Effect of Saccharomyces cerevisiae and Spirulina platensis on Suppressing Root-knot Nematode, Meloidogyne incognita Infecting Banana Plants under Greenhouse Conditions

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ABSTRACT

Banana production is severely hindered by plant-parasitic nematodes (PPNs) worldwide. Chemical nematicide, oxamyl is a systematic compound widely applied for the control of PPNs. Because of increase pests' problems, harmful effects on human and environment caused by chemical pesticides and also increased consumer requirements for safe crops have encouraged research on bio-pesticides production. So, the effect of different stress of the yeast, Saccharomyces cerevisiae and blue green algae (cyanobacteria), Spirulina platensis were examined on banana plants infected with root-knot nematodes, Meloidogyne incognita. All tested treatments were effective methods to manage M. incognita in soil samples and banana roots. Notably, the usage of S. cerevisiae and S. platensis in combination treatment revealed the greater antagonistic action on M. incognita in potted banana. In addition it has an avail advantage over artificial nematicides by having several means to attack the RKN and mainly enhanced plant growth compared to any other treatments. Yet, the previous treatment had increased catalase (CAT) enzyme activity and reduced pectin methyl esterase (PME) activity in banana plant that leads to inhibit the count of the RKN. Generally, our results supply a novel knowledge to understand of PPNs management as a new approach for applying bio-fertilizer and bio-control of the parasitic nematodes.

Keywords: RKN, *Meloidogyne incognita*, banana, yeast, cyanobacteria, PME, CAT, bio-control.

Introduction

Bananas (*Musa* spp.) are planted as a main crop in many tropical and sub-tropical countries (De la Torre-Gutierrez et al., 2008). Plantain crop production is affected by many pathogenic and non-pathogenic agents (Rahman et al., 2010; Gamliel and van Bruggen, 2016), especially plant-parasitic nematode (PPN) that cause rhizome damage leads to reduce water, micro and macro-nutrient compounds uptake (Moens et al., 2004; Mwauraa et al., 2017), resulting in decreased growth criteria, yield and

finally plant death (Karssen et al., 2013). Many synthetic nematicides actually used to inhibit PPN, have created to possess highly toxicity affecting human health and environment (Gupta, 2011; Rani et al., 2017). Therefore, the use of these components have created undesirable effects, high costs and restrictive (Rockström et al., 2016). Hence, it is remarkable to discover an effective alternative methods to include safety food production and environmentally ecofriendly crop production (Naz et al., 2016). Applicability of some yeasts are used for controlling fungal diseases on fruits (Nally et al., 2012; Pesce et al., 2018), and also using as bio-control agents for post-harvest diseases of many crops (Punja 1997; da Cunha et al., 2018). In a similar study by Karajeh, 2013, high concentration of total phenolics compounds in roots of some yeasts-treated plants and hydrogen peroxide (H₂O₂)-treated plants provides a sign on the function of the yeast to induce resistance of the plants against pests. In addition the yeasts are considered as a natural enhancement of plant growth due to presence of compounds such as cytokinin and tryptophan, which playing an important role in plant cell division (Nassar et al., 2005; El-Tarabily and Sivasithamparam, 2006; Ignatovaa et al., 2015). In contrast, application of some cyanobacteria (blue green algae) in soil may inhibit PPN infestation (Chandel, 2009; Holajjer et al., 2012) by producing hydrolytic enzymes and secondary metabolites called cyanotoxins (Gupta et al., 2013). For example, stimulation of plant defense occurs by producing of both hydrolytic enzymes and anti-microbial metabolites (Babu et al., 2015). Microalgae, especially cyanobacteria have abilities of the atmospheric nitrogen fixationas a good biofertilizers (Prasanna et al., 2013; Hamouda and El-Ansary, 2017; El-Ansary and Al-Saman, 2018), due to of some mineral nutrient compounds in cynobacteria (Faheed and El-Fattah, 2008; Rana et al., 2012; Prasanna et al., 2016). Notable, Spirulina platensis have a vigorous inhibitor on root-knot nematodes (RKN) and also possess a powerful stimulating effect on the plant growth (Sharaf et al., 2016). A novel study based on yeast and cyanobacteria for controlling the RKN, Meloidogyne incognita through potted banana, was conducted.

MATERIALS AND METHODS

Nematode analysis

Eggs of *Meloidogyne incognita* were extracted from pure culture propagated on tomato (*Solanum lycopersicum* cv. Castle Rock) roots infected with the nematode using sodium hypochlorite, NaOcl solution (Hussey and Barker, 1973). Second-stage juveniles (J2s) were collected daily from eggs and were stored at 15°C. The J2s used in the experiments were less than 5 days old.

Yeast preparation

Saccharomyces cerevisiae (SH02) was obtained from Microbiology Lab of the Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat City, Egypt. One gram of *S. cerevisiae* was inoculated in 200 ml YPD medium (Yeast extract peptone dextrose – 1% yeast extract, 2% peptone, 2% glucose, 2% agar, 0.01% ampicillin, and 0.01% nalidixic acid dissolved in 1 L of distilled water) under sterilized conditions and incubated at 30°C for 24 (Azeredo et al., 1998). The culture was centrifuged for 5 min at 5000 rpm and washed two times with sterilized distilled water then the pellets were collected and dried in oven until constant weight.

Algal preparations

Blue green algae (Cyanobacteria), *Spirulina platensis* were obtained from Microbiology Lab of the GEBRI. *S. platensis* (15ml) was inoculated in 100ml Zarrouk medium (Zarrouk, 1966) at 25±1°C with continuous illumination using cool white fluorescent tubes (2500 Lux). Cells were collected by filtration using filter paper and was washed with distilled water and then dried in oven.

Treatments

The treatments are used at the following: **A**, *S. cerevisiae* ($\frac{1}{2}$ S= 2g/4kg soil); **B**, *S. cerevisiae* (S= 4g/4kg soil); **C**, *S. platensis* ($\frac{1}{2}$ S= 2g/4kg soil); **D**, *S. platensis* (S= 4g/4kg soil), **E**, *S. cerevisiae* ($\frac{1}{2}$ S) + *S. platensis* ($\frac{1}{2}$ S), **F**, Nematicides check, Oxamyl (Vydate 24% L); **G**, Check with infection; **H**, Check without infection.

Greenhouse infection assay

Two months old banana plants cv. Grande-Naine were obtained from the Tissue Culture Lab of the GEBRI. Plantlets were planted in 30 cm diameter plastic pots containing about 4.0 kg of sterilized soil (1:3 mixture of clay: sand) with pH 7. Twenty pots were treated with previous treatments after preparation, with treatment "A" and "C" in concentration of 2g/4kg soil; treatment "B" and "D" in concentration of 4g/4kg soil; and finally No. "E" is a mixture between "A" and "C". Four number of inoculated pots was treated with 1ml of oxamyl (nematicies check) per pot. Bananas were treated by current applications every month for two month. The remaining four inoculated pots served as inoculated and untreated-nematode control (check with infection). Moreover, four more pots served as control without nematode (check). The tested materials were added to the soil in 3 holes around the plantlet and followed by the addition of 50 ml of water. The pots were arranged in a completely randomized design in a greenhouse of the GEBRI. Plants were evaluated after 60 days of inoculation. The roots were washed carefully by tap water to remove the soil particles and then stained with Phloxine B (3.5 g in 750 ml distilled water + 250 ml acetic acid 5%) solution for 5 min to facilitate counting of females and egg masses in the root. Nematode variables observed per the total root weight were galls, females and egg masses. Also, juveniles per 250 g soil were extracted according to Cobb's sieving and decanting method, using sieves (60 mesh and 325 mesh) (Cobb, 1918). The banana growth variables shoot and root length (cm); also shoot, root and corm fresh weight (g) were also recorded.

Determination of enzyme activities

One gram of banana tissue leaves was homogenized in 20ml of 50mM potassium phosphate buffer (ppb), pH 7.0 at 25 °C. These triturated tissues were removed through four layers of cheese cloth and the filtrate was centrifuged at 20,000 rpm at 4 °C for 15 min. The supernatant served as an enzyme extract for enzyme assay of catalase and pectin methyl esterase using the UV-Vis spectrophotometer (Shimadzu Corporation-MultiSPec-1501).

A- Catalase (CAT) activity

The CAT activity was determined according to the method given by Beers and Sizer (1952). The reaction mixture was formed by mixing 2.90 ml of hydrogen peroxide solution 0.036% (w/w) in phosphate buffer (the absorbance of the solution

should be about 0.5 absorbance units at 240nm) to 0.1ml of enzyme solution. The time required for the A_{240} to decrease from 0.45 to 0.40 absorbance units was recorded. Catalase activity was calculated as: Units/ml enzyme= (3.45)*(df) / (t)*(0.1)

Where:

3.45= corresponds to the decomposition of 3.45 µmoles of hydrogen peroxide in a 3.0 mL reaction mixture producing a decrease in the A_{240} from 0.45 to 0.40 df = dilution factor

t = min(s) required for the A_{240} to decrease from 0.45 to 0.40 absorbance units 0.1= enzyme solution volume

B- Pectin methyl esterase (PME) activity

The PME activity was determined according to the continuous spectrophotometric method given by Hagerman and Austin (1986). The reaction mixture was formed by mixing 2.3 ml of 0.3% pectin solution in 0.1 M NaCl, 0.5 ml of 0.01% of bromothymol blue in 3 mM sodium phosphate buffer (pH 7.5), and 0.1 mL of enzyme extract. The corresponding control sample contained enzyme extract obtained from untreated tissue.

The change in absorbance at 620 nm was monitored for 10 min in a UV/VIS spectrophotometer (UV-200-RSLW scientific) with a constant temperature working at 20° C. PME activity was determined as units of absorbance min⁻¹ per g fresh tissue. Units = $(Ab_{(620)} \text{ sample} / Ab_{(620)} \text{ control})*(df)/(t)*(0.1)$

Where:

df = dilution factor; t = min(s); 0.1 = enzyme solution volume

Statistical analysis

All data were subjected to analysis of variance (ANOVA) (Sokal and Rohlf, 1995). Significance of the variable mean differences was determined using Duncan's multiple range tests ($p \le 0.05$). All analyses were carried out using SPSS 16 software.

RESULTS

Bio-nematicidal action

Influence of the treatments on the RKN, M. incognita in banana plants resulted in a clear depression of the nematode numbers. Counts of galls, females and egg masses in the roots and second stages (juveniles2, J2s) in the soil were recorded compared to the nematode check (check with infection) (Table 1). In general, all of the used applications stimulated the greatest curative results and significantly ($p \le 0.05$) inhibited the count of J2s in soil; galls, females and egg masses in root. With regard to "D" treatment exhibited the highest inhibition of nematodes numbers in soil (84.51% reduction, %R), while the lowest effective treatment in decreasing J2s in soil was recorded in "A" treatment (74.93% R) compared to "G" treatment (nematode check). No significant differences were achieved among "B", "C" and "E" treatments in number of galls and recovered parasitic stages in roots (Fig. 1).

Table 1. Numbers of *Meloidogyne incognita* in soil and root samples under different stresses of the yeast *Saccharomyces cerevisiae* and cyanobacteria, *Spirulina platensis*.

Treatments	Count p	Count per 250 g soil		
	Galls	Females	Egg masses	Juveniles 2
A	471.37d	452.10c	369.15c	1728.75c
В	382.37cd	391.52bc	333.48bc	1227.5b
С	296.11bc	363.64bc	311.85bc	1674.5c
D	279.49bc	308.72ab	239b	1067.75b
Е	231.28ab	257.55ab	299.04bc	1115.25b
F	153.45a	186.79a	122.51a	767.25a
G	912.38f	1054.08d	897.31d	6895d

Means followed by the same letter(s) within a column are not significantly different ($p \le 0.05$) according to Duncan's multiple range test. Each value represents means of four replicates.

A= S. cerevisiae ($\frac{1}{2}$ S= 2g/4kg soil); B= S. cerevisiae (S= 4g/4kg soil); C= S.platensis ($\frac{1}{2}$ S= 2g/4kg soil); D= S. platensis (S= 4g/4kg soil), E= S. cerevisiae ($\frac{1}{2}$ S) + S. platensis ($\frac{1}{2}$ S), F= Oxamyl (Vydate 24% L); G= Check with infection.

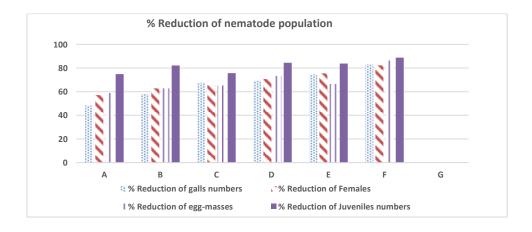


Fig. 1. Incidence (%) of nematode counts after application with different stresses of yeast and cyanobacteria.

A= S. cerevisiae ($\frac{1}{2}S = \frac{2g}{4kg}$ soil); B= S. cerevisiae ($S = \frac{4g}{4kg}$ soil); C= S. platensis ($S = \frac{4g}{4kg}$ soil); D= S. platensis ($S = \frac{4g}{4kg}$ soil), E= S. cerevisiae ($\frac{1}{2}S = \frac{2g}{4kg}$ soil); D= S. platensis ($S = \frac{4g}{4kg}$ soil), E= S. platensis ($S = \frac{4g}{4kg}$ soil); D= S. platensis ($S = \frac{4g}{4kg}$ soil); D=

% Reduction= percent reduction compared with nematode only (check with infection)

Evaluation of banana growth criteria

Growth parameters of tested plants i.e., weight and length of shoots and roots and weight of corms are listed in Table (2). All tested applications as well as control without infection enhanced slightly plant health with no significant differences compared to control (G treatment). Notable, the greatest outcome on the all plant

measurements was given by treatment "E". Relatively, the treatment "B" had lesser effect in increasing corm weight and shoot length compared to "F" treatment. No notable increase in root weight measurements was commented

Table 2. Effect of different stresses of *Saccharomyces cerevisiae* and *Spirulina platensis* on banana growth infected with *Meloidogyne incognita*.

Treatments	Shoot		Root		Corm
	Length	Weight	Length	Weight	Weight
A	42.25ab	33.62b	13.25a	12.21a	13.65a
В	41.25ab	36.73b	17.00a	9.83a	11.23a
С	48.00ab	41.63b	11.25a	8.75a	14.45a
D	44.75ab	44.11b	28.00ab	18.67a	17.06ab
Е	49.00ab	51.86b	15.00a	22.06a	32.41ab
F	42.00ab	38.73b	17.25ab	10.53a	13.23a
G	39.50a	29.25a	18.50ab	16.14a	11.29a
Н	50.00b	50.26b	20.25ab	27.19a	43.08b

Means followed by the same letter(s) within a column are not significantly different ($p \le 0.05$) according to Duncan's multiple range test. Each value represents means of four replicates.

A= S. cerevisiae ($\frac{1}{2}$ S= 2g/4kg soil); **B**= S. cerevisiae (S= 4g/4kg soil); **C**=S. platensis ($\frac{1}{2}$ S= 2g/4kg soil); **B**= S. cerevisiae ($\frac{1}{2}$ S) + S. platensis ($\frac{1}{2}$ S), **F**= Oxamyl (Vydate 24% L); **G**= Check with infection; **H**= Check without infection.

Activity of catalyze (CAT) and pectin methyl esterase (PME) enzymes to depress RKNs, *M. incognita*

The data presented in Figure (2), demonstrate that all applications increased the CAT enzyme especially in the "E" treatment. Consequently, it is obvious that increasing the activity of CAT enzyme had inhibited the count of J_2 s in soil; galls, females and egg-masses of the RKN, *M. incognita* in banana plants. Likewise the same "E" treatment had efficiently increased plant growth. In general the highest CAT activity (3081.5 μ /g) was obtained in "E" treatment, while the least enzyme activity was recorded in "A" treatment (1997.66 μ /g) as compared to check with infection, "G" (1738 μ /g).

Also, the data presented in Figure (3), revealed that the activity of PME was in parallel with the infection severity where the PME activity increased with the increasing of nematode infection severity. "G" treatment (check with nematode) had the highest PME with averages of 0.49 Δ Abs min⁻¹. At the same time both "E" and "G" treatments had the least PME activity with averages of 0.28 and 0.31 Δ Abs min⁻¹, respectively.

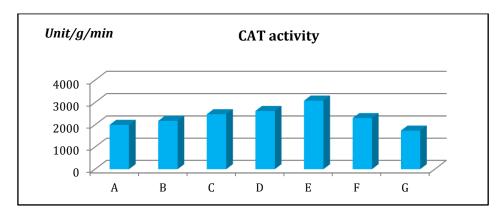


Fig. 2. Activity of CAT in banana plant as affected by RKN infection and applications of yeast, *Saccharomyces cerevisiae* and cyanobacteria, *Spirulina platensis*

A= S. cerevisiae (½S= 2g/4kg soil); B= S. cerevisiae (S= 4g/4kg soil); C= S.platensis (½S= 2g/4kg soil); D= S. platensis (S= 4g/4kg soil), E= S. cerevisiae (½S) + S. platensis (½S), F= Oxamyl (Vydate 24% L); G= Check with infection.

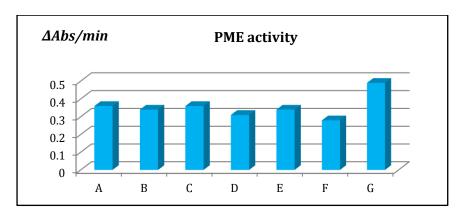


Fig. 3. Activity of MPE in banana plant as affected by RKN infection and applications of yeast, *Saccharomyces cerevisiae* and cyanobacteria, *Spirulina platensis*

A= S. cerevisiae ($\frac{1}{2}$ S= 2g/4kg soil); B= S. cerevisiae (S= 4g/4kg soil); C=S.platensis ($\frac{1}{2}$ S= 2g/4kg soil); D= S. platensis (S= 4g/4kg soil), E= S. cerevisiae ($\frac{1}{2}$ S) + S. platensis ($\frac{1}{2}$ S), F= Oxamyl (Vydate 24% L); G= Check with infection.

DISCUSSION

Antagonistic effects of the yeast and cyanobacteria on nematode development

Many chemical nematicides used to control PPNs, have undesirable effects, high costs and restrictive (Crow, 2005; Rockstrom et al., 2016). So, yields production is required to make natural compounds affecting pathogenic pests, especially PPNs in many crops. In this study, we have tested the effect of different stress of yeast, *S. cerevisiae* and cyanobacteria, *S. platensis* compared to the nematicides check, oxamyl against RKN, *M. incognita* in banana plants. In general, counts of *M. incognita* in the soil, J2s and in the roots, females and egg-masses as well as galls per root were significantly inhibited compared to the check with nematode infection. For instance, "D' treatment revealed the greatest inhibition of J2s number in soil (84.51% R). In contrast, the lowest effective agent in reducing J2s count in soil was "A" treatment (74.93% R) compared with of "G" treatment (untreated check with nematode). Blue

green algae (cyanobacteria), S. platensis have a potent inhibitor on RKN and possess powerful stimulating effect on the plant growth (Sharaf et al., 2016). Hence, cyanobacteria have some mechanisms for inhibiting the development of RKNs. For example, many forms of cyanobacteria are informed to create a large number of toxic compounds like, nodularins, microcystins, neurotoxins (Holajjer et al., 2013; Gaget et al., 2017) and benzoic acid which produced by microalgae (Uzaira et al., 2018). Cyanobacteria can also depress pathogenic agents by distraction of the cytoplasmic membrane and suppression of the protein creation (Swain et al., 2017). Furthermore, the effect of some yeasts on RKN might be due to the potency of these yeasts to change carbohydrates to produce ethyl alcohol and CO₂ that are highly toxic to RKN (Azhar et al., 2017). The other mechanisms of yeast to control activity for pests may introduce competition for some nutrients, parasitism and may induce resistance in plants; and also make physical and chemical soil structures undesirable for pathogenic agents to grow (Zhou et al., 2018; da Cunhaa et al., 2018; Pesce et al., 2018). Consequently, the treatment with the yeast, S. cerevisiae could inhibit of RKNs community and galls formation on cucumber root through its effects on the nematode life cycle to induce plant resistance (Karajeh, 2013).

Efficacy of the yeast and blue green algae on banana growth-promoting

Relatively, most treatments with yeast, *S. cerevisiae* and cyanobacteria, *S. platensis* promoted banana growth and this impact was highest on shoots and corms than on roots. The maximum outcome on the all banana-growth developments was given by treatment "E". Many studies recorded that the plant girth may be enhanced by yeasts (El-Tarabily and Sivasithamparam 2006; Botha, 2011; Ignatovaa et al., 2015). Yeast produce an assortment of naturally active components such as phytohormones, enzymes and auxin groups (indole-3-acetic acid, IAA) that have effectual promoting affect the plant health and help to enhance their yield production (Nassar et al., 2005; Ignatova et al., 2015; Moller et al., 2016). Furthermore, enhancement of plant growth and development is due to, the blue green algae, cyanobacteria having some mineral nutrient compounds and some active compounds like, auxins and gibberellins groups which affect plant health (Rana et al., 2012; Prasanna et al., 2016). In addition some species of cyanobacteria have abilities of the nitrogen fixation as a biofertilizer to promote plant growth (Holajjer et al., 2013; Genuárioa et al., 2017; Raia et al., 2018).

CAT and PME activities in banana plant diseased with RKN

All tested applications on banana leaves, increased the activity CAT enzyme particularly in "E" treatment (3081.5 μ /g). Consequently, the highest CAT enzyme activity in the previous treatment inhibited the count of all nematode parameters in soil and plants. Our results match with previous studies in which CAT enzyme is responsible for scavenging the excess of reactive oxygen species (ROS) in plant cells (Das and Roychoudhury, 2014). In addition CAT plays a vital role in scavenging the toxicity of hydrogen peroxide (H_2O_2) under nematode infection (Asada, 1992). In contrast the activity of PME was in parallel with the infection severity whereas the enzyme activity increased with increasing of nematode infection. For example, untreated infected plants had the highest PME with averages of 0.49 Δ Abs min⁻¹. While, "F" and "E" treatments had the least PME activities with average of 0.28 and 0.31 Δ Abs min⁻¹, respectively. Pectinolytic enzymes are a heterogeneous group of enzymes such as PMEs that catalyse the pectic substances present in plants releasing

acidic pectins and methanol. These enzymes allow pectin hydrolysis during plant cell growth through the infection by pathogenic agents (Gainvors et al., 1994). So, PME play a crucial role during plant-pathogen interactions and affects plant resistance for diseases. Thorpe et al. (2014) reported that the carbohydrate binding module (CBM) in PPN interacts with a host PME and over expression of either CBM or the PME increased through infection by parasitic-nematode.

Overall, in conclusion, results of the current *in vivo* trial established the effect of different stresses of yeast, *S. cerevisiae* and cyanobacteria, *S. platensis* on banana plant diseased with RKN, *M. incognita*. All applications are effective methods to control RKN in soil samples and banana roots. The use of *S. cerevisiae* and *S. platensis* in combination as a treatment showed highly antagonistic action on *M. incognita* in potted banana. It also has an avail over chemical nematicides by having several means to attack the previous pest and mainly enhance plant health compared to any other treatments. In addition increasing of CAT enzyme activity and reducing of PME activity in banana plant as a result of the used applications had inhibited the count of the RKN, *M. incognita*. In general these results supply an extra extensive to understand of PPNs management as a new approach for applying bio-fertilizers and bio-control agents of the current pest.

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الملخص العربي

تاثيرSaccharomyces cerevisiae وSpirulina platensis لتثبيط نيماتودا تعقد الجذور من نوع Meloidogyne incognita والتى تصيب نباتات الموز تحت ظروف الصوبة

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على المستوى العالمي فان عملية انتاج محصول الموز تتاثر كثيرا بخطورة النيماتودا المتطفلة على النبات. و حيث أن استخدام المبيدات الكيميائية النيماتودية و منها المركب الجهازي الأوكساميل و الذي يستخدم على نطاق واسع في مقاومة النيماتودا. و بسبب انتشار الأفات و التاثيرات الضارة على كل من الانسان و البيئة من استخدام المبيدات الكيميائية، بالاضافة الى زيادة الطلب على استخدام المحاصيل الامنة تطلب ذلك على التشجيع على انتاج مركبات طبيعية لمكافحة هذه الافات. لذلك فانه تم اختيار تاثير بعض المعاملات المختلفة من الخمائر من نوع S. cerevisiae على بنباتات الموز نوع S. platensis على نباتات الموز المصابة بنيماتودا تعقد الجذور من نوع M. incognita أطهرت كل المعاملات المختبرة فاعليتها في مكافحة من المحاملة بنيماتودا تعقد الجذور من نوع M. incognita أله المكونة من مخلوط ال S. cerevisiae و الجذور جدير بالذكر أن المعاملة المكونة من مخلوط ال S. cerevisiae واضح عن المبيدات الكيميائية في تثبيطها على النيماتودا، انها أيضا لها القدرة على تحفيز نمو النبات تمثلك تاثير واضح عن المبيدات الكيميائية في تثبيطها على النيماتودا، انها أيضا لها القدرة على تعفيز نمو النبات مقارنة بالمعاملات الأخرى. علاوة على ذلك فان المعاملة السابقة تمتلك القدرة على زيادة تنشيط كل من انزيم الكتاليز و البكتين مثيل استيريز في نبات الموز والذي بدورة يؤدي الى تقليل أعداد نيماتودا. عموما فان النتائج التى حصلنا عليها ساهمت باضافة واسعة في فهم مكافحة النيماتودا المتطفلة على النبات كطريقة جديدة في التسميد الحيوي و المكافحة الطبيعية ضد النيماتودا.