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Evaluation of mushroom compost for the bio control root-knot nematode

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Abstract

The collection of infected roots to *Meloidogyne javanica* nematode of tomato cherry variety, in a greenhouse of Neyshabor city, Iran, carries to laboratory, then sectional and insemination to amount one gram of infection roots to *Meloidogyne javanica*in African violet Media to treatments oyster mushroom compost *Pleurotuso streatus* variety in 30%, 50% and 70% density for control *Meloidogyne javanica* in African violet (*Saintpaulia ionantha*) media. The present research in format trial on base plan randomize complete block to with 5 treatments and 3 replications was conducted in 15 plots. Qualitative and quantitative traits such as shoot fresh weight, dry weight, number of leaves and effect compost concentration of oyster mushroom cultivation in beds of violets on nematode egg number and nematode number. The result showed that increasing concentration of compost fungal nematode population dropped so significantly reduce the nematode population at 70 percent. Also in this study, egg number, nematode number and properties of shoot fresh weight significant but root fresh weight, dry weight and leaf number not significant.

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Introduction

Nematodes are the most abundant multicellular animals on the face of earth. Several hundreds species of nematodes are known to feed on living plants and cause a variety of plant diseases worldwide. Root-knot nematodes are capable of harshly damaging a broad range of crops, in particular vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture (Sikora and Fernandez, 2005).

Tactics for multiple management systems designed to control losses from plant-parasitic nematodes have increased remarkably in the past thirty years (Duncan, 1991). Impetus for the search for innovative pest management practices has arisen from the public concern that new methods do not pollute or otherwise degrade the environment. Only by invoking chemical nematode management when it is determined to be effective, or needed, will the load which synthetic agrichemicals place on the environment be reduced (Kimpinski *et al.*, 2001). At the forefront of environmentally sensitive pest management strategies are integrated pest management (IPM) strategies that have been closely aligned to sustainable systems of agriculture (Weil, 1990). Methods to manage nematodes have evolved in response to IPM principles with a view to determining how the life cycle of a pest must be modified to reduce its economic impact to tolerable levels (Geier, 1966). In this regard, numerous biocontrol agents aimed at interrupting the life-cycle of nematodes (Dusenberry, 1987) or suppressing plant-parasitic nematode populations, have been characterized, including nematode trapping fungi, predacious nematodes and other antagonistic or competitive soil fauna (Kerry, 1990; Stirling, 1991). During the search for more natural nematode controls several plants have been found to possess nematicidal properties (Sukul, 1992) and have been incorporated into crop rotations, or as green mulches, so reducing population densities of plant-parasitic nematodes.

At least 10 species of gilled fungi belonging to the genera *Hohenbuehelia, Pleurotus* and Resupinatus can attack nematodes by adhesion or toxins (Tzen and Liou, 1993). Thorn and Barron (1984) found that when nematodes came in contact with the hyphae of *Pleurotus*, they became inactive very quickly and were subsequently colonized and digested by the fungus. *Pleurotus* species display a kind of nematode capture that appears to be unique to this genus. They produce tiny appendages on the vegetative hyphae and these secrete droplets of a potent toxin (Thorn and Barron, 1984; Barron and Thorn, 1987). The toxin produced by *Pleurotusostreatus* has been identified as trans-2-decenedioic acid (Kwock, *et al.*, 1992). Barron and Thorn (1987) reported that rhabditid nematodes touching such droplets showed a sudden response, the head region shrinked, hyphae attracted to the body orifices and homed in on the head. This mode of nematode attack has been observed in *P. cornucopiae, P. cystidiosus, P. Levis, P. pulmonarius* (Thorn and Barron, 1984; Thorn and Tsuneda 1993), *P. tuberregium, P. dryinus, P. euosmus, P. eryngii* (Hibbet and Thorn, 1994) and *P. sajor-caju* (Sharma, 1994). The toxin produced by *P. cystidiosus* is present either at much lower concentration or is structurally different from that of *P. ostreatus* (Barron and Thorn, 1987). *Aphelenchoides composticola*, one of the most damaging mushroom nematodes, immobilized in *P. sajor-caju* culture filtrates within 2 to 4 h. of the exposure (Sharma, 1994).It was suggested (Barron and Dierkes, 1977; Thorn and Barron, 1984) that nematodes may be an important nitrogen supplement for fungi, especially in wood substrates where the C:N ratio is very high and that the predatory capability of wood decay fungi has evolved to satisfy their nitrogen requirement. The host range for *Pleurotus* has never been fully established. Toxin droplets can be instrumental, not only in supplying a nitrogen source to *Pleurotus*, but can also function as an antifeedant that protect hyphae from fungus feeding microfauna (Barron, 2003). This study is the first investigation in relationship on controll rootpknot nematod on African violate in Iran. In this study, the nematoxiceffects of mushroom compost of *Pleurotus* has been tested *in vivo*.

Materials and methods

Nematode inoculum preparation

Infective juveniles (J2) of the root-knot nematode were prepared by adopting the process from Somasri (2001). Tomato roots with the root-knot symptom were washed with sterile water. Brown egg mass was collected from washed gall root of tomato using forceps. The egg mass was stored on a fine net nylon cloth mounted in a PVC pipe with 3 centimeters diameter and 1 centimeter height. The pipe was sealed with a fine net nylon cloth. The pipe was immersed in 0.5 % of sodium hypochlorite (NaOCl) for 3 minutes, and rinsed with sterilized distilled water 3 times, for 5 minutes each time. The pipe was placed on a cone with 15-centimeters width. The cone had a rubber tube connected at the end with a clamp. Water was poured just to the end of the pipe. After 2 days, J2 would hatch out of the eggs. They congregated at the end of the rubber tube ready to be tested.

In vivo studies (greenhouse experiments)

Inoculation substrate was prepared by boiling of wheat grains in water for 15 min till they were soft. Water excess was drained off and the substrates were spread on the surface of a clean blotting paper and air dried for 15 min to remove the excess water. Then Ca^{2+} was added and the substrates put into milk glasses. The glasses were autoclaved for 60 min at 121°C and cooled. Straws were chopped into small pieces (1±2 cm), soaked in water overnight, and then boiled in water for 60 min. Water excess was drained off and allowed to cool up to 25°C and sterilized. Wet straw (~85% moisture) was mixed with 20% grain spawn of each fungal species. The spawned substrates were then put into 30 cm×42 cm polyethene bags. The bags were tightly closed and pin holes were made on the surfaces. The bags were subsequently kept in a spawn running room at 25±1°C under dark conditions till primordia were formed. After primordial formation, large holes were made in the polythene bags to allow the normal development of fruiting bodies. The bags were then kept at 22±1°C with a 12 h photoperiod and 85–90% of relative humidity. Mushrooms were manually harvested in clusters from the substrates three days after primordial initiation. Spent oyster mushroom composts were used in this trials. The amount one gram of infection roots to *Meloidogyne javanica*in African violet Media to treatments oyster mushroom compost *Pleurotus ostreatus* variety in 30%, 50% and 70% density for control *Meloidogyne javanica* in African violet (*Saintpauliaionantha*) media. After 2–3 weeks, the plants were inoculated with 1000 second-stage juvenils of *Meloidogyne javanica*per plant. Sterile wet straws (without fungus) were used as controls. Plants were maintained in greenhouse at 24±26°C. Plants were extracted from pots after 70 days, fresh and dry weight index and population of egg mass and juveniles were evaluated. Each treatment had four replications.

Measurement of Nitrogen

The sample is automatically digested with a sulfuric acid solution containing potassium sulfate and mercuric sulfate as a catalyst to convert organic nitrogen to ammonium sulfate. The solution is then automatically neutralized with sodium hydroxide solution and treated with alkaline phenol reagent and sodium hypochlorite reagent. This treatment forms a blue color designated as indophenol. Sodium nitroprusside, which increases the intensity of the color, is added to obtain necessary sensitivity for measurement of low level nitrogen.

Measurement of EC and pH

Amount of pH and electrical conductivity (EC) was measured in verdonck method (1992).

Measurement of Organic Carbon

Amount of organic C was measured in Paye *et al.* (1984).

Statistical analysis

Analysis of data was carried out by SAS. Data were subjected to the analysis of variance, and means were compared and grouped according to the Duncan's multiple range test.

Results

In vivo studies (greenhouse experiments)

In vivo studies indicated that the application of completely spawn run compost could reduce some indices of the disease caused by *Meloidogyne javanica*. This application could reduce population of *Meloidogyne javanica*in roots in pot cultures which is one of the important indices in disease diagnosis (Fig. 1).The number of egg mass per gram of soil was significantly lower in all soils treated with spent oyster mushroom composts than that in the non-treated, suppressive control, despite that plants in all pots had received the same nematode inoculum (Fig. 2). Plants weight was not significantly different among each other (Fig. 3). Compost reduced fresh weight plant compared to control. *Pleurotus ostreatus* competition with African violets reduces the growth index. The lowest growth rate was observed in compost treatments.

Table 1. Chemical and physical properties in the substrates used.

No.	Sample	EC	pH	Organic carbon (%)	N (%)	K (mg/kg)	P (mg/kg)
1	Common the substrate grow African violets	0.9	6.9	15.7	27.1	448	260
2	Cocopeat	0.57	5.6	16.9	37.4	2100	80
3	50% substrate commonly growing violets + 50% cocopeat	2.9	6.9	12.3	17.8	1080	280
4	30% Compost	2.7	7.2	13.4	15.9	960	200
5	50% Compost	1.5	7.4	14.5	17.8	1200	220
6	70% Compost	2.7	7.8	19	39.3	1288	220
7	Oyster mushroom compost	1.3	6.3	20.0	47.7	2100	525

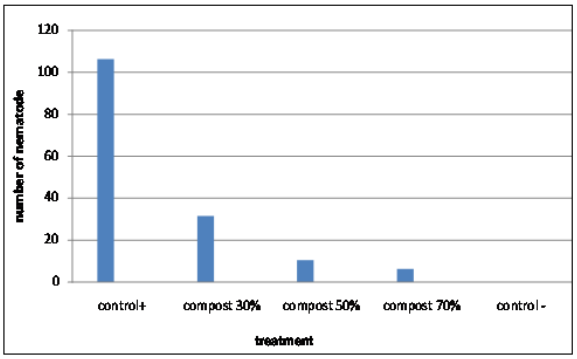

Fig. 1. The Effect of Oyster mushroom compost in the number of root-knot nematode African violets.

Measurement of Elements, EC and pH

Application of SMS to the soil increased the pH and EC. Oyster mushroom compost, with the highest levels of macronutrients containing N 69.47 percent, phosphorus 525 mg kg / g, Potassium 2100 mg kg/kg organic carbon20% and the salinity level acceptable to with 1.32 ds/m and pH 6.37 as a biological fertilizer or farming may be considered in combination with other common substrates. Intensive elements including nitrogen, phosphorus and potassium in compost Fungal compared with conventional the substrate cocopeat grow violets and above the effects of compost 30 percent, 50 percent and 70 percent is evident. The values of these elements increased with increasing compost to. Oyster mushroom compost coco peat and organic carbon compared to conventional culture the substrate was high time that with increasing amounts of compost, organic carbon is also increased (Table 1).

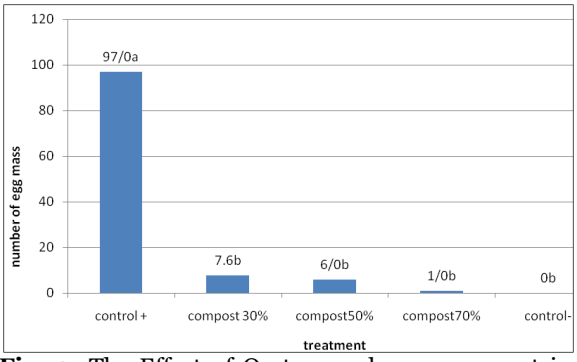

Fig. 2. The Effect of Oyster mushroom compost in the number of egg mass nematode African violets.

Discussion

Experimental results showed the effects of *Pleurotu* sspp. on *Meloidogyne javanica* population densities

in vivo conditions. The study confirmed the ability of *Pleurotus* to capture, kill and digest the nematode. The mode of infecting nematodes by all strains of *Pleurotus* species was consistent with that of *P. ostreatus* described by Thorne *et al.* (2000), Hibbet and Thorne (1994), Sharma (1994) and Thorne and Barron (1984). Barron and Thorne (1987) indicated that the ability to infect nematodes was because of a toxin released as droplets by secretary hyphal cells. Tiny secreted droplets were commonly observed on the hyphae of all tested strains of *Pleurotus* species on water agar (Barron and Thorne 1987; Chitwood 2004). The mentioned results were confirmed in 2006 (Heydari*et al.* 2006; Palizi*et al.* 2006; Palizi*et al.* 2007). Also Barron and Thorne (1987) indicated the oriented/directed growth of the hyphae which then entered with a great precision the head of nematode as directed hyphae. Oriented hyphae were commonly observed on dead nematodes attacked by the *Pleurotus* species. *Pleurotus* spp. killed the root knot nematode after only a short period of exposure to their hyphae. Nematodes were immobilized as soon as they approached the fungal colony. Paralyzed nematodes were characterized by their immobilized, and straightened bodies.The hyphae of these fungi colonized paralyzed nematodes after prolonged exposure of the nematodes to the fungal colonies. Hutchison *et al.* (1996) reported the interaction between the lawn inhabiting agarics *Conocybe lacteal* and fungal feeding nematode *Aphelenchoide* ssp. in which the hyphae killed nematodes with an anti feedant. Results from the multiplication experiments were in accordance with the nematophagous ability of basidiomycetous fungi (Chitwood 2004). The root knot nematode could not reproduce in the cultures of these species of oyster mushrooms.Compost has a lot of elements like nitrogen, phosphorus and potassium, this material increases salt and soil acidification. These results are similar to other studies.It tends to be high in phosphorous and potassium while relatively low in nitrate nitrogen. It has a very high cation exchange capacity, relatively high levels of soluble salts, a slow mineralization rate, and it is "light in weight yet bulky in volume" (American 1995). It has also been shown to have high water holding capacity (Guo 2004) SMS has also been shown to suppress various soil fungi (Davis 2005) and soil- borne plant diseases (Segarra 2007), as well as to increase microbial densities in soils (Perez-Piqueres 2007). Further, composts such as SMS can increase soil organic matter (Perez-Piqueres 2007). Finally, our experiments revealed that *Pleurotus*s pp. could reduce *Meloidogynejavanica* population densities under *in vivo* conditions and it could therefore be regarded as one of the best potential bio control agents in the country.

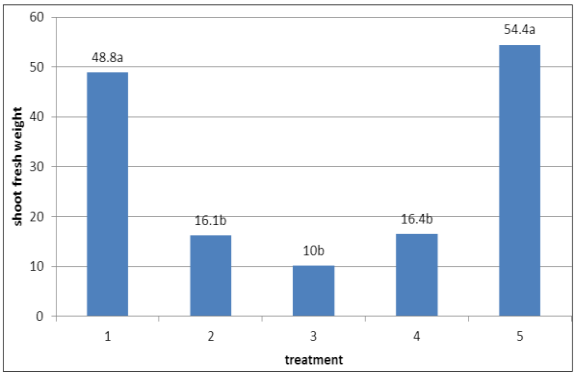

Fig. 3. Fungal Compost effect on shoot fresh weight African violets.

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