tomato plants. Our results are in accordance with Zhao *et al.,* (2008) who reporetd that tomato fruits inoculated with *Pichia guilliermondii* strain CNM2.1801 showed an activation of several plant defense enzymes, i.e., catalase, chitinase, polyphenoloxidase, peroxidase, superoxide dismutase, phenylalanine ammonia lyase and b-1,3-glucanase. Also, Similar previous results by (Nikoo *et al.,* 2014; Khajuria and Ohri, 2018) in the case of tomato

plants. Similarly, Ragaa *et al.,* (2019) stated that by increasing the activity of CAT enzyme the number of J_2s of *M. incognita* decreased in soil, in addition to galls number, females and egg-masses in banana plants roots.

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