

Biological Control of Root-Knot Nematode (*Meloidogyne javanica*) by Using Commercial Dry Yeast on Eggplant *Solanum melongena* in Halabja Province, Iraq

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Abstract: The fungal biocontrol agent using dry yeast was evaluated for its potential to control the root-knot nematode (*Meloidogyne javanica*) on eggplant *Solanum melongena*. In open field, soil samples were taken from a depth of 30 cm under soil surface for nematode analysis and the results showed that the soil was epidemic with root-knot nematodes, therefore, a plan was applied to use four concentrations of dry yeast (3, 6, 9 and 12 g/l) comparing with control which used only water. These treatments were applied in a factorial R.C.B.D. design on eggplant (Jawaher F1) with triplication. Statistical analysis was done by XLSTAT program. Water quantities were controlled by an irrometers and kept around field capacity. Root galling and larvae were measured by sieve method and the results showed that nematode activity in concentrations of 9 and 12 g/l had been inhibited by decreased number of second stage juveniles from 879 to 411 and from 879 to 274 juveniles/kg, respectively with significant differences ($P \leq 0.05$). Also, a significant results increasing was obtained in fresh weigh of shoot system (2050 g), fresh weigh of root system (845 g), plant height (102 cm) and yield (97 ton/hectare) when nematode used in concentration of 12 g/l.

Keywords: Biological control, Eggplant, *Meloidogyne javanica*, Yeast, Plant-parasitic nematode

Introduction

Using nematicides to decrease the activities of plant-parasitic nematodes is an expensive way and may cause a high environmental risk to non-target organisms and applicators (Thomason, 1987). So, there was a need to develop alternative control measures to manage plant-parasitic nematode under field conditions (Jansen et al., 2002; Karajeh, 2013). The use of natural and safe agents may promote growth of crops and induce their resistance to many diseases. *Saccharomyces cerevisiae* in dry yeast is considered as a promising yeast fungus

for promoting plant growth of different crops (Shalaby & El-Nady, 2008). In the last decades, it became an effective alternative to chemical fertilizers safely used for human, animal and environment (Omran, 2000). According to ITIS (2020), *Saccharomyces cerevisiae* Hansen, 1883 belongs to the family Saccharomycetaceae, order Saccharomycetales, class Saccharomycetes, phylum Ascomycota, and kingdom Fungi of the domain Eukarya. Table 1 demonstrates the elements and the contents of the dry yeast.

Table 1: Elements and contents of the dry yeast.

Compound	mg/g	Compound	mg/g
Carbohydrates	82	Magnesium	2
Total Nitrogen	90	Phosphate	1-13
Nitrogen humid acid	40	Potassium	30
Magnesium	2	Sodium	56
Copper	0.05	Zinc	0.05
Calcium	0.1	Cobalt	0.005
Iron	0.05		

This effective role due to its content of cytokinins and tryptophan (a precursor of indole acetic acid), the yeast suggested playing a beneficial role in cell division and enlargement and on vegetative plant growth and fruit yield (Nassar et al., 2005).

El-Tarabily (2004) found out that *Rhizoctonia solani* infection of sugar beet plants was suppressed by yeasts. Yeast were tested for biological control of post-harvest diseases of fruits and vegetables (Punja, 1997; Zheng et al., 2003) as well as against molds of stored grains (Pettersson et al., 1999) and to control powdery mildews (Urquhart & Punja, 1997). Use of yeast fungi as biocontrol agents of soil-borne plant pathogens and plant growth promoters has been recently investigated (El-Tarabily & Sivasithamparam, 2006; Azzam et al., 2012).

The yeast fungus (*S. cerevisiae*) reduced infection of the nematode *Meloidogyne incognita* on Egyptian henbane and increased its growth (Youssef & Soliman, 1997). Yeast also is considered as a natural growth stimulator because of its richness in nucleic acid proteins, carbohydrates, vitamins, lipids and different minerals as well as its improvement of phosphorus and manganese uptake by the plant roots (Mekki & Ahmed, 2005).

Infective juveniles of nematodes are capable of finding their host in response to chemical stimuli from the insects. The bacteria multiply within the body cavity of the infected host insect and cause rapid death of the insect. The nematodes feed on the bacteria within the insect cadaver and reproduce several times before emerging to find new insect hosts. That the nematode and the bacterium cannot survive independently of one another which plays a part in the decision to exempt this microbial pesticide (Nickle & Welch, 1985).

Overcoming nematode environmental sensitivity appears to be the most critical factor restricting wide use of insect-pathogenic nematodes as nonchemical insecticides. Nematode effectiveness will be greatest where moisture and temperature conditions can be optimized for example in greenhouses suppression of soil-inhabiting insects has been most consistent when nematodes have been applied as a drench to potted plants and containerized nursery stock (Bedding & Miller, 1981) under conditions where insects and nematodes are confined when the soil surface is moist and shaded from sunlight and where competition with other organisms is minimized. Nematodes remain effective in conjunction with most insecticides such as pyrethrins and methoxychlor and with rotenone and diatomaceous earth (Pettersson et al., 1999). However, some organophosphates and insecticides such as phenamiphos, carbofuran and oxamyl, adversely affect nematode development and reproduction (Hara & Kaya, 1982) and this incompatibility must be considered when using nematodes in an integrated pest management program.

In the present research, we try to give an understanding about the importunity of protecting plant and soil biologically by using commercial dry yeast to control the negative activation of root-knot nematodes.

Material and Methods

The study included five treatments each with three replicates; T1 = control used only water, T2 = dry yeast 3 g/l, T3 = dry yeast 6 g/l, T4 = dry yeast 9 g/l and T5 = dry yeast 12 g/l.

These treatments were applied as shown below.

T1 = Using of only water for irrigation and spring.

T2 = Spray the transplants with 3 g/l yeast after 15 days of transplanting.

Spray the plants with 3 g/l yeast after flowering.

Spray the plants with 3 g/l yeast after flower setting.

Spray the plants with 3 g/l yeast after first harvesting.

T3 = Spray the transplants with 6 g/l yeast after 15 days of transplanting.

Spray the plants with 6 g/l yeast after flowering.

Spray the plants with 6 g/l yeast after flower setting.

Spray the plants with 6 g/l yeast after first harvesting.

T4 = Spray the transplants with 9 g/l yeast after 15 days of transplanting.

Spray the plants with 9 g/l yeast after flowering.

Spray the plants with 9 g/l yeast after flower setting.

Spray the plants with 9 g/l yeast after first harvesting.

T5 = Spray the transplants with 12 g/l yeast after 15 days of transplanting.

Spray the plants with 12 g/l yeast after flowering.

Spray the plants with 12 g/l yeast after flower setting.

Spray the plants with 12 g/l yeast after first harvesting.

For chemical and physical soil properties, analyzing soil samples was undertaken from the research location. The results of the physico-chemical properties of the soil are presented in Table 2. The analyses of the organic matter were performed according to Schnitzer & Khan (1978). Total nitrogen of the soil was determined by the Kjeldahl method (Kjeldahl, 1883). Phosphorus was measured according to Olsen et al. (1954). Extractable Calcium, Potassium, Magnesium and Sodium were measured according to Thomas (1982).

Table 2: Soil analysis before treatments.

Soil texture	Organic matter (ppm)	pH	E.C. (ds/m)	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Na (ppm)	Cl (ppm)
Clay loam	1295	7.0	0.39	821	12.3	2.1	9.3	19.4	2.3	7.9

Study Area

Halabja province (Figure 1) is located at 35°12'N 46°00'E in Kurdistan region, Iraq, 90 km east of Sulaimaniyah province and 714 km northeast of Baghdad province. With an elevation of 900 m, this area is considered as one of the most fertile areas in whole Iraq.

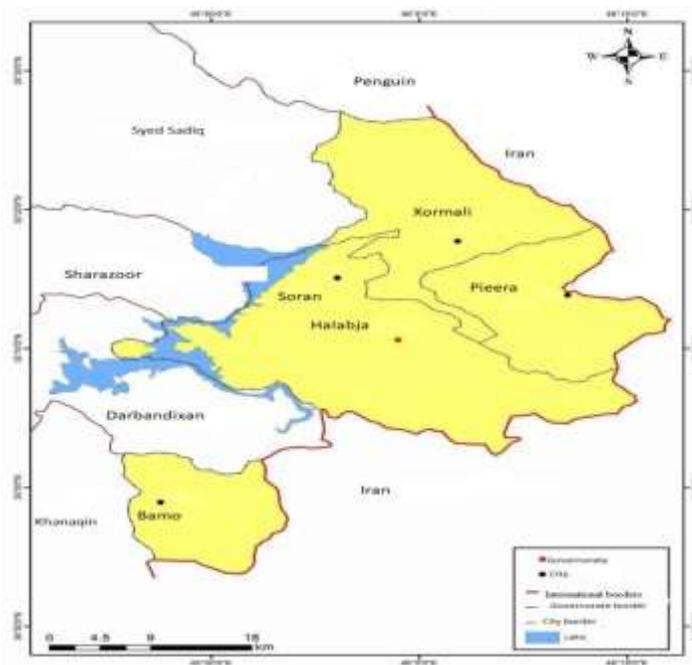


Figure 1: Map of the study location in Halabja province.

Field Distribution of Treatments

- Type of the research design (R.C.B.D.), numbers of treatments = 5
- Statistical analysis was done by XLSTAT program.
- Duncan's multiple ranges test at 0.05 was used for comparison among treatments

- Numbers of replications = 3, distance of treatments (sow line) = 6 m.
- Width of terraces = 0.6 m, high of the terraces = 0.3 m.
- Area of plot = 11 m², numbers of sow lines for each treatments = 3 lines
- Numbers of guard lines = 2, numbers of plants in each plot = 30 plants.
- Numbers of plants selected for parameters and data's = 9 plants.
- Eggplant varieties (Jawaher F1), Treatments Field distributions
- Nematode larvae were isolated and counted from soil as indicated in Table 3.

Table 3: Procedure of nematode analysis.

No.	Materials	Procedure and Source
1	20-mesh sieve (833 µm aperture)	Howard Ferris, Departments of Entomology and Nematology, University of California (from Zuckerman et al., 1981)
2	200-mesh sieve (74 µm aperture)	
3	325-mesh sieve (43 µm aperture)	
4	Coarse sieve (1 cm aperture)	
5	Two stainless steel bowls or plastic buckets	
6	250 ml beaker, 600 ml beaker	
7	Coarse spray wash bottle or tube attached to faucet	

Results and Discussion

The observed larval density of soil in treatments T1, T2, T3, T4 and T5 was recorded before the initiation of the experiment at soil depth of 25-30 cm (Table 4). Such density is higher than the recommended density for cucumber (200-250 larva/1 kg soil). The soil was contaminated with the nematode *M. javanica*.

Table 4: Soil analysis for nematode larvae of *Meloidogyne javanica* before cultivation.

Treatments	Population of nematode before treatments/kg soil
T1 Control	730
T2 3 g/l yeast	844
T3 6 g/l yeast	905
T4 9 g/l yeast	879
T5 12 g/l yeast	781

The results showed a significant effect in the numbers of juveniles that decreased from 1320 in control treatment to 274 when using 12 g/l dry yeast and 411 in 9 g/l dry yeast after 75 days from transplanting (Table 5). This result was returned to the fungus *S. cerevisiae* in the commercial yeast which made some changes in the roots antagonistic to root-knot nematodes because high level of humic acid and sugars in the plant roots increasing the resisting plant for nematodes (Karajeh, 2013). Also, the two humic acid serine and phenylalanine work as nematodes inhibitors and improve the adsorption capability of roots and produce some lignin's and phenols as suggested by Attyia & Youssry (2001).

Table 5: Effect of dry yeast to control number of nematode juveniles biologically.

Treatments	No. of nematodes before transplanting/kg	No. of juveniles/kg soil after 30 days from transplanting	No. of juveniles/kg soil after 75 days from transplanting
Control	730 ^b	890 ^d	1320 ^d
3 g/l yeast	844 ^a	712 ^b	623 ^c
6 g/l yeast	905 ^a	774 ^{bc}	577 ^c
9 g/l yeast	879 ^a	605 ^b	411 ^b
12 g/l yeast	781 ^b	453 ^a	274 ^a

Means with different letters are significantly different according to Duncan's multiple ranges test at $P \leq 0.05$.

Table 6 showed significant effects on numbers of galls that decreased from 38 galls/plant roots when using 12 g/l to 5 galls/plant roots after 75 days and 5 galls/plant roots when using 9 g/l after 75 days. The negative effect of yeasts on *M. javanica* might be due to the ability of the yeast fungus to convert carbohydrates to ethyl alcohol and CO₂ that are toxic to nematodes (Hashem & Abo-Elyousr, 2011). Commercial product, containing cells of *S. cerevisiae*, at the rate of 5 g/plant significantly affected J2s of *M. incognita* in soil and root galling in squash and increased yield of the plant under field conditions (Noweer & Hasabo, 2005).

Field application of *S. cerevisiae* progressed tomato and crop growth and increased nematode resistance to tomato authenticity by increasing their total phenolic root content in a manner similar to the externally applied hydrogen peroxide (Karajeh, 2014).

Table 6: Effect of different concentrations of dry yeast on the number of galls.

Treatments	No. of galls/plant roots before transplanting	No. of galls/plant roots after 30 days from transplanting	No. of galls/plant roots after 75 days from transplanting
Control	14 ^a	26 ^d	38 ^c
3 g/l yeast	15 ^a	12 ^c	10 ^b
6 g/l yeast	16 ^b	9 ^b	6 ^a
9 g/l yeast	15 ^a	10 ^b	5 ^{ab}
12 g/l yeast	18 ^a	12 ^a	5 ^a

Means with different letters are significantly different according to Duncan's multiple ranges test at $P \leq 0.05$.

Increasing in eggplant growth and yield of *S. cerevisiae* that treated plants may be due to the indirect effect of the yeast on the nematode infection besides the yeast direct role in promoting plant growth and development (Akhtar & Alam, 1990). Also, this investigation showed that the growth enhancing effect of yeast application

might be due to the yeast that produced cytokinins which are responsible for increasing the production and accumulation of soluble metabolites. Alam et al. (1977) reported that mechanism of dry yeast as biocontrol activity may involve competition for nutrients, site exclusion parasitism and induced resistance or make physical and chemical soil properties unsuitable for plant pathogens.

Table 7 showed a significant effect on fresh weight of shoot system which increased from 710 g when using 12 g/l to 2050 g and to 1670 g when using 9 g/l. Also, this table indicated that using commercial dry yeasts (9 and 12 g/l) was more effective than using yeasts 3 and 6 g/l in reducing *M. javanica* infection on fresh weight of root system of eggplant and their final population in field soil phytotoxicity accompanied to human when using dry yeast up to 736 and 845 g, respectively compared to control treatment which was 300 g.

Using of dry yeast had significant effect on plants as higher rises compared to control were recognized in the treatments 9 and 12 g/l in the lengths of the plants which reached 89 and 102 cm, compared to the control which was 60 cm (Table 7). This table also showed that using yeast with concentrations of 9 and 12 g/l has significant effect on mild root system weights (736 and 845 g, respectively). Yield reached 86 and 97 ton/hectare respectively, compared to control treatment which was 70 ton/hectare. Such positive effects on the root plant resistance against the plant parasitic nematodes led to improve root health and weight gain (Hashem et al., 2008). Originality the vegetative growth parameters of *Azadirachta indica* (plant height, stem and root and fresh and dry weights) were significantly affected by all foliar uses with yeast extract at 5, 10 and 15 g. (Taha et al., 2016). The effect of promoting yeast extract can be attributed to its effect on metabolism, biological activity, photosynthetic pigments and enzyme activity that in turn promotes vegetative growth (El-Sherbeny et al., 2007).

Table 7: Effect of dry yeast on shoot and root systems weight, plant height and yield of eggplant.

Treatments	Fresh weight of shoot system (g)	Fresh weight of root system (g)	Plant height (cm)	Yield (ton/hectare)
Control	710 ^e	300 ^e	60 ^d	70 ^c
3 g/l yeast	850 ^d	390 ^d	64 ^{cd}	74 ^c
6 g/l yeast	1120 ^c	575 ^c	74 ^c	78 ^{bc}
9 g/l yeast	1670 ^b	736 ^b	89 ^b	86 ^b
12 g/l yeast	2050 ^a	845 ^a	102 ^a	97 ^a

Means with different letters are significantly different according to Duncan's multiple ranges test at $P \leq 0.05$.

Conclusion

Application of the yeast *S. cerevisiae* was used as a treatment to affect population of the nematode *M. javanica* and root gall formation on eggplant through its effects

on nematode infection and reproduction and also through inducing plant resistance and enhancing fruit production of eggplant under field conditions.

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