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Original article

Poultry litter delays the development and reduces the population of *Meloidogyne javanica* in papaya

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ABSTRACT

The Carica papaya, is widely grown as a fruit crop in the all world. However, it suffers from an attack of root-knot nematodes. So the objective of this study was to evaluate the biology and control of Meloidogyne javanica in Carica papaya treated with neem oil, poultry litter, papaya seed extract, and papaya seed meal. Papaya (cv. Hawaii) seedlings were produced and maintained in a greenhouse in a completely randomized design. All treatments were incorporated into the soil. Two experiments were conducted, including one to assess nematode penetration into and development in the root system. Two thousand M. javanica juveniles/plant were inoculated for this assessment. The assessments were performed at 2, 4, 7, 9, 11, 18, 23, 28, 34, and 45 days after inoculation (DAI). Nematode penetration into the root system was not observed at 2, 4, or 7 DAI; second-stage juvenile (J2) penetration only occurred at 9 DAI. The second experiment consisted of 10 replicates. Seedlings were infested with 4.000 M. javanica eggs and J2 per plant. After 90 days, M. javanica stem diameter (mm), stem height (cm), and root fresh weight (g) were assessed in the soil and root; the data showed that poultry litter reduced the nematode population density, leading to the best results for the plant morphometric variables among the treatments tested.

1. Introducion

Papaya is a tropical plant that belongs to the family Caricaceae and has high commercial interest because of its fleshy fruit. This plant is widely grown in tropical and subtropical American countries because its fruit has high nutritional value. Brazil is the second largest producer of papaya in the world, with a production of 1.6 tons/year, trailing only India. Bahia (368.875 tons), Espírito Santo (311.150 tons), Ceará (115.525 tons), and Rio Grande do Norte (86.342 tons) are the Brazilian states with the largest papaya production (CNPMF, 2017).

High papaya production is only possible because of a number of measures, including soil management, irrigation, seed selection and treatment, and mechanization, among others. However, problems with diseases and pests also have increased with the expansion of papaya cultivation. The main papaya phytosanitary problems are caused by viruses, fungi, and mites, including the papaya meleira virus, ringspot, black spot, Phytophthora spp. damping off, red spider mite, broad mite, green leafhopper, whitefly, and cochineal. The root-knot nematode Meloidogyne javanica (Berkeley, 1855) is also a papaya pathogen, penetrating the plant's root system and affecting plant growth, quality, and quantity, thereby causing significant damage to papaya plants (Ferraz et al., 2010).

The life cycle of the root-knot nematode begins with the egg stage, with the formation of the first-stage juvenile (J1). The J1 will undergo ecdysis, reaching the second juvenile stage (J2), which occurs inside the egg. After hatching, the J2 begins to search for its host. Once the J2 finds the host, it penetrates the root and begins to feed, forming the feeding site. Subsequently, the nematode injects esophageal secretions into cell tissues, leading to cell hyperplasia and hypertrophy and the formation of giant cells, which are called galls. Then, the nematode becomes sedentary, undergoes three additional ecdyses, thereby yielding J3 and J4, and the last ecdysis, which yields the female or male. The male is not phytopathogenic and has an elongated, wormlike body and soon leaves the root. Conversely, the female remains inside the root and becomes globose and whitish. The oviposition period begins as soon as maturity is reached, and 500 eggs are laid in each egg mass, on average, over a 30-day biological cycle (Ferraz & Monteiro, 2011).

Chemical nematicides are the most used control method for these plant nematodes. However, these chemicals

are rather expensive and toxic to the environment, animals, soil microorganisms, and even human health (Ferraz & Freitas, 2008). In addition to the aforementioned disadvantages, no chemical nematicide for the papaya crop has been registered in the Brazilian Ministry of Agriculture, Livestock, and Food Supply thus far (MAPA, 2016).

Cultural control is effective against phytonematodes when substances toxic to these plant pathogens are employed. In addition, this type of control favors the soil chemical and biological structure and does not harm the environment (Gardiano, 2009).

Neem oil, extracted from the Indian plant Azadirachta indica, has insect-repelling and nematicidal activities (Baldin et al., 2012). Poultry litter incorporated into the soil is able to create unsuitable conditions for the growth of these plant pathogens because the nitrogen present as uric acid in this organic compost is subsequently converted into ammonia nitrogen, which acts as a microbial antagonistic that will control some plant parasitic nematodes (Lima et al., 2011).

Papaya seed extract and ground papaya seeds reportedly release toxic compounds, including the substance benzyl isothiocyanate, which has anthelmintic activity (Kermanshai, 2001), and studies have shown that papaya seed extract has a nematicidal effect (Neves, 2008). Thus, the objective of the present study was to assess the development and reproductive capacity of M. javanica in the papaya root system when treated with neem oil, poultry litter, papaya seed extract, or papaya seed meal.

2. Materials and methods

2.1 Set-up and performace of the first experiment

The experiment was set up and conducted in a greenhouse and at the Laboratory of Agricultural Nematology of the Federal Institute of Goias (Instituto Federal Goiano – IF Goiano) - Urutaí Campus, Urutai – Goias, Brazil. The experiment was arranged in a completely randomized design.

The inoculum was obtained from tomato plants of the (Tomate-Debora) type with a root system containing Meloidogyne javanica galls kept in an IF Goiano greenhouse at Urutai Campus. M. javanica eggs were extracted using the method of Hussey and Barker (1973) and placed in a hatching chamber consisting of a container with water and an immersed sieve serving as support for facial tissue, which was also in contact with water. The egg suspension was poured over the paper, and the J2, after hatching, passed through the paper and the sieve and were deposited at the bottom of the container by gravity. This suspension was collected with a Pasteur pipette, placed again in the Peters chamber, and observed under an optical microscope to allow counting of the eggs (Cliff & Hirschmann, 1985). Every 24 hours, the suspension in the hatching chamber was poured onto the Peters' chamber attached to the microscope and quantified. Every 24 hours, the suspension from each hatching chamber was retrieved for J2 collection, and water was added. In cases where the minimum number of 10,000 nematodes was not reached, the J2 collected were kept in a refrigerator until the next day. When the necessary number was obtained, the J2 were immediately inoculated into the plants.

The seeds were collected from fully ripe papaya (cv. Hawaii). The seeds were washed, and the mucilage removed manually. Subsequently, the seeds were dried in the shade for 4 days.

The planting substrate (two parts sand and one part soil) was autoclaved and then placed in polyethylene bags with a capacity for 680 g of substrate. One week later, fertilization was performed with NPK (04-30-10) at a dose of 1 g/bag. Planting was performed shortly thereafter, with two seeds being placed in each container. Ten days after the seedlings had emerged, thinning was performed, leaving only one seedling per bag.

Five treatments were used:

- Control—papaya seeds without any treatment;
- Neem oil—commercial insecticide used at a dose of 20 mL/seedling;
- Poultry litter—litter from egg laying poultry farms used at a dose of 0.5 g/ seedling;
- Papaya seed meal—Seeds retrieved from fully ripe papayas were washed to remove the mucilage, and then placed in trays to dry in a nature environment and then ground (Neves, 2008). The dose used was 0.83 g/seedling; and
- Papaya seed extract— A total of 10 g of papaya seed meal was measured, and 100 mL of boiling water was added. The infusion was left standing for 24 hours and then filtered (Neves, 2008) and used at a dose of 0.7 mL/ seedling. The dosages used are references from previous studies.

Papaya (cv. Hawaii) seedlings were inoculated 4 days after application of the aforementioned treatments. Each seedling was inoculated with 2 mL of M. javanica, containing 2,000 J2. The assessments were performed at 2, 4, 7, 9, 11, 18, 23, 28, 34, and 45 days after inoculation (DAI).

Shoots were discarded, and roots were taken to the laboratory, where they were cut, washed, placed in a solution of 1.5% sodium hypochlorite (NaOCl) for 4 minutes, washed again, and left in water for 15 minutes. Then, the water was removed, and 1 mL of acid fuchsin and 20 mL of water were added. Subsequently, the roots were microwaved for 45 seconds and allowed to cool naturally. Then, the cold roots were washed, drained, and placed in 100-mL beakers, and 20 mL of glycerine and 2 drops of hydrochloric acid (HCl) were added (Byrd et al. 1983). Each sample was placed in a 6-cm-diameter Petri dish for observation under a stereomicroscope. The parts of the roots that showed the presence of nematodes were placed on slides with a drop of pure glycerol and taken to the light microscope, where they were examined and photographed.

2.2. Set-up and performance of the second experiment

The experiment was set up and conducted in a greenhouse and at the Laboratory of Agricultural Nematology of the IF Goiano - Urutai Campus, Urutai - GO. The experiment was arranged in a completely randomized design, with 10 replications for five treatments.

Papaya (cv. Hawaii) seedlings were obtained after planting seeds in 1-L polyethylene bags with sterilized soil substrate. The substrate consisted of a mixture of one part soil to two parts sand.

Meloidogyne javanica inocula were obtained from populations collected from roots of tomato cv. Kada, Santa Cruz group, planted in the IF Goiano greenhouse on the Urutai Campus. Meloidogyne javanica eggs were extracted using the method of Hussey and Barker (1973). The concentrations of eggs were adjusted to 4,000 eggs and J2/mL for use as inoculum.

The seeds used for planting and for the five treatments used in this experiment were the same as those used in experiment 1. Three seeds were sown per bag. After the seedlings had emerged, thinning was performed to allow one plant per bag. The plants were fertilized with NPK (04-30-10) and irrigated daily. The inoculation with nematodes was performed when the seedling reached approximately 10 cm in height. Initiated infestation with 2 mL of the aqueous suspension containing 4,000 eggs and J2 M. javanica. The suspension was placed near the neck of the plant in 2 holes with depth of 1 cm that were used for each bag.

The following traits were assessed at 90 days after inoculation: plant height (H); stem diameter (SD), measured using a digital caliper; shoot fresh mass (SFW) and shoot dry weight (SDW); root fresh mass (RFW); gall index (GI); egg

mass index (EMI), where 0 = no gall or egg mass, 1 = 1 to 2 galls or egg masses, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100, and 5 > 100 (Hartman & Sasser, 1985); and nematode reproduction factor (RF), which is the ratio between the final population (number of eggs collected at 90 days after inoculation) and the initial population (4,000). An RF higher than 1 showed that the treatment allowed the nematode to multiply in the root system, with no effect on its control. An RF lower than 1 indicated that the nematode population decreased, and thus, the treatment was effective in controlling the nematode.

The method of Coolen and D´Herde (1972) for root and Jenkins (1964) for soil was used to extract the nematode. This variable was termed population density (PD). The nematodes obtained were quantified on Peters' chamber, with an optical microscope.

To identify which treatment had the best result, Canonical Redundancy Analysis was performed to assess which treatments had the strongest effect on the variables. R software was used for the canonical analysis.

3. Results and discussion

In the first experiment, no nematodes were found in the root system at 2 or 4 DAI. At 7 DAI, J2 were found inside the root of the control plants. At 18 DAI, M. javanica could already be observed at J3. At 28 DAI, adult females were observed in the plants treated with neem oil, papaya seed extract, and papaya seed meal.

In the plants treated with poultry litter, penetration only occurred at 9 DAI, and adult females were only found at 45 DAI (Figure 1). This result occurred because of the high concentration of nitrogen (Koenning et al., 2003), which was converted into ammonia nitrogen when it came in contact with the soil. The nitrogen present in the chicken bed is found in the form of uric acid, which in contact with the soil is converted to ammoniacal nitrogen (Sims & Wolf, 1994). Nitrogen and ammoniacal nitrogen (anomaly) have a nematicidal activity, thus interrupting the life cycle of the nematode (Gitaitis & Beaver, 1990). According to Stirling (1991) when organic matter is incorporated into the soil, microbial growth, directly interfering with phytonuthoid growth. According to work done by Riegel et al. (1996) due to the increase of chicken litter in the soil, a significant production of bacteria was observed, thus negatively affecting the development of phytonematoids in the soil.

In the other treatments, adult females had already been found at 28 DAI due to their cycle closure in 21 days.

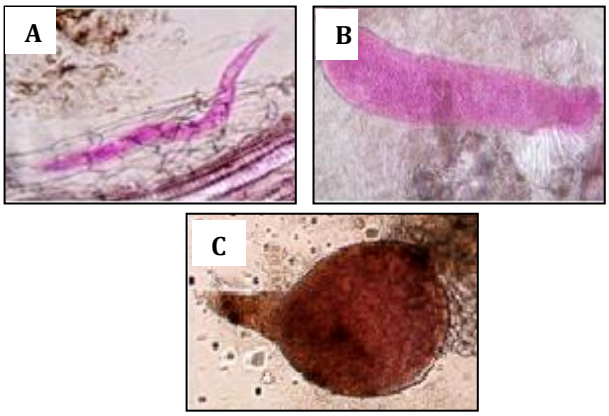

Figure 1. Development of Meloidogyne javanica on papaya root treated with poultry litter. A: At 9 DAI juveniles pentream at root; B. To 23 DAI fed juveniles J3; C: At 45 DAI Female adults.

In the second experiment, at 90 days after planting, the data analysis showed significant interaction between treatments in the morphometric variables. No significant difference was observed among the treatments applied (Figure 2). However, the M. javanica population density (PD) values were lower in the treated plants compared to the control, although the difference was not significant.

The biplot analysis of treatment applications indicated a significant linear behavior between treatments: papaya seed meal, papaya seed extract, and neem oil. However, the lowest nematode PD was observed in the treatment with poultry litter (Figure 2).

The treatments were significantly different regarding the morphometric variables. Treatment 3 (poultry litter) was superior to treatment 5 (papaya seed extract), as higher stem height, root fresh weight, shoot dry weight, and shoot fresh weight were observed in the former (Figure 2).

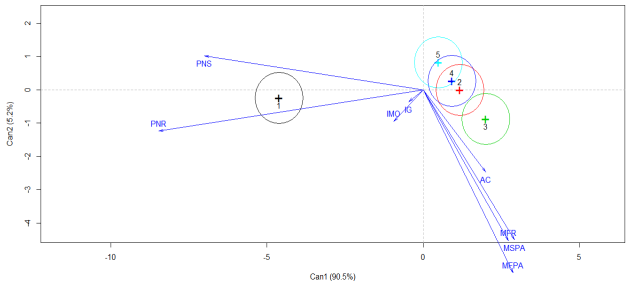

Figure 2: Biplot ellipse containing 95% confidence treatments, built by canonical correlation analysis treatments (top) and variable (lower) in Urutai, GO, 2016. Being treatments 1 (Control), 2 (Papaya Seed Meal), 3 (Poultry Litter), 4 (Papaya Seed Extract), 5 (Neem Oil), and the variables, PNS (nematode population in the soil), PNR (Population nematode the root), IMO (egg mass index), IG (Galha Index), AC (Stem height), MFR

The results indicate that the application of poultry litter is effective for controlling M. javanica, corroborating Everts et al., (2006), who found that the application of poultry litter has a suppressive effect on plant nematodes. This effect has been attributed to the release of toxic substances during organic matter decomposition and, especially, to the presence of ammonia nitrogen (Lima et al., 2011). Another explanation for the effect of poultry litter on plant nematode control may be associated with the microbial activity in this organic compost, especially the activity of fungi and bacteria, which may act as plant nematode control agents (Lima et al., 2011).

Such results also corroborate Ritzinger and Fancelli, (2006), who used poultry litter to suppress plant nematodes in bananas and hypothesized that the addition of organic materials may be used to control nematodes because some materials, especially green manure, are already being applied to the soil.

4. Conclusion

The incorporation of poultry litter, papaya seed meal, papaya seed extract, and neem oil into soil controls the nematode M. javanica.

The incorporation of poultry litter improves the development of papaya plants infested with M. javanica by increasing the stem height, root fresh weight, shoot fresh weight, and shoot dry weight.

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