ORGANIC MANAGEMENT OF ROOT KNOT NEMATODES IN TOMATO WITH SPENT MUSHROOM COMPOST

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ABSTRACT

This study was carried out to assess the effect of spent compost of button and oyster mushrooms on the management of root knot nematodes and to measure total phenolic compounds in the spent composts. Higher levels of phenolic compounds were found in spent of oyster than button mushroom. Total phenolic compounds contained in button mushroom spent compost (BMSC) and oyster mushroom spent compost (OMSC) were 8.75µg/mg and 10.75µg/mg, respectively. Different concentrations of the spent mushroom compost were applied to the M. incognita eggs. OMSC was found more effective in reducing the egg hatching and killing juveniles of root-knot nematodes than button compost. In pot studies at greenhouse, the stimulatory and inhibitory effects of the two composts were also studied. Oyster spent compost was found more effective in inhibiting the galling and production of egg masses in the roots followed by button compost thereby stimulating the plant growth as compared to button compost.

Key Words: **Spent Mushroom Compost, Meloidogyne Incognita, Tomato, Organic Amendments**

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INTRODUCTION

Plant parasitic nematodes have a greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination (Ploeg, 2001). The Endo-parasitic nematodes include some of the economically important species such as root knot nematode (*Meloidogyne* spp*).* The genus includes more than 60 species with some species having several races. Four *Meloidogyne spp. (M. javanica, M. arenaria, M. incognita, M. hapla*) are the major crop pests and distributed worldwide ranging from tropical to temperate regions (Eisenback and Triantaphyllou, 1991).

About 2000 plants are susceptible to infection by root-knot nematodes all over the world. Root-knot nematode damage results in poor growth, a decline in quality, yield of the crop and have the ability to break the resistance of host plant and make it more susceptible to other pathogens (Back, 2002; Castello, 2003; Manzanilla-Lopez, 2004). A high level of root-knot nematode damage can lead to total crop loss. Nematode damaged roots do not utilize water and fertilizers effectively, leading to additional losses for the grower. Most of the currently used methods for the management are not effective against root knot nematodes as they are soil inhabiting. Usually farmers apply chemicals to the soil which is harmful to other micro flora as well as to the environment. Further, these chemicals pollute ground water. Cultural practices such as crop rotation are commonly used, but such practices are not effective as root knot nematodes have a wide host range and they remain in soil for years. Even weeds are attacked and hosts are available in every season. Further, our farmers cannot afford rotation or fallow rotation. Due to their wide host range they are difficult to control by other strategies like rotation and resistant cultivars because of the virulent strains and species mixtures (Roberts, 1992). The existing management procedures could be improved by the development of organic strategies (Siddiqui and Shaukat, 2003). Nematicides are used to control root knot nematodes but are difficult for subsistence farmers at small scale in developing countries. Since most nematicides are not only expensive but also dangerous to human health and environment. The addition of organic matter in the form of compost or manure will decrease nematode population and damage to crops (Walker, 2004; Akhtar and Alam, 1993; Stirling, 1991). This could be a result of improved soil structure and fertility, increase of plant resistance, release of anti-nematode-toxins, or increased populations of fungal and bacterial parasites and other nematode-antagonistic agents (Akhtar and Malik, 2000).

Spent mushroom compost (SMC) is the residual Byproduct produced by the mushroom industry. It is readily available and its formulation generally consists of a combination of wheat straw, dried blood of animals, horse manure and ground chalk, composted together. It is an excellent source of humus, although much of its nitrogen content would have been used up by the composting and growing mushrooms. It remains, however, a good source of general nutrients (0.7% N, 0.3% P, 0.3% K plus a full range of trace elements), as well as a useful soil conditioner (Bradley, 2004). The phenolic compounds present in SMC have antimicrobial activity, which could be an effective biocontrol of RKN, *Meloidogyne spp* on tomato. The antimicrobial properties of water extracts from

edible mushrooms such as *Lentinus edodes, Boletus edulis, Pleurotus ostreatus* and *Agaricus bisporus* have also been reported (Santoyo and Susana, 2009).

Keeping in view the importance of root knot nematodes management and to save environment, the present study was conducted to assess the effect of spent compost of button and oyster mushrooms on egg hatching and larval mortality of *Meloidogyne incognita* and to detect total phenolic compounds in the spent composts.

MATERIALS AND METHODS

Collection of Root Galls

Galled roots were collected from infested fields of Dargai (Jaban). Samples were collected on the basis of root knot symptoms on plants. The samples were collected in plastic bags, appropriately labeled and brought to the laboratory of Department of Plant Pathology Agricultural University Peshawar for further studies.

Pure Culture through Single Egg Mass Inoculation

Nursery of tomato cultivar Rio Grande was raised in sterilized sandy loam soil in the greenhouse of Plant Pathology Department. Two to three weeks old tomato seedlings were transplanted to 15 cm diameter earthen pots. Single egg mass inoculation was done ten days after transplantation.

Identification of Nematode Species Through Perineal Pattern Morphology

Identification of the species of *Meloidogyne* maintained in greenhouse was done by applying perineal pattern method (Eisenback *et al*. 1981). Mature females were collected from galls on the roots of tomato plants. Females were placed in 45 percent lactic acid solution. Perineal patterns (10-15 female) from each sample were mounted in glycerin. Glycerin-infiltrated specimens were examined under light microscope with oil immersion to study their characteristics. The species were identified on the basis of perineal pattern characteristics.

Preparation of Inoculum

Egg masses of root knot nematodes were collected from the galled roots obtained from greenhouse under sterio-microscope with the help of forceps and needle and were preserved in 1% saline solution at 4°C.

Collection of Eggs

Galled roots of tomato were blended in an electrical blender for two minutes in NaOCl solution (5%). Nematode eggs were collected in a beaker after passing the suspension through 36µ mesh size. The eggs were collected in distilled water and counted in a counting dish as eggs per one ml. The procedure was repeated three times and average number of eggs was recorded.

Preparation of Spent Compost Filtrate

Spent compost of button and oyster mushroom were collected from mushroom house of Plant Pathology Department Agricultural University Peshawar and were soaked in distilled water at 1:1 and were stored for 24 hours at room temperature. After 24 hours, the extract was filtered through Whatman filter paper (20 µm).

Preparation of Serial Dilutions and Application

Serial dilutions i.e. 1:1, 1:10 and 1:100 of the two composts were prepared. Eggs inhibition and Juvenile mortality bioassay were performed with the above filtrate. The data were recorded after 24 and 48 hrs.

Determination of the total Phenolic Compounds by Folin-ciocalteau Spectrophotometric Method in Spent Compost of Button and Oyster Mushroom

The chemicals used:

Chemicals

ethanol 200 ml (97%), iron chloride FeCl3 test strips, pyrocatechol (standard, distilled water (46 ml)), sodium carbonate 2%, folin- ciocalteau reagent

Extraction from Samples

One fifty gram of each SMC was oven dried at 40°C before analysis; dried samples of 20g of spent

compost were extracted with 200 ml of ethanol (97%) at 30°C and were centrifuged at 150 rpm for 24 hours, filtered through whatman-4 filter paper. The residue was extracted with addition of 200 ml ethanol twice.

Rotary Evaporation

The combined ethanolic extract was rotary evaporated at 40^{o}C to dry out. It was re-suspended in ethanol to a concentration of 10 mg/ml and later stored at 4°C till further use.

Determination of Soluble Phenols in spent Compost

Total soluble phenols in compost ethanolic extract were determined with Folin-ciocalteau reagent according to the method of Slinkard and Singleton, 1977, using pyrocatechol as a standard. The following steps were carried out.

Step-1: One ml of extract solution was taken in volumetric flask and was diluted with 46 ml of distilled water.

Step-2: One ml Folin-ciocalteau was added and was mixed thoroughly.

After this 3 ml Na_2 CO_3 (2%) was added. The mixture was allowed to stand for 2 hrs with continuous shaking.

Step-3: Standard solutions of phenolic compound pyrocatechol at concentration 1, 2, 3, 4 and 5 ppm was prepared.

Step-4:

Standard Curve

The standard curve was obtained by applying the Folin-ciocalteau Spectrophotometric method. The absorbance was measured at 760 nm. The concentration of total phenolic compound in the spent compost extract was determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph (Gezer and Duru, 2006. Mariela, 2003).

Folin –`Ciocalteau Reagent

The reagent consisted of 100 ml of sodium tungstate dehydrate, 25 g of sodium molybdate dehydrate, 50 ml of 85% phosphoric acid solution and 100 ml of 36% hydrochloric acid solution.

Greenhouse Study

Tomato nursery was raised. SMC at the rate of 10, 20 and 30/g (Khan *et al.*, 1997), was incorporated into earthen pots (15 cm diameter), containing 1kg sterilized sandy loam soil. After one week two to three weeks old tomato seedlings were transplanted to earthen pots. After one week, tomato plants in the pots were inoculated with root knot nematodes except the controls. Twenty egg masses of root knot nematodes were applied to each pot. The plants were kept in the greenhouse of Plant Pathology Department under keen observation. Normal agronomic practices were applied to the plants as well. The plants were uprooted after 45 days of inoculation.

The data were recorded on the following parameters

Number of galls per root system, Galling index, Number of females per gram of root, Number of egg masses per gram of root, Number of eggs /egg mass, Plant height (cm), Fresh shoot and root weight (g), and Dry shoot and root weight (g).

Experimental Design and Data Analysis

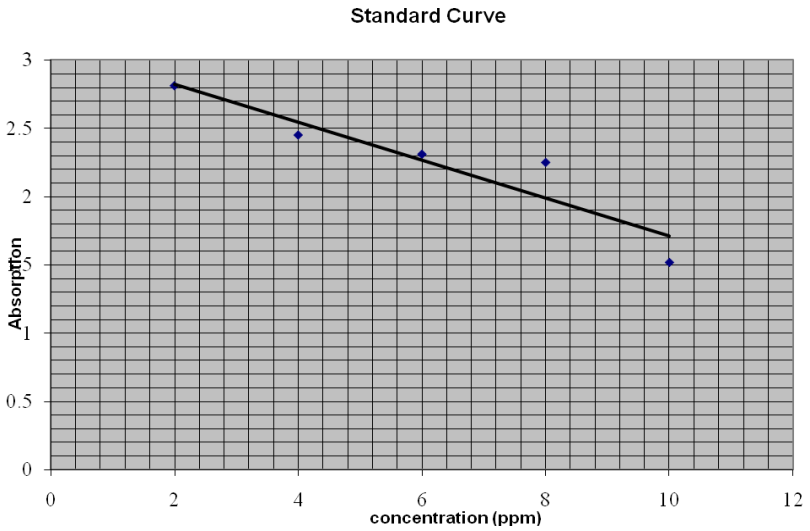
Completely randomized design (CRD) with factorial arrangement with a total of eight treatments and four replications were used. All recorded data were analyzed statistically using MSTATC software (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Total Phenolic Compounds (µg/mg) in spent Compost of *Agaricus bisporus* and *Pleurotus florida*

Results indicated that total phenolic compounds contained in BMSC and OMSC were 8.75 µg/mg and 10.75 µg/mg respectively (Fig.1). The role of phenolic compounds in roots has been associated with resistance in

some plants against nematodes (Cohn, 1974) like acetylenes, terpenoids, aldehydes, sesquiterpenoids and phenoxypropionic acid derivatives are known to have nematicidal activity against some nematodes (Veech, 1979; Mori *et al*., 1982; Hayashi, Wada and Munakata1983; shaukat and Siddique, 2002). Somasundaram (2003) reported total phenolic content of mushrooms (*A. bisporus*, *P. florida*), are Cinnamic acid, p-Coumaric acid,p-Hydroxybenzoic acid -Ferulic acid, Vanillic acid _ p-Hydroxybenzaldehyde ,Catechol ,Guaiacol ,Syringaldehyde ,Tyrosine . In *A. bisporus* the skin had more phenolics than the flesh; *P. florida* contained fewer phenolics, while the stalks of both mushrooms had low phenolic contents.



Standard curve obtained by applying the Folin-ciocalteau Spectrophotometric method. The absorbance was measured at 760 nm. The concentration of total phenolic compounds in the spent compost extract was determined as microgram of pyrocatechol, used as standard.

Total phenolic compounds in the two compost were determined by using the following formula,

$$\text{ppm } (\mu g/g) = \frac{\text{Graphical reading x volume}}{\text{Weight of sample}}$$

The present study was conducted to investigate *in vitro* and *in vivo* activity of spent mushroom compost against the second stage juveniles of *Meloidogyne incognita* and their effect on its egg hatching. As oxidation products of certain phenolic acids are believed to be more toxic to nematodes (Hung and Rohde, 1973), therefore, spent compost of oyster and button mushroom were studied for nematicidal activity, against root knot nematodes. Results revealed that different concentrations of spent compost of oyster mushroom showed a significant effect on reducing egg hatching of root knot nematodes, as compared to button mushroom (Fig. 2). The *in vitro* studies revealed that concentration 1:1 was more effective than other concentrations i.e. 1:10, and 1:100, whereas control treatment showed minimal number of unhatched eggs. Result showed that extracts from spent compost (SC) of oyster mushroom were found more effective in killing the nematodes larvae followed by button compost. Different concentration of oyster compost significantly killed the juveniles (Fig. 3). The lethal effect decreased thereby increasing egg hatching with decreasing concentration. In pot experiment, at greenhouse the stimulatory and inhibitory effect of the two composts were also studied. Oyster compost was found to be more effective in inhibiting the galling and egg masses in the roots followed by button compost. Consequently, it stimulated the plant growth compared to button mushroom compost and inhibited galling on tomato roots (Fig. 4 - 5).

The effectiveness of spent compost diminished with time. Results are in congruence with the work done by Bridge, (1996), Yen *et al*. (1993) and Glucin *et al*. (2003), Santoyo and Susana (2009), also reported that the efficacy of phenolic compound decreased due to the oxidative loss with the passage of time and exposure to light.

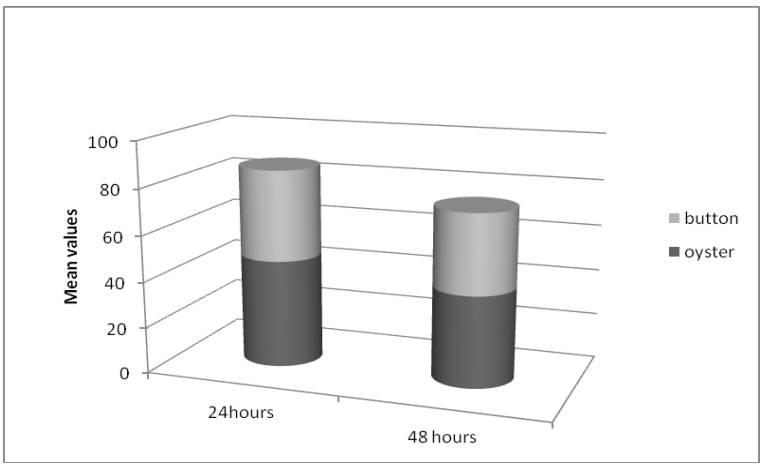


Fig. 2. Effect of spent mushroom compost on egg hatching of M. incognita after 24 and 48 hrs

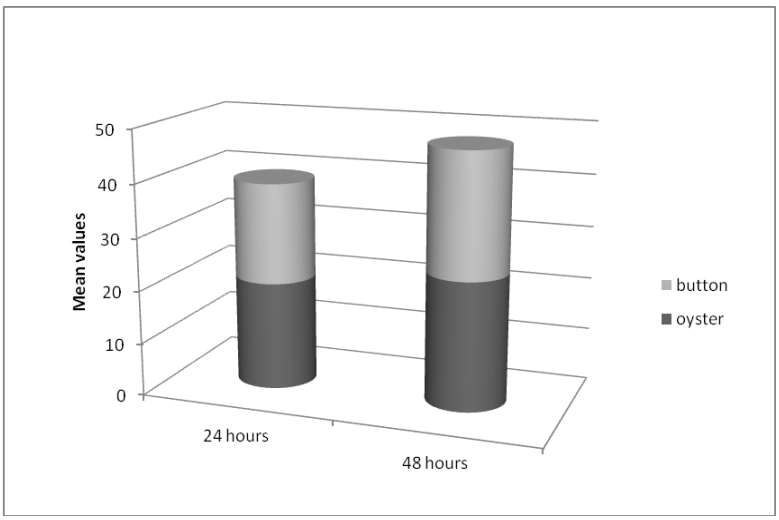


Fig. 3. Effect of spent mushroom compost on juveniles mortality on M. incognita after 24 and 48hrs

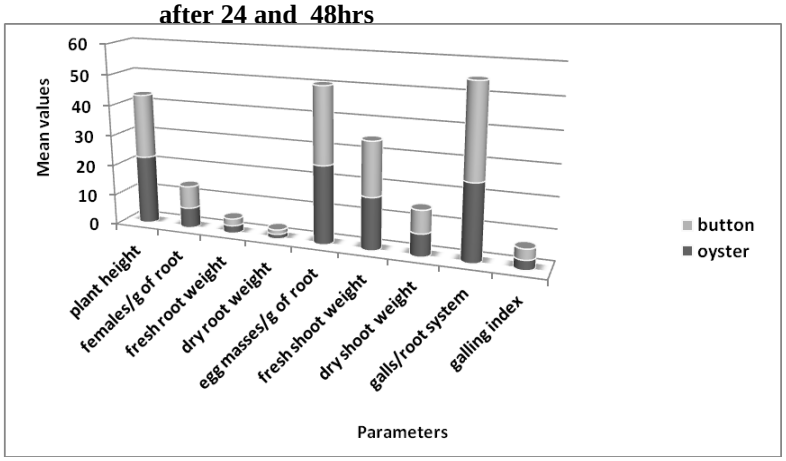


Fig. 4. Effect of spent mushroom compost on M. incognita and the resultant parameters

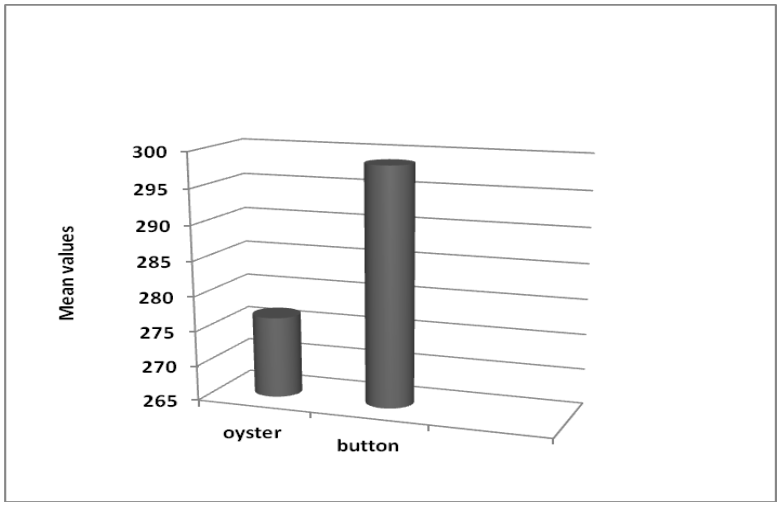


Fig. 5. Effect of spent compost on M. incognita and the resultant number of eggs/egg mass

Spent compost is considered a waste product by the farmers and they usually dispose it. Conversely it is a good source of general nutrients like nitrogen, phosphorus and potassium as well as a useful soil conditioner and can improve the organic matter contents of the soil. Decomposition of organic matter into the soil releases some organic compounds such as acetic acid, propionic and butyric acid that may be toxic to nematodes. Sufficient concentrations of these compounds kill some phytonematodes but not other free living species (Dropkin, 1980). Spent compost of mushroom contains phenolic compounds which have phenol as a primary component in their structure. Its chemical formula is C_6H_5OH and its structure has a hydroxyl group (-OH) bonded to a phenyl ring.

This preliminary *in vitro* study has, however, explored a new and environment friendly way for the management of root-knot nematodes. The method can be used for the management of other parasitic nematodes.

CONCLUSION AND RECOMMENDATIONS

Extracts of oyster and button mushroom composts were found effective for the organic management of root-knot nematodes. The application of oyster compost was found more effective in suppressing the hatching of root-knot nematode eggs and larval mortality. The nematode mortality and plant growth was found positively correlated with the doses of the oyster and button mushroom compost. The spent compost of oyster was also found to have stimulated plant growth.

Spent mushroom compost can be used as an effective tool to manage root-knot nematodes and save the environment from the effect of nematodes. Further research work is needed to investigate the effect of spent mushroom compost on the management of root knot nematodes and other soil born diseases. How spent mushroom compost can help in improving the soil health, also needs an intensive research.

REFERENCES

Akhtar, M. and M.M. Alam. 1993. Utilization of waste material in nematode control: a review. Bio-Resource Technol. 45: 1-7.

Akhtar, A. and A. Malik. 2000. Role of organic soil amendments and soil organisms in the biological control of plant parasitic nematodes. A Rev. Bio-Resource Technol. 74 p.

Barron, G.L. and R.G. Thorn. 1987. Destruction of nematodes by species of *Pleurotus*. Canad. J. Bot. 65:774-778.

Back, M.A., Haydock, P.P.J. and P. Jenkinson. 2002. Disease complexes involving plant parasitic nematodes and soil born pathogen. Plant Path. 51: 683- 697.

Bridge, J. 1996. Nematode management in sustainable and subsistence agriculture. Annual Rev. Phytopath. 34: 201-225.

Castello, P., J.A. Navas-Cortes, D. Goamar-Tinoco, M. Di Vito and R.M. Jimenez-Diaz. 2003. Interactions between *Meloidogyne artiellia*, the cereal and legume root-knot nematodes and Fusarium oxisporum f.sp. ciceris race 5 in chickpea. Phytopath. 93:1513-1523.

Cohn, E. 1974. Relations between *Xiphinema* and *Longidorus* and their host plants. Nematode vectors of plant viruses. Plenum Press, New York. pp. 365-386.

Eisenback, J.D. and H. Triantaphyllou. 1991. Root-knot Nematodes *Meloidogyne* species and races. Manual of Agric. Nematol. pp.281-286.

Eisenback, J.D., H. Hirschmann, J.N. Sasser and A.C. Triantaphyllou. 1981. A more complete characterization of the four most common species of root-knot nematodes (*Meloidogyne spp*.) with pictorial key. IMP Public. N.C. USA.

Gezer, K. and M.E. Duru. 2006. Spectrophotometric determination of phenolic compounds. Afr. J. Biotech. 5: 1924-1928.

Gulcin, I., M.E. Buyukokuroglu, M. Oktaym and Ol. Kufrevioglu. 2003. Anti-oxidant and analgesic activities of turpentine of pinus nigr. Arn. Subsp. Pallsiana (Lamb.) Holmboe . Ethnophar. 86: 51-58.

Hung, C.L. and R.A. Rohde. 1973. Phenol accumulation related to resistance in tomato to infection by root knot and lesion nematodes. J. Nematol. 5: 253-258.

Hayashi, M., K. Wada and K. Munakata. 1983. Synthesis and nematicidal activity of phenoxypropionic acid derivatives. Agric. Biologic. Chem. 47: 2653-2655.

Mariela, G. 2003. Spectrophotometric Determination of phenolic compounds. Latin Amer. J. 22: 243-248.

Manzanilla-Lopez, R.H.E.K. and J. Bridge. 2004. Plant diseases caused by nematodes. CABI Publish. Beijing, China.

Mori, M., S.S. Hyeon, Y. Kimura and A. Suzuki. 1982. The nematicidal activity of acetylene compounds. Agric. Biologic. Chem. 46: 309-311.

Oka, Y. and U. Yermiyahu. 2002. Suppressive effect of compost against the root knot Meloidogyne javanica on tomato. Nematol. 4: 891-898.

Ploeg, A. 2001. When nematodes attack is important. California Grower. pp.12-13.

Roberts, P.A. 1992. Current status on the antiability, development and use of host plant resistnance to nematodes. J. Nematol. 24: 213-227.

Somasundaram, R., M. Nanjarajurs and S. Rashmi. 2003. Biochemical changes associated with mushroom browning in *Agaricus bisporus* (Lange) Imbach and *Pleurotus florida*. J. Sci. Food & Agric. 83: 1531–1537.

Shaukat, S.S. and A.A. Siddique. 2002. Effect of some phenolic compounds on survival, infectivity and population density of *Meloidogyne javanica*. Nematol. Medit. 29: 123-126.

Siddique, A.A. and S.S. Shaukat. 2003. Suppression of root-knot disease by *Pseudomonas fluorescens* in tomato. Importance of bacterial secondary metabolites to 2, 4-diacetylpholoroglucinol. Soil Biol. Biochem. 35: 1615-1623.

Stirling, G.R. 1991. Biological control of plant parasitic nematodes. CABI Publish. Int'l. Wallingford, UK. 275p.

Susana, S.A., C.R. Anguina, G. Reglera and C.S. Rivas. 2009. Improvement of antimicrobial activity of edible mushroom extracts by inhibition of oxidative enzymes. Int'l. J. Food & Sci. Technol. 44: 1057-1064.

Slinkard, K. and V.L. Singleton. 1977. Total Phenol Analysis: Automation and comparison with manual methods. Amer. J. 1:49-55.

Steel, R.G.D. and J.H. Torrie. 1980. Principal and procedures of statistics. McGraw-Hill Co. Book Co. New York. 2nd ed. 633p.

Veech, J.A. 1979. Histochemical localization and nematoxicity of terpenoids aldehydes in cotton. J. Nernatol. 11: 240-246.

Walker, G.E. 2004. Effects of *Meloidogyne javanica* and organic amendments, inorganic fertilizers and nematicides on carrot growth and nematode abundance. Nematologia Mediterranea. 32:181-188.

Yen, G.C., P.D. Duh and C.Tsai. 1993. Relationship between anti-oxidant activity and maturity of peanut hulls. J. Agric. Food Chem. 403.1: 67-70.