



Sustainable Management of Root Knot Nematode *Meloidogyne incognita* through Organic Amendment on *Solanum lycopersicum* L.

Mohd Asif^{1*}, Amir Khan¹, Moh Tariq¹ and Mansoor A. Siddiqui¹

¹Section of Plant Pathology and Nematology, Department of Botany, Aligarh Muslim University, Aligarh, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author MAS conceived the experiment. Author MA performed the experiment, wrote the protocol and wrote the first draft of the manuscript. Authors AK and MT performed the statistical analysis and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the nematicidal potential of wild spinach powder in combination with fresh chopped leaves of different plants viz., Indian mallow, Mexican poppy, Ivy gourd, Trailing eclipta, Wild eggplant and Black pigweed against *Meloidogyne incognita* on plant growth characters of tomato cv. K-21.

Place and Duration of Study: A glasshouse pot experiment was carried out in the Department of Botany, Aligarh Muslim University, Aligarh.

Methodology: A mixture of soil and organic manure was prepared in the ratio 3:1. The pots were treated with fresh chopped leaves of different plants viz., Indian mallow, Mexican poppy, Ivy gourd, Trailing eclipta, Wild eggplant and Black pig weed applied 50 g of fresh chopped leaves of plants were applied combined with seed powder 10 g of seed powder of Black nightshade”.

Results: Combined application of wild spinach powder along with the fresh chopped leaves of all the plants suppressed pathogenic effect of nematode and thereby resulted in significant reduction

*Corresponding author: E-mail: asifgc2616@gmail.com;

in *Meloidogyne incognita* infestation and population density of *Meloidogyne incognita* in soil. The highest reduction in *Meloidogyne incognita* infestation was reported in plants employed with 10 g of wild spinach powder combined with 50 g of Mexican poppy leaves. Plants applied 10 g of wild spinach powder combined with 50 g of Mexican poppy leaves were the best followed by 50 g of Trailing eclipta, 50 g of Wild eggplant, 50 g of Black pigweed, 50 g of Indian mallow, 50 g of Ivy gourd in the descending order. However the lowest reduction was recorded with the application 10 g of wild spinach powder plus 50 g of fresh chopped Ivy gourd. Organic additives of wild spinach powder along with the fresh chopped leaves would work for sustainable management by increasing the nutrient status of the soil which further enhances the plant growth.

Conclusion: The application of Mexican poppy, Trailing eclipta and Wild eggplant in combination with wild spinach powder are quite efficient alternatives of chemical nematicides for the *Meloidogyne incognita* management and yield enhancement. Although the utilization of chopped leaves of Indian mallow and Ivy gourd along with wild spinach powder is not potentially active replacement of synthetic nematicides however further characterization is needed.

Keywords: Tomato; chopped leaves; powder; *Meloidogyne incognita*; sustainable management.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L. Family-Solanaceae) is the most important vegetable crop widely used throughout the world. Tomatoes are consumed in the form of juice, paste, ketchup, soup etc. Indian contribution to the annual world production was 18735912 MT with an area of 882032 HA in 2013-2014 [1]. *Meloidogyne incognita* was found constantly associated with the root of tomato where population of nematode was influenced by clay and silt percentage [2]. The *Meloidogyne incognita* are sedentary endoparasites and are among the most damaging agricultural pests attacking a wide range of crops [3], particularly in vegetables, causing dramatic yield losses mainly in tropical and sub-tropical agriculture [4]. Plant parasitic nematodes attack plants and caused significant damages and yield losses in crops [5]. In the absence of effective control *Meloidogyne incognita* can cause total crop failure [6]. Current management of nematodes includes plant resistance, crop rotation [7] and biocontrol agents [8,9]. Most important way of nematode control is mainly the nematicides. Most of the nematicides are banned due their toxic nature and carcinogenic effect on the fauna; beside this they disturbed the ecological equilibrium of the soil. Thus the development of alternative control strategies and long-term integrated approaches is urgently needed in order to replace chemical nematicides [10,11,12,13].

The incorporation of organic material (plant parts and plant products) into the soil reduces *Meloidogyne incognita* densities, resulting in an increase in tomato yield [14,15]. Various medicinal and antagonistic plants have been

found effective for the control of *Meloidogyne incognita* [16,17,18]. Therefore, this study was conducted with the purpose to evaluate the efficacy of Wild spinach powder in combination with the fresh chopped leaves of different plant species against *Meloidogyne incognita* on tomato.

2. MATERIALS AND METHODS

2.1 Site for the Experiment

A mixture of soil and organic manure was prepared in the ratio 3:1. Glasshouse pot culture experiment was carried out in Department of Botany, Aligarh Muslim University, and Aligarh. It has average temperature ranges from 28–38°C (82–100°F) with the scanty rainfall of 650 to 750 mm is recorded throughout the year.

2.2 Test Plant and Pathogen

Tomato (*Solanum lycopersicum* L.cv.K-21, Family Solanaceae) was selected as a test plant and *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood 1949), was chosen as test pathogen to evaluate the effect of various organic soil amendments on the management of *Meloidogyne incognita*.

Seed powder of Wild spinach (*Solanum nigrum* L., Family Solanaceae), Fresh chopped leaves of Mexican poppy (*Argemone mexicana* L., Family Papaveraceae), Trailing eclipta (*Eclipta alba* L., Family Asteraceae), Wild eggplant (*Solanum xanthocarpum* Schrad. & Wendl, Family Solanaceae), Black pig weed (*Trianthema portulacastrum* L., Family Aizoaceae), Indian mallow (*Abutilon indicum* L. (Sweet), Family

Malvaceae), Ivy gourd (*Coccinia grandis* (L.) Voigt Family Cucurbitaceae) were taken for the study from the campus Aligarh Muslim University, Aligarh. Collected leaves were washed in running tap water to remove dust particles and then processed for chopping with the help of knife.

2.3 Collection of Infected Root

Meloidogyne incognita infected roots were collected from Mathura road, Aligarh, UP, India. Infected roots of the plants gently removed from the soil and kept in polythene bags and then labeled. Further all these roots were brought to the laboratory for the examination.

2.4 Source of *Meloidogyne incognita* Juveniles

Juveniles of *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949) were prepared from a pure culture that was previously cultured by eggmasses and propagated on eggplant (*Solanum melongena* L.) in the glasshouse of Section of Plant Pathology and Plant Nematology, Department of Botany, Aligarh Muslim University, Aligarh, India. Surface attached eggmasses were detached by using sterilized forceps from the infected roots. These eggmasses were placed in 15 mesh sieves (8 cm in diameter) having crossed layer of tissue paper. These were kept in petridishes full of water so that eggmasses may remain in contact with water. These petridishes were then incubated at 28 ±2°C for hatching and for freshly hatched second stage juveniles (J2) of *Meloidogyne incognita*.

2.5 *Meloidogyne* Species Identification

Meloidogyne incognita was identified on the basis of perineal pattern from infected root samples and were examined under stereomicroscope [19].

2.6 Maintenance of Seedlings

The seeds of tomato cv. K-21 were surface sterilized in 0.01% HgCl₂ for three minutes and then rinsed with Double Distilled Water (DDW) three times. For nursery preparation seeds were sown in autoclaved clay pots (30 cm diameter) along with the soil. Three weeks after germination of proper seedlings of tomato cv. K-21 were transplanted to each 15 cm diameter clay pots filled with 1 kg autoclaved soil. These pots were treated with fresh chopped leaves of

different plants viz., Indian mallow, Mexican poppy, Ivy gourd, Trailing eclipta, Wild eggplant and Black pig weed applied 50 g of fresh chopped leaves of different plant combined with 10 g seed powder of Black nightshade per pot. The pots were watered regularly for proper decomposition of the organic additives for two weeks. As the seedlings get established each of them was inoculated with 1500 hatched second stage juveniles (J2) of *Meloidogyne incognita*. Each treatment was replicated four times.

2.7 Inoculation Technique

Inoculation was done by making 3 holes in the pots soil nearby to the roots at the same distance in the manner so that root don't get damage. Then requisite amount of suspension having necessary number of second stage juveniles was procured into the holes and then covered them with the soil.

2.8 Observation

After three months of the inoculation, roots of tomato cv. K- 21 were uprooted carefully from the pots and were washed in running tap water to wash off the soil particles adhered with the root is removed. The water present in the plants was eliminated by pressing them in between the blotting sheets. The plant growth parameters including the length (cm), fresh and dry weight (g) of shoot and root, yield/plant (g) were measured. Biochemical and pathological parameters including percent pollen fertility, chlorophyll content (mg/g), carotenoid content (mg/g), eggmasses /root, eggs/eggmass, nematode population (250 g) and root knot index (RKI) were also examined respectively. The number of plants with galled root system and the root-knot index were evaluated on a 0-5 scale (0 = no galling; 1 = 1-2 galling; 2 = 3-10 galling; 3 = 11-30 galling; 4 = 31-100 galling; and 5 = more than 100 galling per root system according to Taylor and Sasser, 1978). The nematode populations present in the soil was estimated by Cobb's sieving and decanting technique followed by modified Baermann's funnel technique by processing 250 g of soil sample.

2.9 Statistical Analysis

The data presented in the tables was analyzed by one-way analysis of variance (ANOVA) using SPSS 17.00 software (SPSS Inc., Chicago, IL, USA). Least Significant Differences (LSD) were calculated at $P= 0.05$ to test for significant differences between the treatment means.

Table 1a. Effect of chopped leaves of different plant species in combination with leaf powder of Wild spinach on growth of tomato cv.K-21 in relation to root-knot development caused by *Meloidogyne incognita* in pots

Treatment	Length (cm)			Weight(g)					
	Shoot	Root	Total	Fresh			Dry		
				Shoot	Root	Total	Shoot	Root	Total
Indian mallow	31.4ef	15.0cd	46.4de	25.7e	10.3bc	36.0ef	8.3de	3.2cd	11.5ef
Mexican poppy	36. b	17.3b	53.5b	29.0b	12.0b	41.0b	10.0b	4.2b	14.2b
Ivy gourd	30.5f	14.2d	44.7 e	25.2e	10.0c	35.2f	8.0e	3.0d	11.0f
Trailing eclipta	35.0bc	16.7bc	51.7bc	28.0bc	11.6 b	39.6bc	9.5bc	4.0b	13.5bc
Wild eggplant	33.8cd	16.2bc	50.0bcd	27.2cd	11.2b	38.4cd	9.2bc	3.7bc	12.9cd
Black pig weed	32.4de	15.8bc	48.2cde	26.de	10.7bc	37.2 de	8.8cd	3.5bc	12.3de
UUC	53.6a	24.6a	78.2 a	52.0a	20.0a	72.0a	18.0 a	6.0a	24.0a
UIC	24.7g	11.8e	36.5f	20.0f	8.0d	28.0g	7.1f	1.4g	8.5g

Each value is the mean of four replicates; Initial inoculum = 1500 (J_2) of *Meloidogyne incognita* per pot; Means in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$; UUC- Untreated Uninoculated Control; UIC- Untreated Inoculated Control

Table 1b. Effect of chopped leaves of different plant species in combination with leaf powder of Wild spinach on tomato cv. K-21 In relation to root knot development and multiplication of *Meloidogyne incognita* in pots

Treatment	Chlorophyll content (mg/g)	Carotenoid content (mg/g)	Pollen fertility (%)	Yield / plant (g)	Eggmasses /root	Eggs/ Eggmass	Nematode population (250 g)	Root-knot index
Indian mallow	1.51 cd	.400 e	62.0c	160ef	132bc	209bc	1170 c	2.8bc
Mexican poppy	1.73 b	.495 b	67.0b	228b	114e	188e	1042 g	2.0d
Ivy gourd	1.45 d	.390f	61.0c	144g	140 b	215 b	1204 b	3.0b
Trailing eclipta	1.68b	.474 c	65.6b	200 c	118de	193de	1069 f	2.2cd
Wild eggplant	1.62bc	.446 d	64.0bc	186 d	123cd	197de	1100e	2.3cd
Black pig weed	1.55cd	.418 e	63.5bc	167e	128c	202cd	1142 d	2.5bcd
UIC	1.12e	.252g	47.0f	112.0g	175 a	266 a	1608 a	5.0 a
UUC	2.78 a	.880 a	89.5 a	355 a	0 f	0 f	0 g	0 e

Each value is the mean of four replicates; Initial inoculum = 1500 (J_2) of *Meloidogyne incognita* per pot; Means in each column followed by same letter are not significantly different according to Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$; UUC- Un treated Uninoculated Control; UIC- Untreated Inoculated Control

3. RESULTS

The present experiment was carried out under glasshouse conditions to test the effectiveness and nematostatic potential of organic soil amendments with chopped leaves of Indian mallow, Mexican poppy, Ivy gourd, Trailing eclipta, Wild eggplant and 50 g of Black pig weed applied combined with 10 g seed powder of Black nightshade per pot on the root-knot development caused by *Meloidogyne incognita* and plant growth character of tomato cv. K-21. It was noted that there was significant improvement in plant growth parameters in all the treatments but chopped leaves of *Argemone mexicana* combined with Black nightshade seed powder elucidate highest improvement in plant growth parameters and maximum reduction in nematode population and root-knot indices. The total length of tomato cv. K -21 was found highest as 53.5 cm when tomato plants treated with leaves of Mexican poppy followed by 51.7, 50.0, 48.2, 46.4 and 44.7 cm in Wild eggplant, Black pig weed, Indian mallow and Ivy gourd respectively as compared to 36.5 cm in untreated inoculated and 78.2 cm in untreated uninoculated control plants. The total fresh weight of tomato cv. K -21 was found highest as 41.0 g when tomato plants treated with leaves of Mexican poppy followed by 39.6, 38.4, 37.2, 36.0 and 35.2 g in Trailing eclipta, Wild eggplant, Black pig weed, Indian mallow and Ivy gourd respectively as compared to 36.5 g in untreated inoculated and 78.2 g in untreated uninoculated control plants. The total dry weight of tomato cv. K -21 was found highest as 14.2 g when tomato plants treated with leaves of Mexican poppy followed by 13.5, 12.9, 12.3, 11.5 and 11.0 g in Trailing eclipta, Wild eggplant, Black pig weed, Indian mallow and Ivy gourd respectively as compared to 8.5 g in untreated inoculated and 24.0 g in untreated uninoculated control plants (Table 1a).

The total chlorophyll and carotenoid content was significantly increased. The chlorophyll and carotenoid content was 1.73 & 0.495 mg/g, 1.68 & 0.474 mg/g, 1.62 & 0.446 mg/g, 1.55 & 0.418 mg/g, 1.51 & 0.400 mg/g and 1.45 & 0.390 in Mexican poppy, Trailing eclipta, Wild eggplant, Black pig weed, Indian mallow and Ivy gourd respectively as compared to 1.12 & 0.252 mg/g in untreated inoculated and 2.78 & 0.880 mg/g in untreated uninoculated control. The percent pollen fertility was significantly increased in all the treatments. Maximum pollen fertility (67.0%) in treated plants, when the tomato was treated

with Mexican poppy it was followed by as 65.6, 64.0, 63.5, 62.0 and 61.0% in Trailing eclipta, Wild eggplant, Black pig weed, Indian mallow and Ivy gourd respectively as against 47.0% in untreated inoculated and 89.5% in untreated uninoculated control. The chopped leaves along with the seed powder significantly increased the yield. Maximum yield (228 g) was observed in treated one, when the tomato was treated with Mexican poppy it was followed by as 200, 186, 167.0, 160.0 and 144.0 g in Trailing eclipta, Wild eggplant, Black pig weed, Indian mallow and Ivy gourd respectively as against 112.0 g in untreated inoculated and 355.0 g in untreated uninoculated control (Table 1b). Significant reduction was observed in eggmasses, eggs and nematode population of *Meloidogyne incognita* when tomato was treated with chopped leaves in combination with seed powder of different plant species. Highest reduction in eggmasses, eggs and nematode population 114.0, 188.0 & 1042 was observed in Mexican poppy leaves followed by 118.0, 193.0 & 1069 in Trailing Eclipta, 123.0, 197.0 & 1100 in Wild eggplant, 128, 202 & 1142 in Black pig weed, 132, 209 & 1170 in Indian mallow and 140, 215 & 1204 in *C. grandis* respectively as against 148.0, 254.0 & 1558.0 in untreated inoculated control. Fresh chopped leaves in combination with seed powder significantly reduced the root knot development. The highest reduction in the root-knot development was detected when tomato plants were treated with leaves of Mexican poppy and root knot index was recorded as 2.0 followed by 2.2, 2.3, 2.5, 2.8 and 3.0 in Trailing eclipta, Wild eggplant, Black pig weed, Indian mallow and Ivy gourd respectively. In untreated inoculated pots the root-knot development was highest (5.0) (Table 1b).

4. DISCUSSION

Results presented in the study clearly indicated that all the treatment were found to be potent for the tomato plants as they promoted of growth by enhancing the shoot and root growth of the plants thereby results in decreasing the nematode infestation. Among all the treatments Mexican poppy combined with wild spinach leaf powder recorded highest improvement in plant growth and maximum reduction in the nematode multiplication and root knot indices. Other treatments viz., Trailing eclipta, Wild eggplant, Black pig weed, Indian mallow and Ivy gourd combined with spinach leaf powder also showed significant enhancement in plant growth of tomato and reduction in the population of

Meloidogyne incognita. A beneficial relationship was detected in the combined treatment of fresh chopped leaves plus wild spinach leaf powder with the tomato plants in terms of morphological and biochemical parameters as compared to inoculated control. The potential of all treatments to inhibit nematode activity resulted in increased number of fruits and yield of tomato. This beneficial relationship and improvement in plant growth parameters may be due to release of allelochemicals and nutrients on the decomposition of the amendment that enhance the beneficial microflora of the soil rhizosphere that act as promoter of growth activity. While the reduction in nematode infestation may be due to release of some toxic chemicals which either disrupt the life cycle of the nematode or lesser down the infectivity of the juvenile. Reported that incorporation of rock fleabane plant (*Inula viscosa*) powder at a concentration of 0.1% in soil reduced second stage juveniles of root-knot nematodes (*M. javanica*) [20]. The mechanisms of plant extract action may include denaturing and degrading of proteins, inhibition of enzymes and interfering with the electron flow in respiratory chain or with ADP phosphorylation [21]. Sensitivity of plant-parasitic nematodes varies from plant to plant. Generally, J₂s of *Meloidogyne* spp. are very sensitive to plant-derived nematicides [22].

Organic farming is an area of great concern and interest for growing safe and healthy food in sustainable manner without disturbing the ecological diversity of the soil and maintaining the environmental and soil behaviour. The outcome of the study state that the application of Mexican poppy, Trailing eclipta and Wild eggplant in combination with wild spinach powder is quite efficient alternative of chemical nematicides for the nematode management and enhancing the yield. The leaf powder of *I. viscosa*, at a lower concentration, showed nematicidal activity against *Pratylenchus mediterraneus*, although this activity was much lower than that against *M. javanica* J₂ [22]. Reported that neem seed cake improved growth of tomato and reduced the reproduction factor and root galling of nematodes [23]. Some herbal powders and their aqueous extracts result in increasing plant growth and reduction in the infection rate [24]. The observation of the findings are in conformity [11,13,15]. Addition of soil amendments results in a considerable increase in the liberation of CO₂ through the saprophytic activities of soil saprophytes which can suppress the activities of pathogen [25]. The

use of phytochemical compounds and natural plant extracts has substantially reduced the number of galls and the extent of nematode reproduction on tomato plants [26,27]. More specifically, the decomposition of organic residues from some oily plant residues, such as cottonseed meal, or from meal from certain types of mustard have been reported as releasing toxic ammonia, organic acids and other compounds as a byproduct that can kill nematodes [28,29,30]. The use of plants as nematicidal or nematostatic products has been regarded as effective, economical and eco-friendly by numerous researchers [7]. Amendments not only change physical and chemical soil properties, but also support a wide variety of antagonistic microorganisms like fungi, bacteria, etc [31,32]. That through the competition and parasitism may reduce the population of nematode. Usage of *A. indica* (neem) caused maximum reductions in the number of galls, egg masses and reproduction factor (Rf) of the nematode and increases all growth parameters of okra [12,33]. The plant parts/products are safer friendly than synthetic nematicides and may be economic and easily available. Moreover, they are also responsible to increase in soil nutrient status, soil loosening and texture, and to maintain the soil fertility. The present study also elucidate that the reduction in nematode population could be due to the decomposition of plant material in soil and release of secondary metabolites.

5. CONCLUSION

The application of Mexican poppy, Trailing eclipta and Wild eggplant in combination with wild spinach powder is quite efficient alternative of chemical nematicides for the nematode management and enhancing the yield. Although the utilization of chopped leaves of Indian mallow and Ivy gourd along with wild spinach powder is not potentially active replacement of synthetic nematicide however for this further characterization is needed. Exaggeration of the fertility of the soil and enhancement of microbial diversity of the rhizosphere may be the results of the suppression of *Meloidogyne incognita* infestation due to the release of nematotoxic chemical constituents after decomposition thereby ameliorate the plant growth character of the crop. All the plants explored during the course of studies are locally available at large scale without any toxicological impact on the environmental flora and fauna. It can work for fulfill the demand of global food production

through sustainable nematode management by the involvement of organic farming system.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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