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Spent Mushroom as Eco-friendly Management Strategy of Root-knot Nematodes, *Meloidogyne* spp. Infecting Eggplant

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ABSTRACT

Eggplant production was limited by many plant pathogens all over the world. Root-knot nematodes (RKN) considered one of the most warning pathogens. In this study, the potential nematicidal effect of different spent mushroom (SM) doses i.e:10,20,30,50 and 100 g/pot were evaluated against *Meloidogyne* spp. infecting eggplant under greenhouse conditions. Results showed that all used SM doses significantly reduced nematode parameters compared to that contain nematode alone. Reduction in gall numbers reached 96% by adding SM at 100g/pot, followed by 91% at 50g/pot comparing with untreated control. Additionally, reduction of egg mass numbers and females / root system recorded 94.78 and 96.65 % respectively. Also, number of second stage juveniles/ 250g soil was affect by using the treatments. Moreover, plant growth parameters were significantly enhanced by most treatments in comparison with untreated control plants. Therefore, using of SP might be included in the integrated nematode management programs as a sustainable eco-friendly strategy.

Key words: Biological control, *Meloidogyne* spp., management, spent mushroom, Eggplant.

INTRODUCTION

Eggplant (*Solanum melongena L.*) Family Solanaceae listed as one of the most growing economic vegetable crops in Egypt and all over the world. Eggplant offers an important nutrient source for human consumption and protect from many diseases. Eggplant is widely grown throughout the year under greenhouse and open field conditions. In 2019 the total Egyptian cultivated area of Eggplant was 43818 ha with a final production of 1180240 tons (FAO,2019). This crop attacks by one or more of plant pathogens such as bacteria, fungi, viruses, and plant parasitic nematodes (PPNs). Nematodes also can play as a vector for other plant pathogens (Nykyri *et al*.,2014).

Recently, more than 4100 species of PPN were registered (Decraemer and Hunt, 2006). Root-knot nematodes (*Meloidogyne* spp.) listed of the most ten frequently genera of PPNs worldwide as reported by Jones *et al*., (2013). About 3000 plant species from different plant families forms the host range for the 98 species of *Meloidogyne* genus (Jones *et al*., 2013) concerning with varying yield losses in both tropical and sub-tropical agriculture lands (Sikora and Fernandez,2005). So that, *Meloidogyne* recorded as limitation and destructive factor for many crop cultivations in the new reclaimed lands in Egypt (Bakr *et al*., 2011,2017and 2020). According to study by Sasser and Freckman (1987) the International annual loss in eggplant causing by nematodes were 16.9%. While, in 2011-2012 the Egyptian annual yield losses in

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eggplant due to damage by PPNs reached 20% equal 298.41 million L.E. (Abd-El Gawad, 2014). Plants infected by RKN not only showed galled root system but also little vegetative plant growth and a less yield with low-quality grade (Al-Hendy *et al*.,2021).

No one can deny that chemical nematicides offer effective and quick method in controlling of plant-parasitic nematodes. On the other hand, they cause dangerous and un-preferred effects on different biological systems. Thus, searching for sustainable alternatives strategies for PPNs control takes a great interest worldwide to avoid the negative impacts on the environment and support the clean technologies (Bakr and Ketta, 2018). Recently, various studies were confirming the use of organic matter as a sustainable environmental strategy for the control of different plant disease and minimizing the agrochemical synthetic inputs and reducing the farming activity residues (D'addabbo *et al.*, 2011; Bakr, 2017and 2018; Varo-Su_arez *et al*., 2018; Bakr,2021).

Edible mushroom is recommended as rich sources of different minerals, proteins, and vitamins with other pharmaceutical and medicinal properties. After mushroom harvesting process, a huge amount of solids waste (spent mushroom (SM) remains. Ater cultivation ,5–6 kg of by-product coms from every kilogram of mushrooms (Ma *et al*., 2014). For sanitation, grower must rapidly discard these wastes to avoid contamination and infestation and of new cultivation cycles by pests and pathogens. (Marques *et al*., 2014). Different uses of SM substrates were mentioned (Rinker, 2017). Spent mushroom can used as soil improver, fertilizer and for bioremediation of soil pollutants (Kulshreshtha *et al*., 2014; Orluchukwu and Adedokun, 2015; Mostafa *et al*., 2019). Also, SM material of mushrooms may have nematicidal potential thus, there is a possibility of using it as sustainable tools to control plant parasitic nematodes (Aslam and Saifullah,2013; Hahn *et al*.,2019; Castañeda-Ramírez *et al*.,2020; Tanimola and Adedokun, 2020).

Therefore, the present study was planned to determine the nematicidal potential of SM substrate on root-knot nematodes infecting eggplant under greenhouse conditions.

MATERIALS AND METHODS

Multiplication of *Meloidogyne* spp.:

For root-knot nematodes, *Meloidogyne* spp. maintenance, tomato plant (*Lycopersicon esculentum* cv. Beto-86) grown in plastic pots 50 cm in diameter previously filled with mixed clay: sandy soil (1:2 v/v). Then, tomato plants were infected with egg masses of *Meloidogyne* spp. and kept under greenhouse conditions (25 ± 3 ºC with relative humidity 85%) at the experimental farm, Faculty of Agriculture, Menoufia University, Egypt.

Meloidogyne spp. inoculum preparation:

The severity galled tomato roots infected with *Meloidogyne* spp. were carefully uprooted and gently washed to remove all adherent soil particles. Small pieces of roots were macerated using blender (Monilinex) at high speed twice for 10 seconds. Produced root solution was transfer into a glass bottle containing 0.5% sodium hypochlorite (NaOCl) for complete extraction of nematode eggs according to the technique described by Hussey and Barker (1973). Bottle was carefully shaken for 3 minutes to increase the ability of NaOCl to remove the gelatin matrix from egg masses to free eggs. Also, the obtained solution was pass-through serial sieves to remove extra root tissue. Extracted Eggs were received on the 20 micrometer (µm) sieve and washed several times using tap water until totally remove residual NaOCl.

Resulted eggs were collected in jar containing tap water where number of eggs per one millimeter was counted under dissecting microscope.

Effect of SM on eggplant plants infected with *Meloidogyne* spp.:

An experiment was carried out to evaluate the effect of SM at different doses on root-knot nematodes in eggplant under greenhouse conditions at the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt. Plastic pots (15 cm in diameter) were filled with nonsterilized mixed sand / clay soil (2/1 v/v). Different doses of SM at 10, 20, 30, 50 and 100 g/pot were mixed thoroughly with soil pots. Pots were daily irrigated to allow SM for decomposition process. One week after, eggplant seedling (*Solanum melongena* L.) cv. Balady 3 weeks old was transplanted in each pot (one seedling/pot). After three days, 3000 eggs and second stage juveniles were inoculated/plant by pipetting into 3-4 holes around the plant roots. Each treatment was replicated three times. Plants ware irrigated as needed and weekly fertilized with 5 ml of 2g /l of N: P: K (20:20:20) obtained from International Egypt company for Agricultural and Industrial Developing. After two months of nematode inoculation, plants were removed, and their roots were carefully washed under running tap water to remove soil particles. Vegetative parameters i.e., plant and shoot lengths (cm), fresh shoot and root weights (g), dry shoot weight (g), were recorded. Nematode characters i.e., second stage juveniles (Js_2) /250 g soil, number of galls, females and egg masses/ root system were also recorded. Egg masses were counted by staining the root system with Phloxine-B as described by (Daykin and Hussey, 1985) by dipping the root system in 0.015% Phloxine-B solution for 20 minutes. Females were collected as mentioned by Mahdy (2002).

Peroxidase activity was measured according to the method described by Fehrman and Dimond (1967). In addition, phenoloxidase was determined by the method described by Broesh (1954).

Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA) and comparisons of means at the 5% level of significance using costat 6.3 version program according to Duncan's multiple range test.

RESULTS

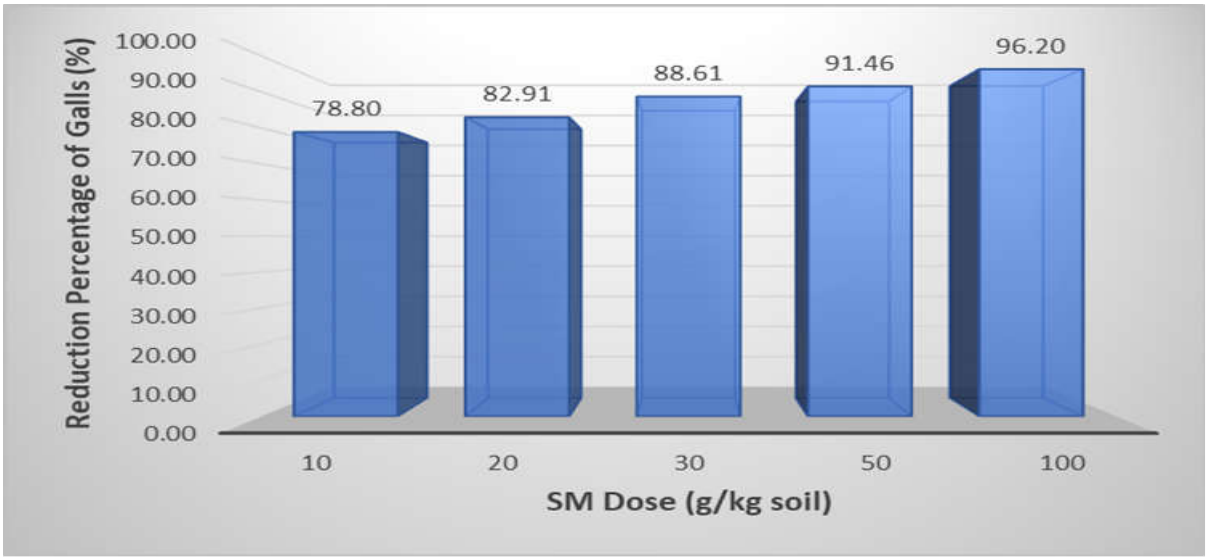
Results presented in table (1) showed that all applied doses of SM significantly reduced all nematode parameters compared to plants treated with nematode alone. Data showed that the lowest number of galls was recorded with SM at 100g / pot, followed by 50g/ pot, while the highest number of galls was observed with 10g/ pot. Reduction percentage of galls reached 96% at the treatment of 100g SM /pot, followed by 91% at 50g/pot, with significant differences between all SM doses and the treatment of plants with nematode alone as shown in table (1) and fig. (1). Results referred that all doses of SM significantly decreased the mean number of egg masses/ root system compared to untreated plants. Also, results showed significant differences in egg masses number between plants treated with all SM doses and plants treated with nematode alone as presented in table (1) and fig. (2). Results also indicated that the highest dose (100g/ pot) gave a high effect in reducing egg masses/ root system, followed by the treatment of 50g/ pot as the reduction percentage of egg masses was 94 and 76%, respectively, while the lowest one

was obtained with the dose of 10g/ pot. The great effect in reducing female numbers / root system was obtained with the dose of SM at 100g/pot, followed by 50g/ pot resulting 96 and 70%, respectively, while the lowest one was recorded in pots treated with 10g/ pot. Results revealed that SM at 100g/ pot was the best treatment among all doses and the least effective one was recorded with 10g/ pot as illustrated in fig. (3). The same trend of results was recorded with the number of J_2s as the highest decrease was recorded with 100g/pot and the lowest one was observed with 10g/ pot. Reduction percentage of J2s reached 96% with 100g/ pot, whereas it was only 29% at the treatment of 10g/ pot as shown in fig. (4).

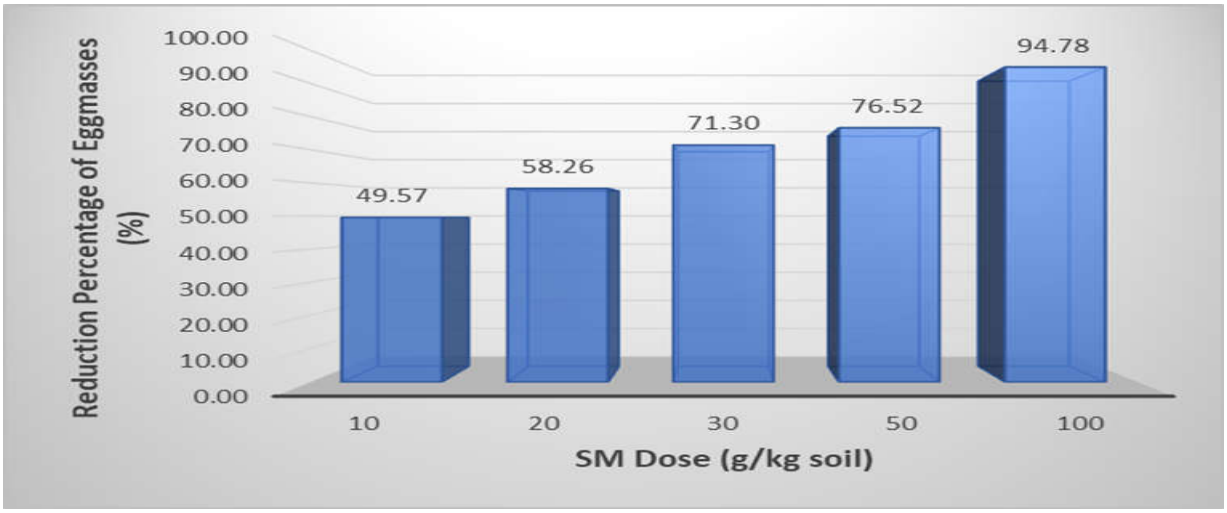
Table (1): Effect of spent mushroom applied at five doses on nematode parameters of eggplant plants infected with *Meloidogyne* spp under greenhouse conditions.

Treatments	Doses g/kg soil	Second stage/250g soil	Nematode parameters/ root system		
			Number of galls	Number of egg masses	Number of females
Spent mushroom + Nematode	10	5995^{b}	22.33^{b}	19.33^{b}	21.66^{b}
	20	4915^{c}	18^{b}	16^{bc}	18.66^{bc}
	30	3500^{d}	12^{b}	11^{bc}	14.33^{bc}
	50	2495^{e}	9^{b}	9^{cd}	11.66^{c}
	100	266.66^{f}	4^{b}	2^{d}	1.33^{d}
Nematode alone		8495^{a}	105.33^{a}	38.33^{a}	39.66^{a}

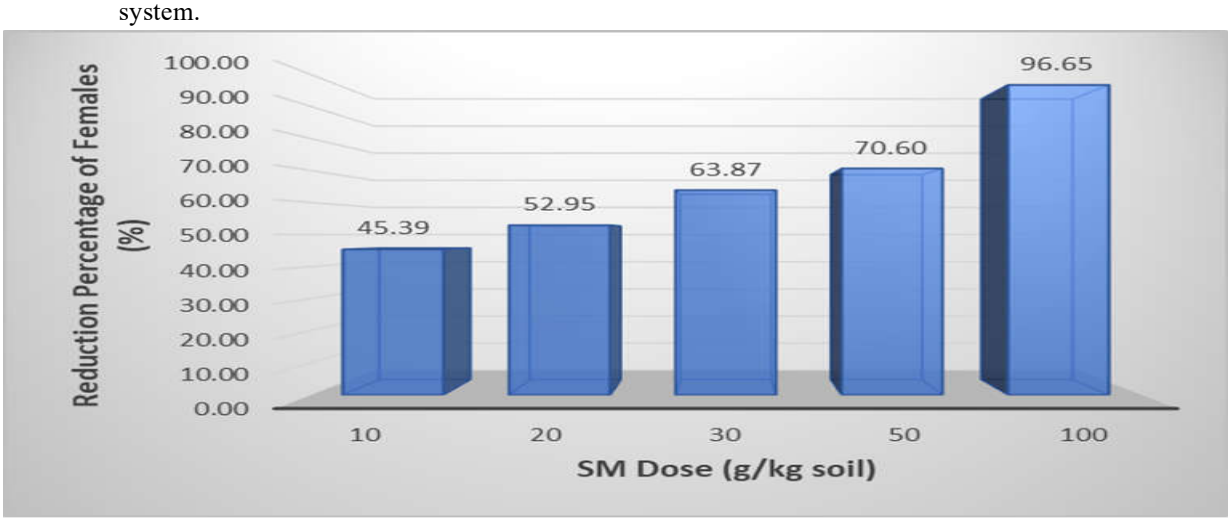
Columns followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05).



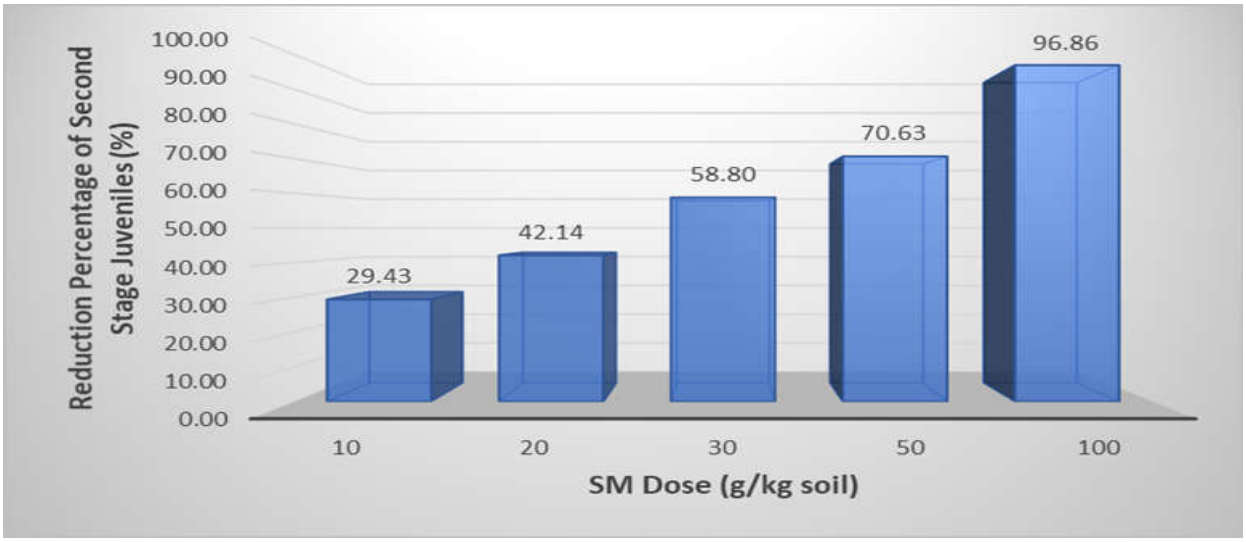
Fig(1): Effect of spent mushroom (SM) at five application doses on reduction percentages of galls/ root system.



Fig(2): Effect of spent mushroom (SM) at five application doses on reduction percentage of egg masses/ root system.



Fig(3): Effect of spent mushroom (SM) at five application doses on reduction percentage of females/ root system.



Fig(4): Effect of spent mushroom (SM) at five doses on reduction percentage of second stage juveniles/ 250g soil.

Effects on plant growth

Data presented in table (2) showed the effect of SM on plant growth parameters of eggplant plants either infected or non-infected by root-knot nematodes (control). In general, data confirmed that all treatments increased growth parameters as compared with untreated plants. Results showed that the SM at 100 g/pot was the effective applied dose in enhancing plant length followed by 50 g/pot, while the lowest effect was recorded with 10 g/pot as shown in fig. (5). Data cleared that the high value of fresh shoot weight was recorded when plants treated with 100 g/pot followed by 50 g/pot for the same treatment. However, the lowest fresh shoot weight was recorded with 10 g/pot as illustrated fig. (6). Data showed that the highest dry shoot weight of eggplant plants was recorded with 100 and 50 g/pot, followed by 30 g/pot. However, the least value of dry shoot weight recorded with 10 g/pot as presented in fig. (7).

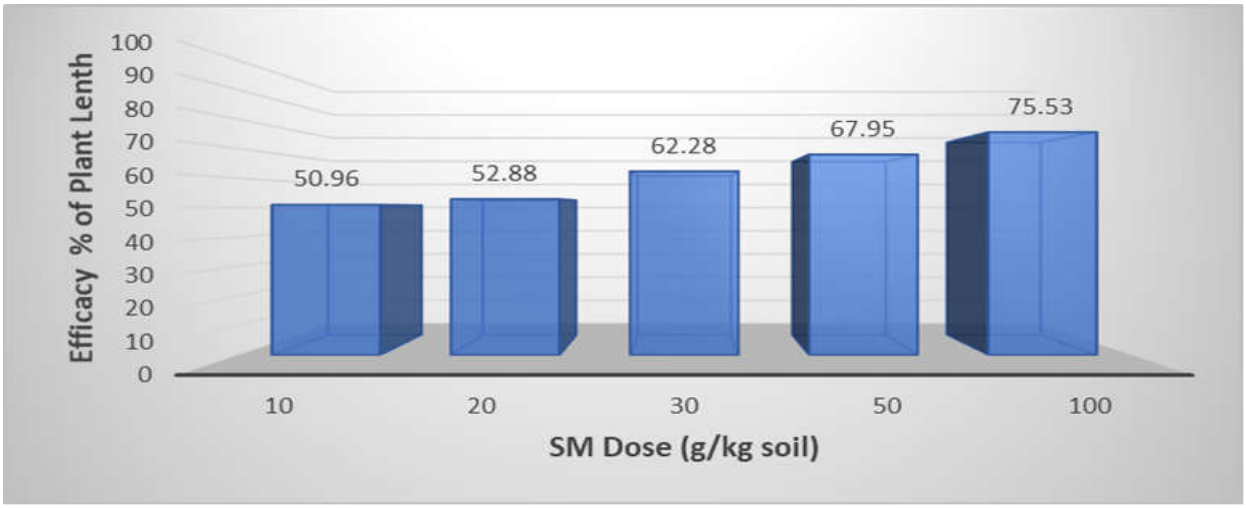
Antioxidant enzymes activity:

The effect of SM application at different doses on antioxidant enzymes activity in fresh leaves of nematode infected eggplant plants are presented in table (3). Results showed the positive effect of SM application at all doses on peroxidase and phenoloxidase compared to plants treated with nematode alone. Spent mushroom at 100g was the most effective, followed by SM 50g, while the lowest effect was recorded with SM 10g.

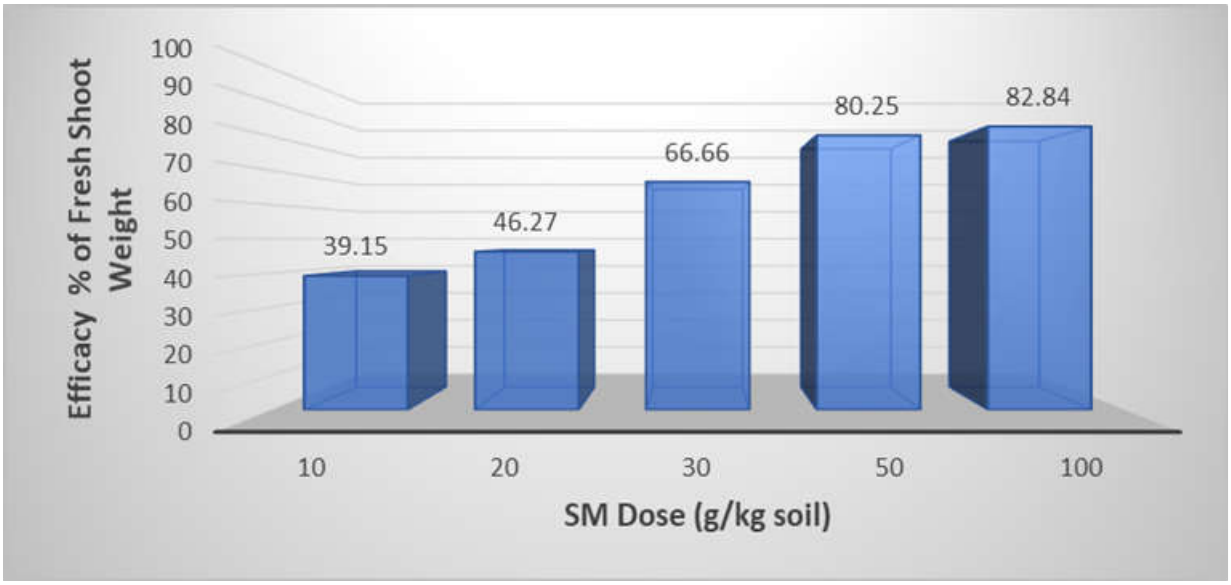
Table (2): Effect of spent mushroom at five application doses on eggplant growth parameters, infected with *Meloidogyne* spp under greenhouse conditions.

Treatments	Doses g/kg soil	Plant length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)
Spent mushroom + Nematode	10	26.66^{b}	4.30^{abc}	0.47^{ab}	2.17^{d}
	20	27.00^{ab}	4.52^{abc}	0.37^{ab}	3.43^{b}
	30	28.66^{ab}	5.15^{abc}	0.34^{ab}	4.13^{f}
	50	29.66^{ab}	5.57^{ab}	0.32^{ab}	4.43^{a}
	100	31.00^{a}	5.65^{a}	0.26^{b}	4.49^{a}
Nematode alone		17.66^{c}	3.09^{bc}	0.53^{a}	2.11^{c}

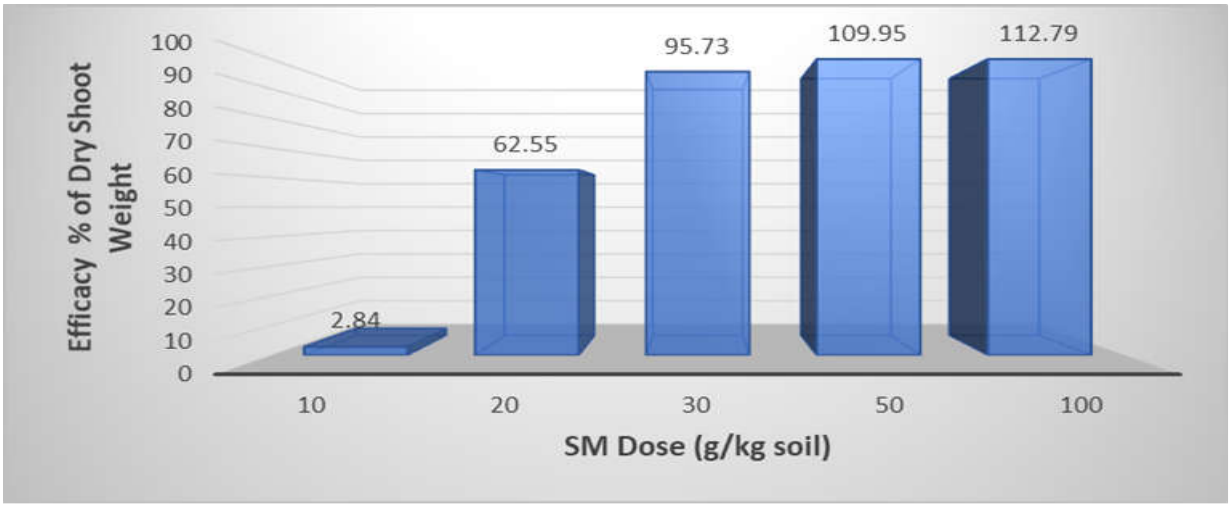
Columns followed by different letters are significantly different according to Duncan's Multiple Test ($P \leq 0.05$)



Fig(5): Effect of spent mushroom (SM) at five application doses on plant length of eggplant plants infected with *Meloidogyne* spp. under greenhouse conditions



Fig(6): Effect of spent mushroom (SM) at five application doses on fresh shoot weight of eggplant plants infected with *Meloidogyne* spp. under greenhouse conditions.



Fig(7): Effect of spent mushroom (SM) at five application doses on dry shoot weight of eggplant plants infected with *Meloidogyne* spp. under greenhouse conditions

Table (3): Effect of SM at five application doses on the activity of some antioxidant enzymes of eggplant plants infected with *Meloidogyne* spp.

Treatments	Doses g/kg soil	Antioxidant enzymes	
		Peroxidase (O.D.g-1 fr.wt. after 2 min)	Phenoloxidase (O.D.g-1 fr.wt. after 45 min)
Spent mushroom + Nematode	10	0.46b	0.20b
	20	0.47b	0.40b
	30	0.70b	0.43b
	50	0.73b	0.53b
	100	1.03a	0.99a
Nematode alone		0.19c	0.09c

Columns followed by different letters are significantly different according to Duncan's Multiple Test (P≤0.05).

DISCUSSION

Numerous studies by researchers worldwide indicated that soil amendment with organic materials reduced disease incidence and considered as a suitable mean of nematode control (Arancon *et al*., 2003; Nahar *et al*., 2006; Renčo *et al*., 2007; Bakr,2021). Current study indicated the efficacy of SM in reducing and managing of RKN under greenhouse conditions. Our results are greatly consistent with those by Sabina and Saifull (2013), who reported that oyster mushroom spent compost (OMSC) was effective in reducing the egg hatching and killing juveniles of root-knot nematodes under laboratory conditions.

Also, OMSC reduced the root galling and egg masses in tomato plants infected with RKN and effectively stimulated the plant growth parameters under pot experiment. Similar results obtained by Abbasi *et al*., (2014) who evaluated the nematicidal effect of spent oyster mushroom composts at different concentrations against *M. javanica* on tomato plants and found that spent oyster mushroom composts at 70% significantly reduced nematode population and egg mass in roots compared with nontreated control. In another study by El-Sherbiny and AwdAllah (2014) they found that, under field conditions, pre-planting soil biofumigation with waste residues of oyster mushroom greatly managed *M. incognita* infection on tomato plants, where number of root galls, nematode egg masses, final population (Pf) and reproduction factor (Rf) in all treatments were significantly reduced compared to control plants. The composts release compounds toxic to plant nematodes like phenols, tannins, terpenes, (Mian and Rodriguez-Kabana, 1982) or derived from decomposition processes in the soil, like ammonia, nitrites, hydrogen sulphide (Rodriguez- Kabana, 1986). Presence the phenolic compounds in spent mushroom compost offer an antimicrobial activity, which may be considered an effective biocontrol of RKN, *Meloidogyne* spp. on tomato plants. Earlier studies confirmed the antimicrobial properties of *Lentinus edodes, Pleurotus ostreatus, Boletus edulis* and *Agaricus bisporus* water extracts (Santoyo *et al*., 2009). However, farmers dispose spent mushroom as a waste product it, save a good source of nutrients such as nitrogen, phosphorus, and potassium besides its ability as a soil conditioner and improve soil organic matter. Interesting results were recorded by El-Saedy, *et al*., (2017) when they found that spent of oyster mushroom significantly reduced second stage and females of citrus nematode, *Tylenchulus semipenetrans* in Valencia orange roots under field conditions.

Spent mushroom has a lot of elements like nitrogen, phosphorus and potassium, these materials increase salt and soil acidification. It tends to be high in phosphorous and potassium, while relatively low in nitrate nitrogen. It is bulky in volume but light in weight with high cation exchange capacity and a slow mineralization rate (Wuest *et al*., 1995), which consider helpful means in improvement of vegetative growth characters such as, plant height, fresh shoot weight, root fresh weight of tomatoes plants. Different mushroom species might be induced plant defenses against plant pathogens by producing some biotic resistance inducers (Silva *et al*., 2013).

CONCLUSION

From the findings of this study, it could be reported that spent mushroom effectively in decreasing RKN population and improving growth parameters and yield of eggplant plants. Therefore, it could be concluded that, SM as an ecofriendly strategy, can be used a part of integrated PPNs management with an advantage to agriculture sustainability.

FUTURE PROSPECTIVE

More conscious concerning with eco-friendly control strategies such as biological methods is necessary for improving safety crop production. Improvement of different methodologies for bio-control multiplication, adaptation, combination with other negative or less environmental impact is suggested. This will develop formulation to maximize their efficacy and stability. These could lead to reduce using of chemical synthetic nematicides then providing environment and food security.

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