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Management of the nematode of the nodule of *Meloidogyne incognita* in tomato (*Solanum lycopersicum* L.) with extracts in a biospace condition

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For the management of the nematode of Meloidogyne *incognita* nodule in plants of *Solanum lycopersicum* L., the effectiveness of plant extracts: *Ruta graveolens, Eucalyptus spp., Ocimum basilicum, Acacia farnesiana,* and *Nicotiana tabacum,* and as a control fungus *Paecilomyces lilacinus* 6.5×10^{13} UFC/g were used. For each treatment, 5000 nematodes J2 of *M. incognita*/plant were used. The extracts were applied per intervals of 10 days in three occasions. The assessed variables were: plant height, performance at eight cuts and efficiency of the extracts at 20 weeks after the transplant. The results show there were no significant differences among treatments for the plant height. Regarding the performance with *A. farnesiana,* it obtained the highest performance (18.46 kg m^{-2}), followed by *P. lilacinus* (6.5×10^{13} UFC/g) with 16.46 kg m^{-2}. Both treatments are statistically different from the treatments, control (12.91 kg m^{-2}), *R. graveolens* and *O. basilicum* (13.03 and 13.8 kg m^{-2}), respectively. Regarding the effectiveness for the reduction of the nematode in soil, *A. farnesiana* reduced it by 57% and *P. lilacinum* fungus by 50.5%. So, the use of vegetable extracts for the management of nematodes populations of the *Meloidogyne* gender is an alternative way because they act as repellents and cause the death of nematodes.

Key words: Oils, nematodes, nodules, tomato, extracts, management, tomato, effectiveness.

INTRODUCTION

S. lycopersicum L. tomato is considered the most important vegetable worldwide. Mexico takes the 12th place as a tomato producer, and the 2nd as an exporter. It is the most important product according to the Mexican farming exports (1.43 million ton). The average worldwide consumption per capita has an increasing tendency from 15.4 kg in 2001 to 20.2 kg in 2011. In Mexico, the tendency of the crop in the protected agriculture system SAGARPA (2016) has increased since 2005, by using different levels of crop technologies. In the state of Oaxaca, there is a register of 757.82 ha cultivated with this vegetable. However, 90% of the surface is grown in greenhouse soils of medium and low technology (Martínez-Gutiérrez et al., 2014).

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It has caused several problems both as such in the management of the crop, and in the phytosanitary aspect. Concerning the phytosanitary problems in the tomato crop there are the nematodes, which are rounded microscopic worms present in the root-knots. They form nodules that affect the growing of the plant and cause economic losses of 40-100% in the performance (Quiroga et al., 2007). *Nacobus aberrans* is the principal nematode species that causes damages to the tomato crops from Mexico, and also to other crops like chilli, beans, spinaches, baby squashes, amaranth (Cristóbal et al., 2001). The species of *Meloidogyne incognita, M. javanica* and *M. arenaria,* common in the protected crop system (Arias et al., 2009), are even more important, and are present in several crop zones in Mexico and in the state of Oaxaca (Cid del Prado et al., 2001).

There have been several evaluated control methods for the phytopathogenic nematodes with the use of microorganisms, with good effects of the growing of the tomato plant (Khalil et al., 2012), the same as the use of plant extracts with nematicide effects such as *Azadirachta indica, Tagetes* spp., *Brassica napus, Chrysanthemun* spp, *Calendula* spp, *Ricinus, Raphanus* (Collange et al., 2011), to which there were extracted their metabolites with diverse techniques. Some lilaceae such as *Allium cepa* and *Allium sativum* which contain sulphur were also used. It is hydrolyzed to form a variety of isocyanates with pesticide effects, fungicides, antibiotics, nematicides and toxic effects (Bekhiet et al., 2010), and others such as *Eucalyptus citriodora* Hook (Choi et al., 2007; El-Rokiek and El-Nagdi, 2011).

Chemical pesticides are expensive and not effective, besides having harmful effects for human health, water, soil, and crop products (Brand et al., 2010). Natural products have been considered an alternative solution to environmental problems caused by chemical pesticides and many researchers have tried to identify the most effective natural products to integrate them as control strategies instead of using the traditional solutions (Kim et al., 2005).

The objective of this research was to evaluate wild plants from the study site, such as aqueous extracts of *Ruta graveolens*, *Eucalyptus* spp., *Ocimum basilicum*, *A. fercian* and *Nicotiana tabacum,* to determine the nematicidal effect against *M. incognita* in the culture of *S. lycopersicum* under greenhouse conditions in a biospace.

MATERIALS AND METHODS

Study place

The research was conducted under greenhouse conditions by using a biospace of 300 m^2 surface in the experimental field of the Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca of the Instituto Politécnico Nacional from Mexico (CIIDIR Unidad Oaxaca IPN). Is geographical coordinates are 17°02' north latitude and 96°44' west longitude, with an altitude of 1,550 m above sea level.

Collection of plants and preparation of the plant extracts

The endemic plants of Ruda (*R. graveolens),* Eucalipto (*Eucalyptus* spp)., Albahaca (*O. basilicum),* Acacia (*A. farnesiana),* and Tabaco de Virginia (*N. tabacum)* were collected in the Central Valleys of Oaxaca, Mexico. The plants were dried in the shade per 10 days. Right away, 200 g of leaves from each plant were individually weighed, and 100 mL of distilled water was included and were blended per 30 s, except *A. farnesiana* where the pre-washed roots were used to remove any dust particles and were manually smashed in a mortar. The mixture of each preparation was put aside for 24 h. After this time, the mixture was filtered by using a multipore filter paper of 0.2 µm, and the concentrate was set in a 1000 mL beaker for its application. The extracts were prepared like the spore suspension of the entomopathogenic fungus *P. lilacinus*. The plants used in the experiments were deposited and identified in the herbarium of the CIIDIR Unidad Oaxaca IPN.

Extraction of the nematodes from infected plants

The extraction of the nematode's eggs and juvenile J2 stage of *M. incognita* of the infected roots was done through the macerate-filtration method (Hooper et al., 2005), by dividing 25 g of roots in pieces of 2-3 cm, which were blended mechanically with 100 mL of distilled water per 30 s. The plant blended material was filtered through a series of sieves of 35, 100, 200 and 400 µm diameter. The particles which retained the sieves of 60 and 100 µm were disposed. The precipitate of the retained eggs in the sieves of 200 and 400 µm was transferred to a beaker by using a pipette. The extracted eggs were hatched in Petri dishes per 9-10 days for the emergence of juvenile J2 stage (Whitehead and Hemming, 1965). Both the eggs and juveniles just hatched eggs were used for the field tests and their application in every treatment.

Experimental design and treatments

The experiment was established with seven treatments: T1: Control treatment (only nematodes); T2: Control treatment *P. lilacinus* fungus at $6.5x10^{13}$ UFC/g, and the extracts T3: *R. graveolens;* T4: *Eucalyptus* spp., T5: *O. basilicum* L.; T6: *A. farnesiana,* and T7: *N. tabacum* at the same doses of 35 mL per plant to each one. There were four repetitions per treatment, under a design per blocks completely aleatorized. There was used a variety of Reserve tomato of undetermined cycle at a planting density of 2 plants$\cdot m^{-2}$, distance among lines and plants of 1.25 m × 0.4 m, guided by a one single stem, and with a common management.

For each treatment, there were applied the amount of 5000 nematode eggs after 15 days of the transplant (ADT) to a depth of 10-15 cm right in the base of each plant. The fungus and the plant extracts were applied with an automated pipette of 1000 mL of capacity in three holes around the stem base of each plant at intervals of 10 days with a total of three applications from the 30 ADT.

The assessed variables were: The plant height, what was performed a destructive test after 20 weeks after the transplant (STD); the performance of the crop kg$\cdot m^{-2}$ considering 8 cuts and an effectiveness percentage of the treatments after 20 (SDT). The effectiveness of the applied treatments (EAT) with plant extracts was calculated as the percentage of reduction of the nematodes in 250 g soil according to the Henderson and Tilton Puntener's formula (Puntener, 1981), as follows:

$$EAT = \frac{(J2\ PTA)(J2\ PCB)}{(J2\ PTB)(J2\ PCA)} * 100$$

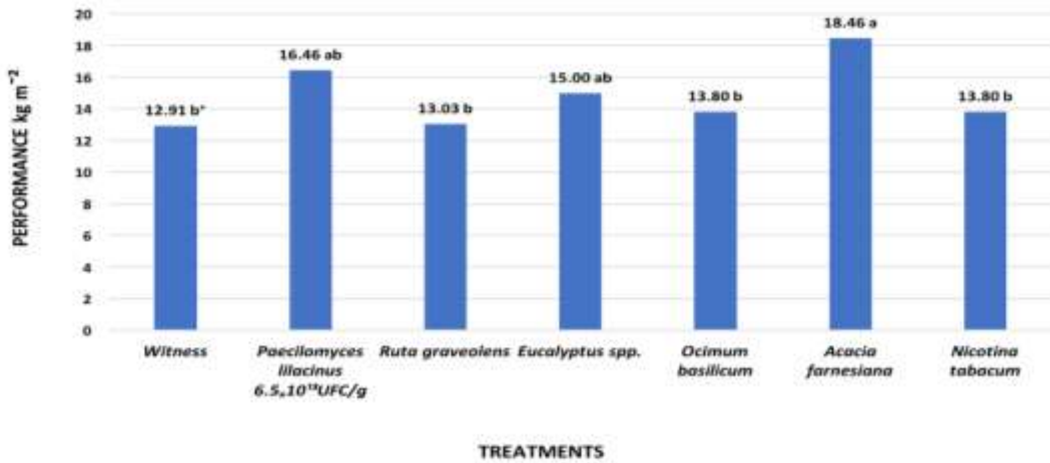

Figure 1. Effect of applications of plant extracts on the performance of *S. lycopersicum* tomato at eight cuts. *Means with same letters are not statistically different (Tukey, 0.05).

Where, *EAT* = Percentage of reduction of the nematodes, in %; *J2 PTA* = Total Population of J2 Nematodes after the application; *J2 PCB* = Total Initial Population of Nematodes of the control treatment, *J2 PTB* = Total Population of J2 Nematodes after the application, and *J2 PCA* = Total Population of Final J2 Nematodes of the control treatment.

Statistical analysis

With the obtained data, variance analysis, and multiple mean comparison tests were done. The efficiency percentage data were transformed by the arcsine function, and later a variance analysis was done to establish the differences among the means of the variables by the Tukey test. All analysis was done using the Statistical Analysis System package (SAS® Institute, 2004).

RESULTS AND DISCUSSION

Effect of plant extracts in the growing of *S. lycopersicum*

The growing of the *S. lycopersicum* plants was homogeneous in all the treatments, which indicates that the presence of nematodes did not affect directly the growing variables until the assessment time. This can be explained based on the Seinhorst's model, which indicates that, in presence of nematodes, the plants can show two effects: one of stimulation, and the other of inhibition or reduction. The plant is able to solve the damage and still to continue with the growing stimulation (Seinhorst, 1965). Niño et al. (2008) evaluated the response of the following population; 100, 200, 500 and 1,000 J2 of *Meloidogyne hapla/*100 cc soil on *Physalis peruviana,* and there were no differences found among treatments during the first samplings at 45 and 245 days. The highest negative effect on height was with 500 nematodes until seven months. Cadete et al. (2005) consider that the nematodes population increases proportionally to the food availability of their hosts, and it is also influenced by the fact that the adult plants with extensive radicular systems provide more food and shelter to the nematodes than the younger plants.

Effect of the plant extracts on the performance of *S. lycopersicum*

Figure 1 shows that the treatment with the application of *A. farnesiana* registered the highest performance with 18.46 kg·m^{-2} which was statistically different from the treatments with *R. graveolens, O. basilicum, N. tabacum* extracts, besides the control. They were overcome by more than 4 kg·m^{-2} of performance. The *P. lilacinus* fungus 6.5×10^{13} UFC/g as a control treatment showed a similar performance with *Eucalyptus* spp., and *A. farnesiana.* They are statistically the same (Figure 1). According to Sikora and Fernández (2005) when any method is used to control the nematodes populations, the performance can be affected until a 41.07% with a presence of 5000 nematodes at the beginning of the crop, and this reduction effect is due to the inhibition of the development of the root-knots which cannot absorb water or nutrients.

Effectiveness of the plant extracts in the reduction of the nematodes population

There were two treatments assessed in this work that showed effectiveness results for the reduction of nematodes in soil higher than 50%, the one with *P. lilacinus* 6.5×10^{13} UFC/g with a 50.5%, and *A. farnesiana*

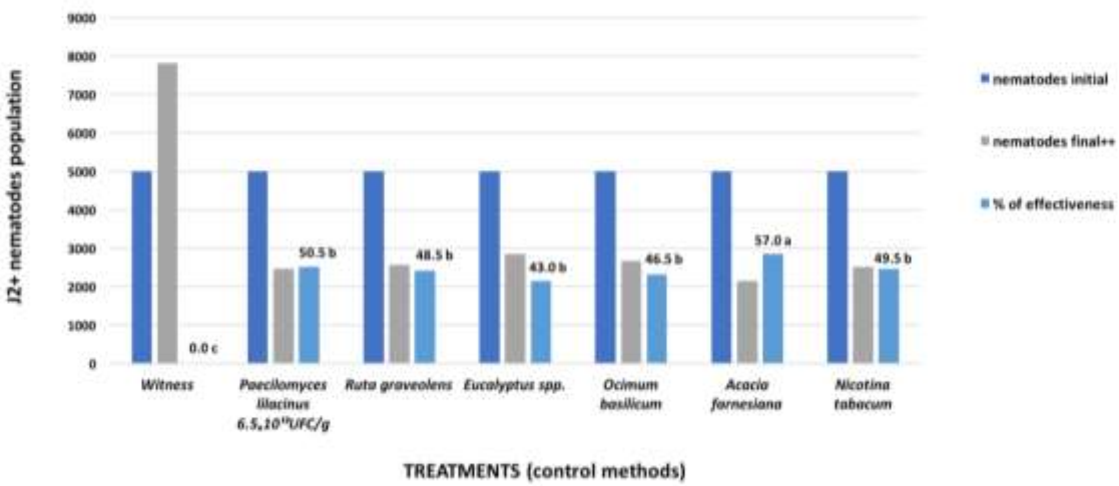

Figure 2. Effectiveness of plant extracts for the reduction of on-soil nematodes populations.

with a 57.0%. This unique one was statistically different from all the treatments. The results of the other assessed treatments had between 40.3-49.5% of effectiveness (Figure 2). It must be mentioned and highlighted that, in the control treatment, the quantity of nematodes increased by 43.28% in the final population of *M. incognita.* The assessment on this study of the treatment with *N. tabacum* presented 49.5% of effectiveness in similar studies with plants having nematicide effects. This is same with the ones done by Wiratno et al. (2009) when they assessed the nematicide activity of extracts from 17 plant species. The results with *N. tabacum* in lab tests obtained a mortality of 94% when testing the extracts from leaves in doses of 5 mg·mL^{-1} over a population of 150 J2 of *M. incognita* exposed for 24 h. Besides, there was registered a mean lethal concentration (LC_{50}) with 3.9 mg·mL^{-1}. The toxic activity of *N. tabacum was rep*orted by Nguyen et al. (2000), who mentioned that it has effects on the inhibition of the acetylcholinesterase just as it is the action of organophosphate and carbamate type of pesticides. Although our results showed that the extract of *A. farnesiana* was the best one compared to the others, it is not recommendable to apply it due to its high toxic activity that affects humans and mammals. Kamal et al. (2009) emphasized the nematicide activity of *Eucalyptus camaldulensis* against young stages of *M. incognita* under assessed in greenhouse conditions.

Hasabo and Noweer (2005) assessed *O. basilicum* for the control of *M. incognita* in *Solanum melongena* eggplant with a mortality percentage of 61% under laboratory conditions assessed at 24 h and of 46.1% in field conditions at 4 months, both to a concentration of 5%. Also, Elbadri et al. (2008) assessed 27 extracts from different plant species to determine their efficiency against juveniles of *M. incognita* in laboratory. As a conclusion, they said that all of them showed a level of toxicity over the nematodes, and specifically with *Acacia nilotica* (the pods extract) there was a percentage of mortality of 94.7%, and for *O. basilicum* 66.5% with the extract of leaves, and 55.5% with the seeds extract assesses after 72 h, both treatments in doses of 500 ppm.

According to the obtained results, the use of the extracts can be useful for the management of the *Meloidogyne* nematodes populations, because they act as growing regulators, in the feeding, repellent inhibitors, distractors, attractors, or to kill them in the *S. lycopersicum* crops. The nematicide effect of the plant extracts could be attributed to its content of certain oxygenated compounds that are characterized by its lipophilic properties, which are capable to dissolve the cytoplasmic membrane from the nematodes cells (Knoblock et al*.,* 1989).

The *P. lilacinus* fungus 6.5×10^{13} UFC/g showed a little higher effectiveness than the extracts (50.0%). The effectiveness of biological organisms, as the one used in this work has been documented on several research, such as Wen-Kun et al. (2016). When evaluating *P. lilacinus* in a mixture with *Syncephalastrum rasemosum*, it was found a 70% of ovicidal activity over *M. incognita,* and as a result, the reduction of gills in the roots in cucumber crops, and in tomato crops (Anastasiadis et al*.,* 2008). So this is one of the most effective organisms in its parasitic action over the *M. incognita* eggs in the tomato crop. Oka (2010) suggests its application in more than one occasion during the crop cycle to keep the nematode population under control and to obtain a better effectiveness (Kiewnick and Sikora, 2006; Udo, et al*.,* 2014).

Conclusion

The use of extract of *A. farnesiana* in at least three times during the early developing of the crop can improve the performance of tomato. With the application of the fungus *P. lilacinus* at concentration of 6.5×10^{13} UFC/g, it is possible to obtain good results regarding positive performance and controlling of nematodes on soil nodules.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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