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INHIBITION ACTIVITY OF CURCUMA, GINGER AND PUMPKIN SEEDS EXTRACTS AGAINST ROOT KNOT NEMATODES ON EGGPLANT

Dhulfiqar L. Al-Sandooq*, Waleed K. Mutleb and Mosab K. Abbas

Department of Plant protection, College of agricultural engineering sciences, University of Baghdad, Iraq.

Abstract

This study was conducted to evaluate the activity of three plant extracts, Curcuma rhizome extract (CE), Ginger rhizome extract (GE) and Pumpkin seeds extract (PSE) against *Meloidogyne javanica* Development in eggplant roots, it was found that all the extracts caused reduction in nematodes penetration and development in roots tissues in protective treatments. (PSE) at 15 % was found to be the more effective with 7 fourth stage juveniles (J4) penetrated the roots followed by (GE) 15% and (CE) at 15% 22, 24 J4 penetrated respectively, compared with 74 J4 penetrated in control. (PSE) at 5%, 15% and (GE) 15% were found the more active in restriction root knots development in protective treatment with gall index (gi)1, compared with 8 in control. In curative treatments the more effective extract was found to be (PSE) 10% with gi1 compared with index 8 in control. (PSE) at 5, 10, 15% and (CE) at 10%, 15% caused Total decomposition of egg shells (zero healthy egg) compared with 1050 in control in lab. Experiment after 28 hours (hrs). After 72 hrs the higher numbers of dead the egg and J2 conserving their shape were found in (PSE) treatment at 5, 10, 15%, at zero compared with 112, 1040, 272 perspectivity in control.

Introduction

Root knot nematodes *Meloidogyne* spp. Are among the most important of nematodes group parasitizing plants. Root knot nematodes are distributed worldwide and considered as the more dangerous pathogens in tropical and moderate areas in the world would annual losses in the yield estimated to be 100millions Dollars (Al-Kubaicy and Al-Sabe½a, 2014). More than 80 species of *Meloidogyne* where identified infection more than 3000 plant species causing high losses in yield (Qiao *et al.,* 2013; [1]AL-sandooq and Fattah, 2017) root knot nematodes became more dangerous when associated with other pathogens Including fungi and bacteria that causing a more lesses than single infection ([2]AL-sandooq and Fattah, 2017; Al-Waily *et al.,* 2018).

It has been reported that formers in north Carolina in usa spend up to 19 billion dollars to control root knot nematodes (Othman, 2008). In iraq 5 species of *Meloidogyne* were identified, *M. javanica*, *M. arenaria, M. thamesi*, *M. incognita*, *M. hapla*, causing high losses in yields on different host plants (Katcho, 1972; Al-Kubaicy and Al-Sabe½a, 2014). Eggplant, *Solanum melongena* L. family Solanaceae is one of the most important vegetables in iraq and in the word cultivated in protected and open fields and considered as main nutritive in tropical and middle east countries (Afshari *et al.,* 2017). Eggplant reported to be infected with many pathogens among these root knot nematodes causing losses in yields estimated to be 20.9-89% in the word (Lamberit and Tylor, 1979).

This study was conducted to evaluate the activity of curcum (*Curcuma longa*), Ginger (*Zingiber officinale*) and yellow pumpkin seed (*Cucurbita maxima*) extracts to control on root knot nematodes *M. javanica* on eggplant.

***Author for correspondence :** E-mail : dhulfiqar_laith@yahoo.com

Materials and methods

Inoculum preparation :

Work was carried out at the Nematology Lab. of the college of Agricultural Engineering Sciences - University of Baghdad - Iraq, eggs of root knot nematodes *M. javanica* were extracted from infected plants as described (Hussey and barker, 1973) With some minor

modifications : roots of eggplants and tomato, highly infected with *M. javanica*, showing root knots were collected and cut to small pieces (1-2 cm). The pieces were surface sterilized with 1% sodium hypochlorite for 4-6 min., the pieces were then transferred successively into sieves respectively 300, 150, 75, 50 and 25 µm. Wash well with current water for 5-10 minutes to get rid of NaOCL residues. The eggs collected from 25 µm sieve in 125ml cylinder and transferred into 20cm dim petri dish and maintained at 25°C for 1-3 days until Hatch the eggs to j2 with the dish and the inoculum a variable amount of oxygen to ensure continuity of the inoculum. Examine the sample under the microscope at an increase of 100X to confirm the activity of J2, and then the plants are inoculation with the J2 and at a concentration of 1000 j2 for each plant.

Glass House Experiences:

Used in this study seedlings of eggplant *Solanum melongena* L., each seedling was planted in a pot volume 1-kilo Mix it with sterile mixed soil and sterile 1:1, Two experiments were carried out for the Glass house, the first was the treatment of the plants before the inoculation by nematodes (protective treatment) and the second: the treatment of the plants with the extracts after the nematodes inoculation (curative treatment).

Plant extract preparation :

Seeds of pumpkin seeds and rhizomes of curcuma and ginger were purchased from local markets and dried with 60°C for 24-48 hrs to get rid of the internal moisture and to reach complete drought. It was well grinded and turned into a powder. Prepare three concentrations of 5, 10 and 15% for each powder of solvation 5g, 10g and 15g in 100ml distilled water. Mix well in the vibrator for 2hrs and leave for 24hrs at 22°C to obtain the largest amount of active soluble substances and the extracts were then shaken well before treatment. The extracts were added irrigation to each plant by 20ml per plant. In protection experiment the plants were treated by extracts and after one month inoculated by nematodes but curative treatment the plants inoculated by nematodes and after one month treated with extracts.

Experiment of penetration : Calculation of J4 number into the roots

This experiment is only for the protective treatment and to make sure that the infection of the plant from the nematode and to ensure the effectiveness of the inoculum in the survival of nematodes effective after the penetration, Where it arrives at this stage and is the last before sexual differentiation to males and females and Indicates the quasi-complete life cycle and composition knots on the roots:

-Root pigmentation after two weeks from nematodes inoculation to check for infection.

-Keep roots infected with nematodes in a high freezing method after storing them with plastic bags of 0°C (Not to be washed with water or rid of the dust adjacent to it) and waiting for the process of staining (not to exceed the conservation process for more than a month) and then the process of staining according to method Sodium - hypochlorite - acid fuschin (Bybd *et al.,* 1983; AL-sandooq and Fattah, 2015; [1]AL-sandooq and Fattah, 2017).

((Dissolve 0.35g of Acid Fuchsin with 25ml Acetic acid and 75ml distilled water to form the base solution. Wash the roots thoroughly with water to remove them from the soil while keeping water running for minutes. Prepare 1% sodium hypochlorite solution to remove root color with immersion of roots for 5 minutes. Wash the roots thoroughly with water and leave under water for minutes to completely remove them from the solution. Add 1ml Acetic acid and complete the volume to 100ml distilled water and put the roots for 15 minutes to acquire the roots of acidity that helps to stabilize the dye. Pour the solution with the roots not washed with water. Prepare 1ml of the concentrated solution of the Acid Fuchsin dye and complete the volume to 30ml of distilled water. Place the solution on a heat source until it is heated. Add the roots for 30 seconds with time commitment (for fear of root damage) and leave the roots inside the solution for half an hour to cool. Wash roots with water to get rid of excess pigmentation. Preparation of 700ml glycerol 300ml distilled water and 2mL HCL and shake the solution well to blend, Add 20ml of the solution (depending on the root size) in a glass cylinder on a heat source until boiling and place the roots in it for 30 seconds. Place the roots on glass slides and examine them with a microscope on a 64x force and calculate the number of J4 lactates within the roots)).

Root knot gall index (gi) :

Using root knot gall index according to scale (Coyne *et al.,* 2007) and developer by. ([1]AL-sandooq and Fattah, 2017) to be :

1 = Healthy root (no galls), 2 = Galls on 1-10% of roos , 3 = Galls on 11-20% of root, 4 = Galls on 21-30% of root, 5 = Galls on 31-40% of root, 6 = Galls on 41-50% of root, 7 = Galls on 51-60% of root, 8 = Galls on 61-70% of root, 9 = Galls on 71-80% of root, 10 = Galls on 81-100% of root.

The pots were distributed in glass house in complete

randomized design (C.R.D.) with three replication and three plants in each replication for each concentration.

Laboratory experiment :

Two ml of *Meloidogyne* eggs suspension (2000 + 50 eggs) were placed in each of petri plates with 6ml of distilled water. One ml of each extract was separately added to each plate. plates contained the inoculum (2ml of eggs suspension + 6ml distilled water) only were used as control. The plates were maintained at 26°C. Numbers of healthy eggs, active second Juveniles and dead second Juveniles non decomposed were calculated after 48 and 72 hrs. Three replications with 3 plates for each concentration were used and distributed in (C.R.D.).

The results from all the experiments were analysed by Genstat Discovery edition 4 and the significant difference were compared by L.S.D. of p = 0.05.

Results

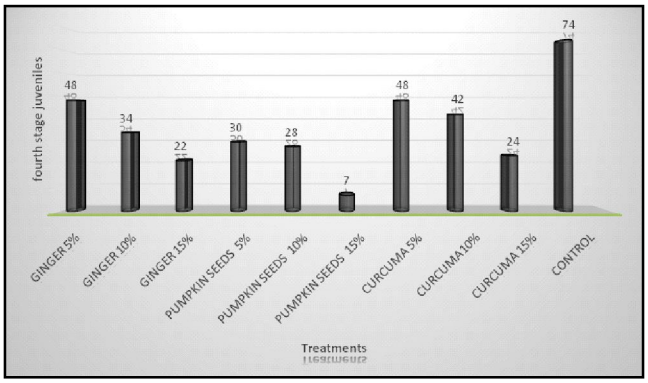

Fig. 1. Effect of plant extracts on growth and development of *Meloidogyne* spp. juveniles (J4) on eggplant in protective treatment.

Pot experiment :

Significant reduction in Nematodes J4 penetrated and developed in root tissue was induced by all the plant extracts used. The more effective extract was found the (PSE) at 15% with 7 juveniles penetrated root tissue followed by (GE) at 15% and (CE) at 15% with penetrated juveniles 22 and 24 respectively and Followed by other treatment, the highest number of juveniles penetrated and development was (GE) 5% and turmeric extract 5% at 48 J4 compared with 47 penetrated J4 in control (Fig. 1).

(PSE) at 5% and 15%, (GE) at 15% were found the more active in restriction root knot nematodes development in root tissue with (gi) 1, followed by (GE) at 10%, (PSE) at 10% and (CE) at 10% and 15% with gi 2 compared with index 8 in control in protective treatment. While in curative treatment, the more effective was (PSE) 10% with gi 1, followed (GE) at 10% and 15%,

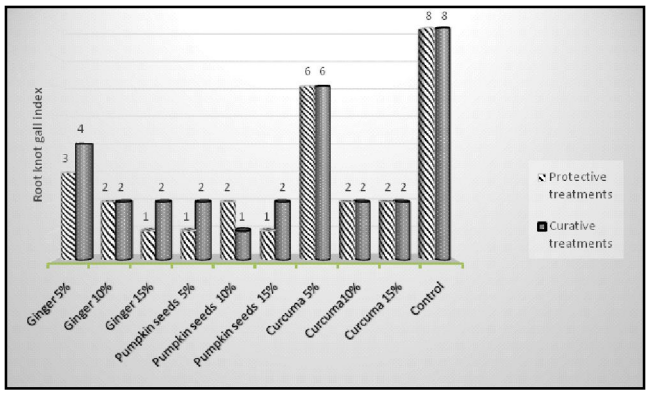

Fig. 2. Effect of plant extracts on root knot gall index on infected eggplant with *Meloidogyne* spp. for protective and curative treatments .

(PSE) and 5% and 15% and (CE) at 10% and 15% with gi 2 compared with 8 in control (Fig. 2).

Laboratory experiments:

After 48 hours of treatment with extracts, the results were depending on shape and activity nematode stages and data were recorded for the egg stage depending on the egg shape (Did the shape of the egg remain completely Or the destruction of walls and Caetinous membranes) or depending on J2 shapes (success of the eggs with hatch and Evolved into J2) and did this J2 active or died anddid the juveniles dead remain conservative in their general shape or decomposed the walls of the Cauticle and scattered contents in thesuspension. The treatments of egg suspension with (PSE) at 5%, 10%, 15%, and (CE) at 10%, 15% caused decomposition of egg shell completely, followed by (GE) at 5%, 15% with healthy eggs 96 and 75 respectively compared with 1050 eggs in control (Fig. 3).

The higher number of dead J2 conserving its shape

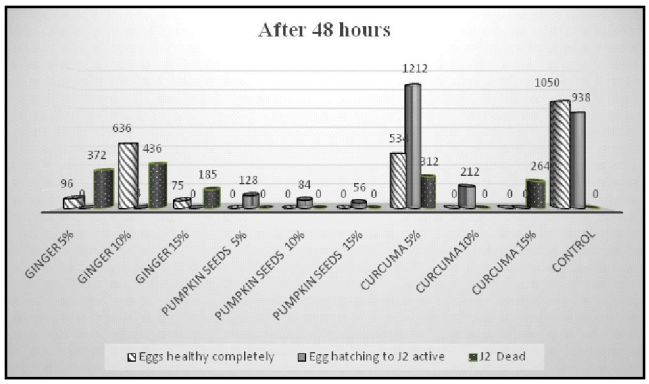

Fig. 3. Effect of plant extracts on the numbers ofhealthy and complete eggs , the numbers of second stage juveniles active and the numbers of the second stage dead (non-decomposed) with *Meloidogyne* spp. in the laboratory after 48 hours of treatment.

was found in (GE) at 5%, 10%, with 372 and 436 J2 respectively. While, in (PSE), the eggs and juveniles were completely decomposed Its contents were scattered with the suspension rendering the estimation of eggs and juveniles number very difficult, Therefore, no juveniles were recorded dead and preserved in her shape (everyone is fully decomposed).

In the all (PSE) treatments recorded death all eggs and juveniles and completely destroyed and all data recorded zero because there are no complete juveniles shapes or eggs shape of dead and (GE) 10%, (CE) 15% the juveniles and eggs were dead and the absence of any living juveniles (Fig. 4).

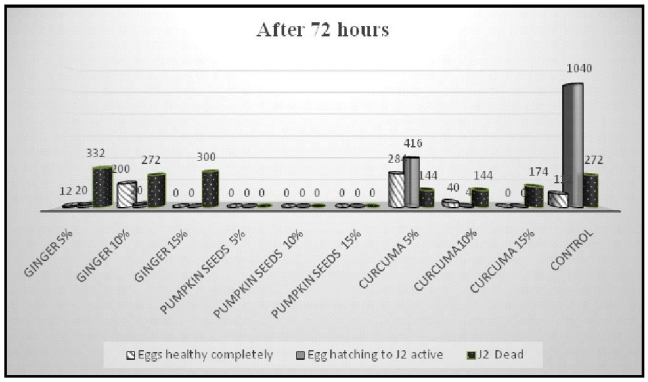

Fig. 4. Effect of plant extracts on the numbers of healthy and complete eggs , the numbers of second stage juveniles active and the numbers of the second stage dead (non-decomposed) with *Meloidogyne* spp. in the laboratory after 72 hours of treatment.

Discussion

The results of this study demonstrated that all the plant extracts used were active in restriction the penetration and development of root knot nematodes juveniles in root tissue. The more active was (PSE). The activity of the extract may attributed to their contents of compounds that exert an inhibition effects against nematodes development. It was reported that pumpkin seeds contain many secondary metabolite, including cucurbitacin B, cucurbitin, cucurmosin, saponins and sterols effects the shape and viability of nematodes and eggs hatching (Al Shahwani *et al.,* 2008; Grzybek *et al.,* 2016) Indicated to the efficiency of oil extracted from pumpkin seeds to inhibited the human pathogenic bacteria *Proteus vulgaris* and other pathogens. (PSE) contain also phytosterols that antagonize with nematodes fats necessary fore their development and reduced their production leading to nematodes cells death (Gutierrez, 2016). Other studies reported that pumpkin seeds contain linoleic acid that effect nematodal intestine and decrease digestion ability leading to gradually decline of intestine cells as well as affecting nervous system and stop nematodes movement (Stadler *et al.,* 1993).

The activity of plant extracts against nematodes may be due to activation of own plant defence mechanisms leading to produce compounds, phytoalexins and proteins around sites of nematode penetration and systematically in the roots that inhibits the juveniles penetration and restrict their development, referred as induced systemic resistance.

It has been reported that plant posses numerous defence mechanisms to protect themselves against pathogens attack, some of these are inducible and become activated after pathogen infection and by non-pathogenic agents leading to synthesis phytoalexins and production anti pathogens performance (Mehdy, 1994; Hackson and Taylor, 1996). Treatment of plants with variety agents including plant extracts can lead to the induction of resistance to subsequent path on attack, both locally and systematically the resistance induced is characterized by restriction of pathon development and suppression of disease symptoms development compared with non – induced plants infected with some pathogens (walters, 2010; Kepenekçi *et al.,* 2016). The activity of plant extracts in restriction the development *Meloidogyne* and decreasing the symptoms manifested on plant may be promising in man agent of disease caused.

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