Research Article

Nematicidal Properties of Some Yeast Culture Filtrates against *Meloidogyne javanica* Infecting Squash Plants (*In Vitro* and *In Vivo*)

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Abstract | The influence of some yeasts culture filtrate of *Candida oleophila, Cryptococcus albidus*, *Pichia guilliermondii*, *Saccharomyces cerevisiae* and *Sporobolomyces roseus* include 10^3, 10^6 and 10^{12} cells, on hatching of second-stage juveniles (J_2) of *Meloidogyne javanica* were estimated in laboratory and greenhouse conditions. Results revealed that all the tested culture filtrates caused significant reduction in number of J_2 in all treatments, compared to the non-inoculated control, where the hatching inhibition and mortality percentages increased with an increase in exposure time. At 24 hours, increased the mean number of J_2 hatched slowly than in first stages of experiment. Similar patterns showed against *M. javanica* infecting squash plants under greenhouse conditions. Data indicated that treatment with *P. guilliermondii* filtrate (10^{12}) caused best percentage of nematode reduction (71.46%). Followed by *S. roseus* at (10^{12}) (42.95 %), then in third treatment with *C. albidus* (10^3) by (40.50 %). On the other hand, all tested treatments decreased the negative effects of nematodes and enhanced growth characters of squash plants. The highest enhancement in the growth characters were recorded in treatment with *P. guilliermondii* filtrate at (10^{12}).

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Introduction

Genus *Meloidogyne* (Tylenchida: Meloidogynidae) has been of interest to nematologists worldwide probably due to their widespread distribution and success as parasites of economically important crops and is considered as one of the most important genera of plant parasitic nematodes. The root-knot nematodes, *Meloidogyne javanica* (Treub) Chitwood (1949) the most common pest nematode species in vegetables greenhouses (Koenning *et al*., 1999), which represented about 10% of total losses caused by plant pests and pathogens combined on vegetables production (Barker and Koenning, 1998). Biological control has become an attractive alternative strategy for the control of plant diseases to reduce the excessive use of agrochemicals and its health hazards, also, multiple beneficial characters such as rhizosphere competence, antagonistic potential. As biocontrol agents many fungi are known to produce nematicidal compounds (Meyer *et al*., 2000). Many experiments have been made to use antagonistic fungi and compounds produced by these microbes its compounds to reduced phytonematodes and promoting of plant growth (Ashraf and Khan, 2010). Some yeasts are reported to reduce effectively various plant pathogenic of fruits (Fan and Tian, 2001). Potential use of yeast culture products fungi as biocontrol agents of soil-borne

plant pathogens and plant growth promoters was recently investigated (Azzam *et al*., 2012). Recently, a great attention has been given to the application of yeasts to inhibition various plant pathogens (Hashem *et al*., 2008). The activity of yeast culture components which have nematicidal properties on viability of phytonematodes through inconstancy of rhizosphere ecology and inhibition of hatching process, juvenile development and root-finding (Fernández *et al*., 2001). Youssef and Soliman (1997) found that the Egyptian isolate of *Saccharomyces cerevisiae* reduced *Meloidogyne incognita* populations and improved the growth on *Hyoscyamus muticus*, also, the application of *Saccharomyces cerevisiae* as a biocontrol agent of the root-knot nematode, reducing the nematode reproduction ability on cucumber under greenhouse and field conditions (Karajeh, 2013). So this study aims to evaluate some yeasts culture filtrate as biocontrol tools for their application in filed conditions.

Materials and Methods

Purification yeasts culture filtrate

Five tested yeasts culture filtrate of *Candida oleophila*, *Cryptococcus albidus*, *Pichia guilliermondii*, *Saccharomyces cerevisiae* and *Sporobolomyces roseus* were grown on the medium using shaking incubator (200 rpm) for 3 days at 30° C then, the cells were harvested by centrifugation at 10000 rpm for 20 min, the culture medium was discarded. The supernatant was filtered by passing the culture broth through a sterile membrane filter 0.2 µm according to the method described by El-Boghdady (1993).

In vitro assay

Effect of culture filtrates on egg-masses and eggs hatching

In vitro test ten egg-masses in uniform in medium size, age and undifferentiated, immersed in 5 ml of three culture filtrate that contains three concentrations of yeasts strains at 10^3, 10^6 and 10^{12} cells prepared in sterile distilled water, egg-masses in plain water in cup supplied with nematode suspension was used as a control. Each treatment was replicated three replications. Cups were loosely covered which slightly permit aeration and reduce evaporation, at 30 ± 5°C. The number of second-stage juveniles hatched was estimated using nematode counting slide in accordance with the Hussey and Barker (1973) technique modified by (Boneti and Ferraz (1981), using an inverted microscope at 40x. The number of second-stage juveniles observed at 6, 24, 48, 72, 96, 168 and 240 hours. Egg-masses hatching inhibition percentage and mean hatching per egg-mass was calculated using the following formula:

$$\text{Mean hatch per egg mass} = \left(\frac{\text{Number of juveniles hatched in treated}}{\text{Initial no. egg} - \text{masses}}\right)$$

$$\text{Hatching inhibition \%} = \left(\frac{\text{Number of juveniles hatched in control- Number of juveniles hatched in treated}}{\text{Number of juveniles hatched in control}}\right) \times 100$$

Effect of yeasts culture filtrate on juveniles mortality

For the extraction of *Meloidogyne* egg-masses and J_2 the method in Hussey and Barker (1973) was used. Egg-masses were handpicked from the galled tomato roots from stock cultures, and incubated in sterile distilled water at room conditions at 30±5°C for 48 hours. Hatched second stage juveniles (J_2s) that had passed through the tissue paper into the petri dish were counted and concentrated until reached contented 1 ml of distilled water approximately 150 second stage juveniles used each for all the treatments including the control, population density of second stage juveniles in stock suspension (150 J_2s/1ml dw) was considered mean population number from 3 times of one ml of stock suspension. 1ml of this juveniles suspension poured in screw-capped test tubes which contained 5 ml of different concentrations of yeasts strains filtrate timely preparation and incubated at 30±5°C for four days and the numbers of dead juveniles were counted at 12,24,48 and 96 hours. Juveniles were considered dead if they did not move when teased with fine needle and body become straight (Cayrol *et al*., 1989). Each treatment was replicated three replications, distilled water served as control. The mortality percentage was calculated according to the Abbott's formula.

$$\text{Mortality \%} = \left(\frac{\text{Number of survived larvae in control-Number of survived larvae in treated}}{\text{100 - Number of survived larvae in control}}\right) \times 100$$

$$\text{Mean hatch /egg} - \text{mass} = \left(\frac{\text{Number of juveniles hatched in treated}}{\text{Initial no. egg} - \text{masses}}\right)$$

In vivo assay

Squash seeds cv. Mabrouka® (Hy 42095) (registration no. 1508 – 1998 in ministry of agriculture and land reclamation by Hytech seed company) were sown in pots (20 cm dia.) sterilized clay filled with mixture of clay: sand (1: 3, v: v). After germination, plants were thinned to one per pot and treated with 3000 freshly J_2/ pot, poured into five holes in the soil around the plant. Pots were treated with highest effecting rate resulted from *invitro* assay of culture filtrate which were *C. oleophila* (10^3), *C.albidus* (10^6), *P.guilliermondii* (10^{12}), *S. cerevisiae* (10^6) and *S. roseus* (10^{12}), as a soil drench after nematode inoculation. Treatments were replicated three times. The pots were arranged in a

randomized complete block design, maintained at 32 ±5 °C and watered as needed. The experiment was terminated six weeks after nematode inoculation. Numbers of galls, egg-masses, developmental stages number, adult female and number of eggs/egg mass were determined, processed for nematode extraction according to methods described by Christie and Perry (1951) and Southey (1986). Nematode final population (Pf) calculated by the following formula:

$$Pf = [\text{no. Egg/masses} \times \text{no. Eggs / Egg} - \text{masses}] + [\text{Developmental stage/root}] + [\text{Juveniles in soil}] + [\text{Adult females/root}].$$

The reproduction rate was calculated by dividing the final population (Pf) by the initial one (Pi). The percentage of nematode reduction calculated by the following formula:

$$\text{Nematode Reduction\%} = \left(\frac{\text{Number of nematode in control - Number of nematode in treatment}}{\text{Number of nematode in control}}\right) x100$$

Also fresh and weights of the root and shoot systems as well as their lengths were determined.

The percentage reduction/ increase in growth parameters were calculated by the following formula:

$$\text{Percent (\%) reduction/increase} = \frac{AxB}{A} x100$$

Where; A= value of control, B= value of treatment.

Statistical analyses

The experiment designed with randomized complete design. All the data were subjected to Analysis of Variance (ANOVA) using Costat package version 6.311. The means were compared according to Duncan's multiple range tests at P ≤0.05 (Duncan, 1955).

Results and Discussion

Effect of some yeasts culture filtrate on hatching of M. javanica in vitro

The influence of some yeasts fungi culture filtrate of *Candida oleophila*, *Cryptococcus albidus*, *Pichia guilliermondii*, *Saccharomyces cerevisiae* and *Sporobolomyces roseus* at concs: 10^3, 10^6 and 10^{12} cells of each, on hatching of egg-masses of *Meloidogyne javanica* were estimated in laboratory conditions. Data presented in (Tables 1 and 2) revealed that, all the tested culture filtrates caused significantly reduction (P≤0.05) in number of second-stage juveniles (J_2) hatched from egg-masses and hatching inhibition percentage at all treatments, compared to the non-inoculated control. Data showed that, in all treatments after six hours of exposure no second-stage juveniles hatching where observed which means that all the treatments have no effect on the egg-masses as compared with control (distilled water only), which achieved mean (1.33) J_{2S} hatched from egg-masses. But with increasing exposure time to 12 hours significant reduction was observed between treatments and control but not significant between treatments, in all concs where in 10^3 best effect recorded in number of hatched second-stage juveniles associated with *C. albidus* followed by *S. cerevisiae* then *P. guilliermondii*, *C. oleophila* and *S. roseus* (0.0, 0.33, 0.0 ,0.0 and 1.0), respectively as compared with control (3.0) in this period while in hatching inhibition percentage best results was achieved in *S. cerevisiae* with (33.34%) followed by *S. roseus* (11.0%), While in second diluation of 10^6 same results was achieved in *P. guilliermondii*, *S. cerevisiae* and *S. roseus* (3.3) in hatched J_{2S} and percentage of hatching inhibition (11.0). Also, in high conc. 10^{12}, *C. oleophila* exibited best data) in hatched J_{2S} and percentage of hatching inhibition flowed by *P. guilliermondii*, *S. cerevisiae* (0.0, 0.33 and 3.3) then *C. albidus* and *S. roseus* (1.33 and 1.33) and (0.0,11.0, 11.0, 44.34 and 44.34), respectively as compared with control (3.0). After 48 hours *S. cerevisiae* recorded best effect in all concs (5.67, 4.0 and 3.67), respectively, followed by *C. oleophila* (6.67, 8.33 and 4.33) and *P. guilliermondii* (8.0, 11.33 and 5.0), respectively as compared with control (20.67). After 96 hours, in conc. 10^3 the best results associated with *S. cerevisiae* hatched J_{2S} and hatching inhibition % (5.77 and 40.0%) followed by *C. oleophila* (6.33 and 21.1%) and *P. guilliermondii* (7.33 and24.44%) and least effect in *S. roseus* (12.0 and19.24%), respectively. In 10^6 *S. cerevisiae* showed best results (5.33 and 82.33%) followed by *S. roseus* (8.33 and 27.77%) and *C. albidus* (8.33 and 2.77%) as compared with control (30.0).

In the end of test at 240 hours, increased the mean number of JS2 hatched slowly than in first stages of experiment. And with increased in time, the effect was decreased; data recorded significant reduction (P≤0.05) in number of J_2 hatched from egg-masses and hatching inhibition percentage at all treatments where, in first conc. (10^3) *C. oleophila* recorded highest reduction in hatched J_2 and hatching inhibition percentage as reduction inhibition over the control in

Table 1: *Evaluation of some yeasts culture filtrate on egg-masses hatching of root knot nematode, Meloidogyne javanica in vitro.*

Treatments	Mean number of juveniles hatched after intervals (Hrs.)														
	6 Hrs.			12 Hrs.			24 Hrs.			48 Hrs.			72 Hrs.		
	10^3	10^6	10^{12}	10^3	10^6	10^{12}	10^3	10^6	10^{12}	10^3	10^6	10^{12}	10^3	10^6	10^{12}
C. oleophila	0	0	0	0.00 B(b)	0.00 B(b)	0.00 B (b)	6.67 AB(bc)	9.33 AB(b)	4.33 B(c)	8.67 B(b)	9.33 BC(b)	5.33 B(b)	8.00 B(b)	9.67 B(b)	6.67 B(b)
C. albidus	0	0	0	0.00 B(b)	0.00 B(b)	1.33 B(b)	2.67 B(b)	6.67 AB(ab)	4.33 B(b)	6.67 B(bc)	8.33 BC(b)	4.33 B(b)	7.33 B(bc)	9.00 B(b)	4.67 BC(c)
P. guillier-mondii	0	0	0	0.00 B(b)	0.33 B(b)	0.33 B(b)	6.00 AB(ab)	9.33 AB(b)	2.67 B(c)	8.00 B(bc)	11.33 B(b)	5.00 B(b)	6.67 B(b)	6.67 BC(b)	3.33 C(c)
S. cerevisiae	0	0	0	0.33 B(b)	0.33 B(b)	0.33 B(b)	4.67 AB(b)	4.00 B(b)	4.00 B(b)	5.67 B(b)	4.00 C(b)	3.67 B (b)	5.67 B(b)	5.67 BC(b)	3.33 C(b)
S. roseus	0	0	0	1.00 B(b)	0.33 B(b)	1.33 B(b)	7.33 AB(ab)	4.33 B(b)	1.00 B(c)	12.67 AB(b)	4.33 C(c)	4.67 B(c)	12.33 B(b)	3.33 C(c)	4.67 BC(c)
Control	1.33			3.00 A(a)			14.67 A(a)			20.67 A(a)			22.67A(a)		
LSD (0.05)	-	-	-	1.10	1.02	1.51	9.54	7.84	7.12	11.12	6.27	5.92	6.19	5.16	5.53
	96 Hrs.			120 Hrs.			168 Hrs.			240 Hrs.					
C. oleophila	6.33 BC(c)	12.33 B(b)	9.00 B(bc)	5.33 D(c)	12.33 B(b)	12.00 B(b)	5.33 C(c)	14.33 B(b)	12.00 B(b)	6.00 D(c)	14.67 B(b)	10.33 B(bc)			
C. albidus	10.33 B(b)	8.33 BC(b)	5.67 BC(c)	10.67 BCD(b)	8.33 BC(b)	9.67 BC(b)	18.67 B(b)	7.67 BC(c)	9.67 B(c)	18.33 BC(b)	7.33 CD(c)	9.67 B(c)			
P. guillier-mondii	7.33 BC(b)	8.67 BC(b)	1.67 C(c)	12.33 BC(b)	13.33 B(b)	8.67 BC(b)	17.33 B(b)	13.33 B(bc)	7.67 BC(c)	17.33 BC(b)	12.67 BC(bc)	8.33 B(c)			
S. cerevisiae	5.77 C(bc)	5.33 C(c)	7.00 B(bc)	14.67 B(b)	5.33 C(c)	8.33 BC(bc)	15.00 B(b)	4.67 C(c)	8.00 BC(bc)	14.33 CD(b)	4.33 D(c)	8.67 B(c)			
S. roseus	12.00 B(b)	8.33 BC(b)	1.67 C(c)	6.20 CD(b)	8.33 BC(b)	5.67 C(b)	18.67 B(b)	12.00 BC(c)	6.33 C(d)	26.67 B(b)	12.33 BC(c)	7.33 B(c)			
Control	30.00 A(a)			36.00 A(a)			40.67 A(a)			47.33 A(a)					
LSD (0.05)	5.93	4.74	4.23	6.42	5.61	6.09	8.07	7.35	6.30	10.39	6.03	6.20			

Each figure represents the mean of three replicates; Capital letters represented significantly between all conc. In all treatments, and small letters represented significantly test between three conc. to alone yeast filtrate according to Duncan's multiple-range test (P < 0.05); Values followed by the same letter are not statistically different according to Duncan's multiple-range test (P ≤0.05).

Table 2: *Evaluation of some yeasts culture filtrate on egg-masses hatching inhibition percentage of Meloidogyne javanica in vitro.*

Treatments	Conc.	*Hatching inhibition %									*Total hatch	Mean hatch per egg mass
		6 Hrs.	1 2 Hrs.	24 Hrs.	48 Hrs.	72 Hrs.	96 Hrs.	120 Hrs.	168 Hrs.	240** Hrs.		
C. oleophila	10^3	-	0	45.44	41.95	35.29	21.1	14.81	13.11	12.68	6.00	0.60
	10^6	-	0	63.58	45.14	42.66	41.1	34.25	35.24	30.99	14.67	1.47
	10^{12}	-	0	29.49	25.79	29.43	30	33.33	29.51	21.83	10.33	1.03
C. albidus	10^3	-	0	18.21	32.27	32.34	34.44	29.64	45.91	38.73	18.33	1.83
	10^6	-	0	45.47	40.3	39.71	27.77	23.14	18.86	15.49	7.33	0.73
	10^{12}	-	44.34	29.52	20.95	20.6	18.9	26.87	23.78	20.44	9.67	0.97
P.guilliermondii	10^3	-	0	40.9	38.71	29.43	24.44	34.25	42.62	36.62	17.33	1.73
	10^6	-	11	63.6	54.82	29.43	28.9	37.03	32.78	26.77	12.67	1.27
	10^{12}	-	11	18.21	24.19	14.69	5.57	24.09	18.86	17.6	8.33	0.83
S. cerevisiae	10^3	-	33.34	49.97	61.3	54.39	40	40.75	36.89	30.28	14.33	1.43
	10^6	-	11	29.52	20.95	14.69	82.33	14.81	11.49	9.15	4.33	0.43
	10^{12}	-	44.34	6.82	22.6	20.6	23.34	23.14	19.68	18.32	8.67	0.87
S. roseus	10^3	-	11	31.84	27.44	25.02	19.24	17.23	45.91	56.35	26.67	2.67
	10^6	-	11	27.24	19.36	25.02	27.77	23.14	29.51	26.06	12.33	1.23
	10^{12}	-	11	27.24	17.76	14.69	5.57	15.75	15.57	15.49	7.33	0.73

**, Increasing inhibition over the control in percentage; **, Total hatch represented mean number of cumulative juveniles hatched after 240. Hrs.- Initial number of egg-masses = 10 egg-masses; uniform size, age and undifferentiated.*

percentage (6.0 and 12.68%) as compared with other treatments; *S. cerevisiae* (14.33 and 30.28%), *P. guilliermondii* (17.33 and 36.62%), *C. albidus* (18.33 and 38.73%) and *S. roseus* (26.67 and 56.35%) and control (47.33). While in second conc. data recorded showed highest reduction in *S. cerevisiae*, followed by *C. albidus*, *S. roseus*, *P. guilliermondii* and *C. oleophila*. While the highest conc. showed no any significant reduction (P≤0.05) in number of second-stage juveniles hatched from egg-masses and hatching inhibition percentage in all treatments, results ranged from (7.33 and 21.83%) in *Sporobolomyces roseus* to 10.33 and 15.49 % in *C. oleophila* compared with control (47.33). Finally, in the end of test data showed that using of yeasts culture filtrate in high conc. 10^{12} showed significantly reduction (P≤0.05) in mean number of second-stage juveniles hatched from egg-masses and hatching inhibition compared with non-treated control.

Evaluation of some yeasts culture filtrate on the mobility and mortality of M. javanica in vitro

The effect of previous yeasts culture filtrate of each on mortality of *Meloidogyne javanica* were evaluated in laboratory conditions. Data presented in (Tables 3 and 4) revealed that, all the tested culture filtrate caused significant differences (P≤0.05) in the dead number of second-stage juveniles and mortality percentages at all treatments, compared to the non-inoculated control. In all treatments and control after six hours of exposure no dead second-stage juveniles were observed. But results showed significant differences in other concentrations. After 12 hours at 10^3 the best result was observed in *C. albidus* that equal with *P. guilliermondii* in dead number of second-stage juveniles and mortality percentages (1.33 and 0.66%) and (1.33 and 0.66%), respectively, and moderate effect was seen in *C. oleophila* (2.33 and1.68%) followed by *S. cerevisiae* (2.0 and1.34%), and maximum effect was exhibited by *S.roseus* (3.0 and 2.34%), respectively as compared with control (0.67). In second conc. 10^6 data showed significantly differences between treatments and control but not found in between treatments where the means ranged between (2.0 and1.34%) in *C. oleophila* shared by *P. guilliermondii* also followed by (3.33 and 2.68%) in *S. roseus*. In third conc. 10^{12} no significant difference was observed between the treatments that differed from the previous conc. Generally, the mortality rates of juveniles increased with an increase in exposure time. Where, increased the exposure time to 24 hours, no difference was observed in the three concs, where in first conc. *C. albidus* recorded (4.67 and 0.35%) and in second conc. (5.33 and 1.04%), while in third conc. *S.roseus* recorded (5.67 and 1.40%) as best results. After 48 hours culture filtrate of *C. albidus* proved most effect with (5.33 and 1.38%) and in second conc. 10^6 the results remain close to the low conc. Where *S. roseus* (3.67 and 0.34%) and in highest conc. 10^{12} *C. oleophila* with (8.33 and 4.51%), respectively as compared with control in this period (4.00).

Table 3: *Evaluation of some yeasts culture filtrate on the mobility and mortality of M. javanica in vitro.*

Treatments	The dead second-stage juveniles after six exposure time (Hours)																	
	6 H			12 H			24 H			48 H			72 H			96 H		
	10^3	10^6	10^{12}	10^3	10^6	10^{12}	10^3	10^6	10^{12}	10^3	10^6	10^{12}	10^3	10^6	10^{12}	10^3	10^6	10^{12}
C. oleophila	-	-	-	2.33 AB(a)	2.00 AB(a)	1.33 A(ab)	5.00 A(a)	5.00 A(a)	7.33 A(a)	6.00 A(ab)	6.00 A(ab)	8.33 AB(a)	6.00 AB(b)	6.33 A(b)	11.67 AB(a)	7.33 AB(b)	7.67 A(b)	15.33 A(a)
C. albidus	-	-	-	1.33 BC(b)	3.33 A(a)	1.33 A(b)	4.67 A(a)	5.33 A(a)	8.00 A(b)	5.33 A(b)	6.00 A(b)	13.00 A(a)	5.67 AB(b)	8.33 A(b)	15.67 A(a)	7.33 AB(b)	9.33 A(b)	17.00 A(a)
P. guilliermondii	-	-	-	1.33 BC(a)	2.00 AB(a)	1.33 A(a)	6.33 A(a)	6.00 A(a)	7.67 A(a)	8.33 A(a)	7.67 A(a)	8.67 AB(a)	11.33 A (a)	6.33 A(b)	11.67 AB(a)	13.00 A(a)	8.00 A(b)	12.00 AB(a)
S. cerevisiae	-	-	-	2.00 ABC(a)	2.00 AB(a)	2.00 A(a)	4.67 A(a)	4.67 A(a)	8.00 A(a)	7.00 A(b)	6.00 A(b)	12.00 A(a)	7.67 AB(b)	6.67 A(b)	14.67 AB(a)	6.67 AB(b)	5.67 A(b)	17.33 A(a)
S. roseus	-	-	-	3.00 A(a)	3.33 A(a)	2.33 A(a)	4.67 A(a)	4.00 A(a)	5.67 A(a)	6.33 A(ab)	3.67 B(b)	9.67 AB(a)	8.33 AB(ab)	4.67 B(b)	10.00 B(a)	8.33 AB(b)	6.33 A(b)	11.00 AB(a)
Control	-			0.67 C(B)(b)c			4.33 A(B)c			4.00 B(b)			5.00 B(C)b			5.67B(A)b(c)		
LSD (0.05)	-	-	-	1.51	1.62	1.72	3.30	3.08	5.35	5.13	3.88	4.01	5.40	4.68	4.37	6.31	5.98	6.20

Each figure represents the mean of three replicates. Capital letters represented significantly between all conc. In all treatments, and small letters represented significantly test between three conc. to alone yeast filtrate according to Duncan's multiple-range test (P ≤ 0.05). Values followed by the same letter are not statistically different according to Duncan's multiple-range test (P ≤0.05).

After 72 hours effects were evident significantly between the treatments and the control, where *P. guilliermondii* achieved best effect on dead number of second-stage juveniles and mortality percentages by (11.33 and 6.66%) flowed by *S. roseus* (8.33 and 3.50%) and in last order *C. albidus* (5.67 and 0.7%). In the end of test at four days (96 h) in low conc. *P. guilliermondii* achieved more significant differences effect with (13.0 and 7.77%) flowed by *S. roseus* (8.33 and 2.81%), in second conc. *C. albidus* achieved (9.33 and 3.87%) as best data followed by *C. oleophila* with (7.67 and 2.12%), while in high conc. the best recorded data was associated with *S. cerevisiae* followed by *C. albidus* (17.33 and 12.36%) and (17.0 and 12.01%), respectively as compared with control in this period (5.67).

Table 4: *Evaluation of yeasts filtrates on percentage mortality of the second stage juveniles of Meloidogyne javanica.*

Treatments	Conc.	Mortality (%)					
		6 Hrs	12 Hrs	24 Hrs	48 Hrs	72 Hrs	96 Hrs
C. oleophila	10^3	-	1.68	0.70	2.09	1.05	1.75
	10^6	-	1.34	0.70	2.09	1.40	2.12
	10^{12}	-	0.66	3.13	4.51	7.02	10.24
C. albidus	10^3	-	0.66	0.35	1.38	0.70	1.75
	10^6	-	0.66	1.04	2.09	3.50	3.87
	10^{12}	-	0.66	3.83	9.37	11.23	12.01
S. cerevisiae	10^3	-	1.34	0.35	3.12	2.81	1.06
	10^6	-	1.34	0.35	2.09	1.75	0
	10^{12}	-	1.34	3.83	8.33	10.17	12.36
S. roseus	10^3	-	2.34	0.35	2.42	3.50	2.81
	10^6	-	2.68	0.34	0.34	0.34	0.69
	10^{12}	-	1.68	1.40	5.90	5.26	5.65
P. guilliermondii	10^3	-	0.66	2.09	4.51	6.66	7.77
	10^6	-	1.34	1.74	3.32	1.40	2.47
	10^{12}	-	0.66	3.50	4.86	7.02	6.71

Initial number of juveniles = 150 second stage juveniles; fresh hatching and undifferentiated.

Greenhouse assay

Measurement of Meloidogyne javanica criteria

The influence of previous yeasts culture filtrates against *M. javanica* infecting squash plants were evaluated under greenhouse conditions. Data presented in Table 5 revealed that, all the tested treatments caused significant reduction (P≤0.05) in the nematode criteria; numbers of root galls, developmental stages, nematodes in soil, number of egg-mass, number of eggs per egg-mass, final population, rate of nematode reproduction and percentages of nematode reduction as compared with control. Where data indicated that treatment with *P. guilliermondii* filtrate at (10^{12}) caused the best effect on the calculated nematode criteria, rate of nematode reproduction and reduction percentages (2.51 and71.46%), respectively as compared with non-treated control (8.79), respectively, except the number of juveniles in soil (226) where that effect not noted compared to control which recorded (324). Followed by treatment with *S. roseus* at (10^{12}) which showed (5.01 and 42.95 %), then in third treatment with *C. albidus* at (10^3) by (5.23 and40.50 %) in same previous criteria with noting obvious significant effect on the number of juveniles in soil (175) as compared to other treatments and control. And in last ranked treatment with *C. oleophila* at (10^3) which caused (5.42 and 38.33 %) and *S. cerevisiae* at (10^6) which caused (5.83 and 33.70%).

Measurement of plant growth

One the other hand, all tested treatments decreased the negative effect of nematodes and an enhancement in the growth characters of squash plants, but the effect varied through all tested culture filtrates. Although the effects varied depending on examined concentrations, generally represent a positive effect to reduce the nematodes that were able to penetrate the plants. Data presented in Table 6 reported that the highest increase percentage in shoot and root length and weight were recorded in treatment with *P. guilliermondii* filtrate at (10^{12}) which caused (37.06% and 50.23%) and highest root length and weight (85.40 and 24.57%) as compared to control treatment followed by treatment with *S. roseus* at (10^6) in shoot length and weight (34.71% and 38.12%) and root length and weight (82.59 and 20.53%) (Table 6).

The results obtained in this study showed the use of some yeasts fungi culture filtrate: *C. oleophila*, *C. albidus*, *P. guilliermondii*, *S. cerevisiae* and *Sporobolomyces roseus* caused significant reduction on the root-knot nematode *M. javanica*, this was explained by the reduced hatching and mortality juveniles *in vitro*. Similar results were obtained by Shawky *et al.* (2006) who reported that all the bioagent candidates *S. uvarum* and *S. ludwigii* proved harmful to *M. javanica* juveniles, egg-masses, but the effect differed from one candidate to another. As general, the lethal action of toxic compounds produced by microorganisms on egg in vitro noted by Meadows *et al.* (1989). Data showed that, after six hours of exposure no second-

stage juveniles hatching were observed which means that all the treatments have no effect on the egg-masses as compared with control (distilled water only), which achieved mean (1.33) J_{2S} hatched from egg-masses, but with increased exposure time to 12 hours significant reduction was observed between treatments and control but no significant reduction between treatments. And with increased exposure time to 24 hours data began to increase showed significantly reduction (P≤0.05) in means number of second-stage juveniles hatched from egg-masses and hatching inhibition percentage in all treatments as compared with control. Similar results were obtained by Mohamed *et al.* (2008) indicated that the application with the yeast isolates *P. guilliermondii* and *C. albicans* treatments significantly reduced the number of juveniles in vitro after both 24h and 48h. This study showed that by using culture filtrate of *P. guilliermondii* as bio-agents to root-knot nematode, as reported by Jijakli and Lepoivre (1998) who noted that *P. guilliermondii* was found to show high effect which may be refer to the activity of β-1,3-glucanase enzyme that could result in the degradation of the cell walls (Jijakli *et al.*, 1999; Masih *et al.*, 2001). On the other side, the influence of previous yeasts culture filtrate against root-knot nematodes, *M. javanica* infecting squash plants caused significant reduction (P≤0.05) in the nematode criteria compared with control, and an enhancement in the growth characters of squash plants, according to Karajeh (2013), who reported that different yeast strains are promising biocontrol agents for different crops against root-knot nematode infection, which reduced nematode reproduction and increased plant growth parameters (Wu, 2015). The increase growth characters in all treatments are partially due to the effect of the tested culture filtrate on the nematode; besides its role in plant nutrition as suggested by Akhtar and Malik (2000). Where data indicated that treatment with *P. guilliermondii* filtrate caused the best effect on the nematode criteriam, followed by treatment with *Sporobolomyces roseus* then in third treatment with *C. albidus*, and in last ranked treatment with *C. oleophila* and *S. cerevisiae*. Similar results were obtained by Shawky *et al.* (2006) who reported that among all the bioagent filtrates, *Saccharomyces* spp. proved harmful to *M. javanica* juveniles, egg-masses and numbers of galls but the effect magnitude differed from one filtrate to another and an enhancement in plants growth. Also, Karajeh (2013) reported that the application of *S. cerevisiae* as soil drench treatment led to an obvious reduction of root galling caused by *M. javanica* and resulted in reducing the nematode reproduction ability on cucumber under growth room and field conditions and the yeast was more effective at increase in concentration. Karajeh (2014) noted that the effect of the yeast fungus *S. cerevisiae* on tomato lead to a significant reduction of root galling and root-knot nematode reproduction ability as compared to the untreated control. Applied the filtrate of *S. cerevisiae* at high dilution (10^{12}) given the best reduction on the root-knot nematode *M. javanica* as hatching and mortality juveniles in vitro, this agreement with Youssef and El-Nagdi (2012). Application of *S. cerevisiae* at the highest concentration caused the highest percentage reduction of juveniles and galls followed by the moderate and the lowest concentrations (Ismail, 2014).

Table 5: *Evaluation of some yeasts filtrate on root knot nematode Meloidogyne javanica infecting squash plants under greenhouse conditions.*

Treatments	Conc.	Nematode criteria								
		No. of galls/root system	No. of nematode			No. of egg masses/ root	No. of eggs/egg mass	Final population	NRR	Nematode reduction %
			In soil (250 g)	In root	Egg laying female					
Candida oleophila	10^{3}	22 b	145 c	153 a	123 b	107 ab	148 bc	16257	5.42	38.33
Cryptococcus albidus	10^{6}	15 c	175 bc	120 bc	94 bc	95 b	161 ab	15684	5.23	40.50
Pichia guilliermondii	10^{12}	14 c	226 bc	76 c	66 c	53 c	135 c	7523	2.51	71.46
Saccharomyces cerevisiae	10^{6}	26 b	238 b	163 a	148 b	99 b	171 ab	17478	5.83	33.70
Sporobolomyces roseus	10^{12}	13 c	197 bc	90 c	81 bc	90 b	163 ab	15038	5.01	42.95
Control	-	76 a	324 a	132 b	163 a	130 a	198 a	26359	8.79	-
LSD	-	7.26	9.72	8.61	3.59	3.98	7.29	-	-	-

Means at each column followed by the same letter are not significantly different at (P≤0.05) according to Duncan multiple range test. NRR: Nematode rate of reproduction = Pf/Pi

Table 6: *Plant growth response to some yeasts filtrate against root knot nematode Meloidogyne javanica infecting squash plants under greenhouse conditions.*

Treatments	Rate	Growth characters							
		Shoot				Root			
		Length (cm)		Weight(g)		Length (cm)		Weight(g)	
		Treated	Increase %	Treated	Increase%	Treated	Increase %	Treated	Increase %
C. oleophila	10^3	40.27**	18.45	24.80ns	11.22	27.90**	56.75	12.63ns	10.79
C. albidus	10^{12}	36.83 ns	8.33	30.05*	34.76	22.70**	27.53	12.30 ns	7.90
P. guilliermondii	10^{12}	46.60**	37.06	33.50**	50.23	33.00**	85.40	14.20*	24.57
S. cerevisiae	10^6	38.00ns	11.77	26.40ns	18.39	24.20**	35.96	12.70 ns	11.41
S. roseus	10^6	45.80**	34.71	30.80**	38.12	32.50**	82.59	13.74*	20.53
Control	-	34.00	-	22.30	-	17.80	-	11.40	-

= Significant at 0.05 level of probability; **= Highly significant at 0.05 level of probability; ns= Non significant at 0.05 level of probability.*

Generally, several yeasts fungi (commercially available products or natural) have shown significant disease reduction through various mechanisms to reduce pathogen development either directly through antagonism of soil borne pathogens or indirectly by eliciting a plant-mediated resistance response (Suzzi *et al.*, 1995; Singh, 2014; Nourani *et al.*, 2015), where Punja and Utkhede (2003), reported that yeasts produce secondary metabolites and enzymes that demonstrate toxicity against plant parasitic nematodes; these results suggested that enzymes or other active compounds produced by the fungal culture filtrates exhibit activity against specific stages in life cycle of nematode (Shinya *et al.*, 2008; Li *et al.*, 2015; Bogner *et al.*, 2016) and including loline alkaloids, pyrrolopyrazine, and organic acids that may account for activity against some phytoparasitic nematodes (Porter *et al.*, 1994; Bush *et al.*, 1997). Youssef and Soliman (1997) reported that the effect of yeast on *M. incognita* might be due to the activity of *S. cerevisiae* to convert carbohydrates to ethyl alcohol and CO_2 toxic to nematodes (Mostafa, 2004; Youssef and El-Nagdi, 2012) Linoleic acid was identified as a nematotoxic compound from *Arthrobotrys conoides* and *Arthrobotrys oligospora* (Meyer, 1995). The nematicidal properties may be referred to the fact that most microorganisms act against plant parasitic nematodes by means of metabolic byproducts, enzymes and toxins (Chernin and Chet, 2002). The effects of these toxins include the suppression of nematode reproduction, egg hatching and juvenile survival, as well as the direct killing impact on nematode itself (Siddiqui, 2005).

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Novelty Statement

The current study demonstrated the ability of yeast culture filtrates to ssuppress root-knot nematodes in addition to increasing plant health.

Author's Contribution

El-Sagheer, A. conceived designed and performed the experiments, analyzed data, wrote, and reviewed the paper. El-Mesalamy, A. reviewed the first draft. Anany, A. designed the experiment and reviewed the final draft. And Mahmoud N. reviewed the final draft.

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References

Akhtar, M., and Malik, A., 2000. Roles of organic soil amendments and soil organisms in the biological control of plant-parasitic nematodes: A review. Bioresour. Technol., 74(1): 35-47. https://doi.org/10.1016/S0960-8524(99)00154-6

Ashraf, M.S. and Khan, T.A., 2010. Integrated approach for the management of *Meloidogynejavanica* on eggplant using oil cakes and biocontrol agents. Arch. Phytopathol. Plant Prot., 43(6): 609-614. https://doi.org/10.1080/03235400801972434

Azzam, S., Karam, N., Hameed, K., Goussous, S., Maraqa, A. and Makhadmeh, I., 2012. Investigation of indigenous plant root associated bacteria and yeast isolates for plant growth promoting ability. Jordan J. Agric. Sci., 8: 1-14.

Barker, K.R. and Koenning, S.R., 1998. Developing sustainable systems for nematode management. Ann. Rev. Phytopathol., 36(1): 165-205. https://doi.org/10.1146/annurev.phyto.36.1.165

Bogner, C.W., Kariuki, G.M., Elashry, A., Sichtermann, G., Buch, A.K., Mishra, B., and Schouten, A., 2016. Fungal root endophytes of tomato from Kenya and their nematode biocontrol potential. Mycol. Prog., 15(3); 30. https://doi.org/10.1007/s11557-016-1169-9

Boneti, J. and Ferraz, S., 1981. Modificação do método de Hussey and Barker paraextração de ovos de Meloidogyneexigua de raízes de cafeeiro. Fitopatol. Brasil., 6(3): 553.

Bush, L.P., Wilkinson, H.H. and Schardl, C.L., 1997. Bioprotective alkaloids of grass-fungal endophyte symbioses. Plant Physiol., 114(1): 1. https://doi.org/10.1104/pp.114.1.1

Cayrol, J.-C., Djian, C. and Pijarowski, L., 1989. Study of the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyceslilacinus*. Rev. Nematol., 12(4): 331-336.

Chernin, L., and Chet, I., 2002. Microbial enzymes in the biocontrol of plant pathogens and pests. Enzymes in the environment: Activity, ecology, and applications, 171-226. https://doi.org/10.1201/9780203904039.ch7

Chitwood, B.G., 1949. Root-knot nematodes. Part 1. A revision of the genus *Meloidogyne goeldi*, 1887. Proc. Helminthol. Soc. Washington, 16(2): 90-114.

Christie, J.R., and Perry, V.G., 1951. Removing nematodes from soil. Proc. Helminthol. Soc. Washington, 18(2): 106-108.

Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11(1): 1-42. https://doi.org/10.2307/3001478

El-Boghdady, M.E., 1993. Integrated postharvest diseases management of certain pome fruits [Anna apple].

Fan, Q. and Tian, S., 2001. Postharvest biological control of grey mold and blue mold on apple by *Cryptococcus albidus* (Saito) Skinner. Postharv. Boil. Technol., 21(3): 341-350. https://doi.org/10.1016/S0925-5214(00)00182-4

Fernández, C., Rodríguez-Kábana, R., Warrior, P. and Kloepper, J.W., 2001. Induced soil suppressiveness to a root-knot nematode species by a nematicide. Biol. Contr., 22(2): 103-114. https://doi.org/10.1006/bcon.2001.0961

Hashem, M., Omran, Y.A. and Sallam, N.M., 2008. Efficacy of yeasts in the management of root-knot nematode *Meloidogyneincognita*, in Flame Seedless grape vines and the consequent effect on the productivity of the vines. Biocontr. Sci. Technol., 18(4): 357-375. https://doi.org/10.1080/09583150801950568

Hussey, R.S. and Barker, K.R., 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Dis. Rep., 57: 1025-1028.

Ismail, A.E., 2014. Growing *Jatrophacurcas* and *Jatrophagossypiifolia* as ainterculture with sunflower for control of *Meloidogynejavanica* in Egypt. Int. J. Sustain. Agric. Res., 1(2): 39-44.

Jijakli, M.H., and Lepoivre, P., 1998. Characterization of an exo-β-1, 3-glucanase produced by Pichiaanomala strain K, antagonist of Botrytis cinerea on apples. Phytopathology, 88(4): 335-343. https://doi.org/10.1094/PHYTO.1998.88.4.335

Jijakli, M.H., Lepoivre, P., and Grevesse, C., 1999. Yeast species for biocontrol of apple postharvest diseases: an encouraging case of study for practical use. In Biotechnological approaches in biocontrol of plant pathogens. Springer, Boston, MA. pp. 31-49. https://doi.org/10.1007/978-1-4615-4745-7_2

Karajeh, M.R., 2013. Efficacy of Saccharomyces cerevisiae on controlling the root-knot nematode (*Meloidogynejavanica*) infection and promoting cucumber growth and yield under laboratory

and field conditions. Arch. Phytopathol. Plant Prot., 46(20): 2492-2500. https://doi.org/10.1080/03235408.2013.799819

Karajeh, M.R., 2014. Enhancement of tomato growth, yield and resistance to the root-knot nematode (*Meloidogynejavanica*) after the field application of Saccharomyces cerevisiae. Hell. Plant Prot. J., 7(1): 35-41.

Koenning, S., Overstreet, C., Noling, J., Donald, P., Becker, J. and Fortnum, B., 1999. Survey of crop losses in response to phytoparasitic nematodes in the United States for 1994. J. Nematol., 31(4S): 587.

Li, J., Zou, C., Xu, J., Ji, X., Niu, X., Yang, J., and Zhang, K.Q., 2015. Molecular mechanisms of nematode-nematophagous microbe interactions: Basis for biological control of plant-parasitic nematodes. Ann. Rev. Phytopathol., 53: 67-95. https://doi.org/10.1146/annurev-phyto-080614-120336

Masih, E.I., Slezack-Deschaumes, S., Marmaras, I., Barka, E.A., Vernet, G., Charpentier, C., and Paul, B., 2001. Characterisation of the yeast Pichiamembranifaciens and its possible use in the biological control of Botrytis cinerea, causing the grey mould disease of grapevine. *FEMS Microbiol. Lett.*, 202(2): 227-232. https://doi.org/10.1111/j.1574-6968.2001.tb10808.x

Mayer, A., 1995. (Please give reference title). Ph. D. thesis, University of Kaiserslautern, Kaiserslauten.

Meadows, J., Gill, S. and Bone, L.W., 1989. Factors influencing lethality of Bacillus thuringiensiskurstaki toxin for eggs and larvae of Trichostrongy luscolubriformis (Nematoda). J. Parasitol., pp. 191-194. https://doi.org/10.2307/3282765

Meyer,S.L.F.,Massoud,S.,Chitwood,D.andRoberts, D., 2000. Evaluation of Trichodermavirens and Burkholderiacepacia for antagonistic activity against root-knot nematode, *Meloidogyne incognita*. Nematology, 2(8): 871-879. https://doi.org/10.1163/156854100750112815

Mohamed, H., Omran, Y.A., and Sallam, N.M., 2008. Efficacy of yeasts in the management of root knot nematode *Meloidogyne incognita*, in flame seedless grape vines and the consequent effect on the productivity of the vines. Biocontr. Sci. Technol., 18(4): 357-375. https://doi.org/10.1080/09583150801950568

Mostafa, E.A.M., 2004. Effect of spraying with ascorbic acid, vitamin B and active dry yeast on growth, flowering, leaf mineral status, yield and fruit quality of grand nain banana plants. Ann. Agric. Sci. Ain Shams Univ., 49(2): 643-659.

Nourani, S.L., Mohammadi-Goltapeh, E., Safaie, N., JalaliJavaran, M., Pourjam, E., Shams-Bakhsh, M., and JahanshahiAfshar, F., 2015. The effects of *Arthrobotrysoligospora* and *Arthrobotrysconoides* culture filtrates on second stage juvenile mortality and egg hatching of Meloidogyne incognita and *Meloidogynejavanica*. J. Crop Prot., 4: 667-674.

Porter, J.K., Bacon, C.W., and White, J.F., 1994. Chemical constituents of grass endophytes. Biotechnol. Endophytic Fungi Grasses, pp. 103-123. https://doi.org/10.1201/9781351070324-8

Punja, Z.K. and Utkhede, R.S., 2003. Using fungi and yeasts to manage vegetable crop diseases. Trends Biotechnol., 21(9): 400-407. https://doi.org/10.1016/S0167-7799(03)00193-8

Shawky, S., El-Shennawy, R. and Shady, A., 2006. Biological control of *Meloidogynejavanica*on tomato plants with isolated bioagent in Egypt. J. Agric. Sci. Mansoura Univ., 37: 6049-6063.

Shinya, R., Aiuchi, D., Kushida, A., Tani, M., Kuramochi, K., and Koike, M., 2008. Effects of fungal culture filtrates of *Verticilliumlecanii* (*Lecanicillium* spp.) hybrid strains on *Heteroderaglycines* eggs and juveniles. J. Invert. Pathol., 97(3): 291-297. https://doi.org/10.1016/j.jip.2007.11.005

Siddiqui, Z.A., 2005. PGPR: Prospective biocontrol agents of plant pathogens. In PGPR: Biocontrol and bio-fertilization, pp. 111-142. https://doi.org/10.1007/1-4020-4152-7_4

Singh, H., 2014. Management of plant pathogens with microorganisms. Proc. Natl. Acad. Sci., 80: 443-454. https://doi.org/10.16943/ptinsa/2014/v80i2/55120

Southey, J.F., 1986. Laboratory methods for work with plant and soil nematodes. London, UK, Her Majesty's Stationery Office, pp. 202.

Suzzi, G., Romano, P., Ponti, I., and Montuschi, C., 1995. Natural wine yeasts as biocontrol agents. J. Appl. Bacteriol., 78(3): 304-308. https://doi.org/10.1111/j.1365-2672.1995.tb05030.x

Wu, B., 2015. Effects of mixed application of microorganisms on vegetable growth. Dissertation of Soil and Environmental Science, Zhongxing University, pp. 1-86.

Youssef, M. and Soliman, M.M., 1997. Effect of integrated management on *Meloidogyne incognita* infecting Egyptian henbane, *Hyoscyamusmuticus* and on subsequent cowpea plant. 1st Scientific Conference of Agricultural Sciences, Fac. Agric., Assiut Univ., Assiut.

Youssef, M.M.A., and El-Nagdi, W., 2012. Effect of biologically active bread yeast on controlling *Meloidogyne incognita* infesting green bean and on the yield quantity and quality. Selçuk J. Agric. Sci., 25(4): 30-33.