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Full Length Article

Sustainable Management of the Southern Root-knot Nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, by Means of Amendments of *Fumaria parviflora*

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Abstract

Greenhouse and field studies were conducted in the spring and autumn of 2010 to test the efficacy of dry amendments of *Fumaria parviflora* as a form of eco-friendly management of *Meloidogyne incognita* in tomato. Various preparations of *F. parviflora* (in the form of dry root, stem, leaf and whole plant powder) at different dose rates (10, 20 and 30 g per kg of soil) significantly reduced levels of *M. incognita* in the roots of tomato *cv*. Rio Grande, and promoted plant growth. The root amendments of *F. parviflora* at the highest application dose (30 g per kg of soil) were the most effective, significantly reducing the number of galls, the galling index, the egg masses per g of the root, and the adult females per g of the root. Shoot and root lengths, the fresh and the dry shoot weight, and the number of branches and flowers per plant were improved in greenhouse trials that were conducted in the spring and autumn. Under naturally infested field conditions, the root´s amendment of *F. parviflora* at the highest application dose was the most effective, and reduced the number of galls, the GI, the number of egg masses per g of root, the adult females per g of root, and the reproduction factor (*Rf*). It also promoted plant, health and increased the number of fruits per plant and the fruit weight per plant in the spring and autumn experiment. Dry amendments of *F. parviflora* have remarkable nematicidal potential and could be used as an effective and environment-friendly management tool against *M. incognita* as an alternative to chemical control. © 2015 Friends Science Publishers

Keywords: *Fumaria parviflora*; *Meloidogyne*; Tomato; Dry amendments; Eco-friendly management

Introduction

Root-knot nematodes (RKNs) (*Meloidogyne* spp.) are obligate endoparasitic polyphagous pests of tomato that are found throughout the world (Sasser and Freckmann, 1987). Their worldwide distribution, extensive host ranges, and their involvement with fungi, bacteria and viruses in disease complexes rank them among the top major plant pathogens, mainly in developing countries (Sasser, 1980). Three species of RKNs, namely *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, are the major RKNs in subtropical and tropical countries (Moens *et al*., 2009). Within these species, the most important, worldwide, is *M. incognita* (Sasser, 1980). These three species are widely distributed in Pakistan, and particularly in the Khyber Pakhtunkhwa province, where the disease is 100% prevalent in tomato fields (Naz *et al*., 2012).

Globally, many synthetic nematicides have been effectively used to suppress RKNs in the soil. However, most of them have now been banned because of the health and environmental issues that are associated with their use and production, as well as with the emergence of resistant RKN strains (Conway, 1995). In addition, synthetic nematicides may affect the agro-ecological system, and have a detrimental effect on other beneficial parasites, predators and other soil-dwelling microbes (Faruk *et al*., 2011). Plant resistance is one of the most effective and economically viable methods of controlling RKN. However, plant resistance is not available in many important crops, and effectiveness is often restricted to a few races of a nematode genus (Whitehead, 1998).

The development of a resistant variety is a long-term and laborious process (Isakeit and Jaster, 2005), with some of them being locally unavailable to the growers in such

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developing countries as Pakistan (Saifullah, pers. comm.). The resistant tomato-carrying *Mi-1* gene has conferred resistance against *M. incognita*, *M. javanica* and *M. arenaria* (Medina-Filho and Stevens, 1980). This resistance breaks down under high (above 28°C) soil temperatures (Dropkin, 1969), and leads to the emergence of virulent strains in the field by way of high selection pressure, as a result of the reiterated use of said gene (Verdejo-Lucas *et al*., 2009), favouring its use within an integrated management context (Sorribas *et al*., 2005). Therefore, nematologists are currently looking for alternative and novel strategies for the effective management of these pests, or for their integration with other strategies, in order to obtain more congruent and economically viable results.

Presently, researchers have diverted their attention to managing plant-parasitic nematodes through the use of plant-derived safe phytochemicals (Pérez *et al*., 2003; Naz *et al*., 2013a), organic amendments and biopesticides (Faruk *et al*., 2011). The use of phytochemical compounds and natural plant extracts has substantially reduced the number of *M. incognita* disease galls, and the extent of reproduction occurring on, tomato plants (Naz *et al*., 2013a, b). More specifically, the decomposition of organic residues from some green manure 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 by-product that can kill nematodes (Oka, 2010; McSorley, 2011; Thoden *et al*., 2011).

Amendments from a number of plants, including the castor oil plant (*Ricinus communis*), velvet bean (*Mucuna* spp.), marigold (*Tagetes* spp.), and sunn hemp (*Crotalaria juncea*), neem cake, Rakshak gold (a neem-based product) and cruciferous plants have been used successfully against RKNs (Randhawa *et al*., 2002; Wang *et al*., 2002; Hooks *et al*., 2010). Plants, in their entirely or in parts, can be used as soil amendments, either fresh or in dry form, as has been demonstrated in one study on chickpea, in which soil amendments with flowers, leaves, roots, and seeds of *Chrysanthemum coronarium*, and with flowers from *Chrysanthemum segetum, Calendula maritime, Calendula officinalis*, and *Calendula suffruticosa*, showed differential effects on *M. artiellia* eggs and juveniles (Pérez *et al*., 2003).

Previously, *in vitro* and *in planta* nematicidal activity of *Fumaria parviflora* Lam (Fumariaceae) extracts was evaluated against the southern RKN, *M. incognita*, and different classes of bioactive nematicidal compounds, namely tannins, saponins, steroids, flavonoids, glycosides, alkaloids, and phenols were detected in the plant roots and stem (Naz *et al*., 2013a). Powdered aerial parts of *F. parviflora* have been shown to possess antibacterial activity (Vahabi *et al*., 2011), whereas almost no information has yet been made available on the nematicidal activity of *F. parviflora* used as organic amendments. In the present paper, nematicidal activities of *F. parviflora* was reported on *M. incognita* populations using tomato plants, and the beneficial increase of plant growth parameters under artificially inoculated greenhouse and naturally infested field conditions.

Materials and Methods

Collection of *Fumaria parviflora* and the Preparation of Dry Amendments

Mature *F. parviflora* plants were collected in the months of March-April 2010 from the wheat fields of the Agriculture Research Farm, Malkandher, the University of Agriculture, Peshawar. The plants were authenticated, and the voucher specimen no. ISH-1732 was deposited in the herbarium of the Department of Botany, the University of Peshawar (Naz *et al*., 2013a). Plants were washed in running tap water and separated into stem, leaves and roots, whereas some plants were left intact. All the plant parts, including those that were intact, were dried in an oven at 45°C for one week, and later gently ground to a particle size of 1 mm (Naz *et al*., 2013a). The powdered plant material was stored at room temperature (25°C) in paper bags prior to use.

In planta Experiment using Dry Powder Application of *Fumaria parviflora* and Tomato cv. Rio Grande during the Spring and Autumn of 2010

The experiments were carried out in a greenhouse of the Plant Pathology Department, the University of Agriculture, Peshawar (30 ± 5°C temperature, 70.0% relative humidity, and a 16 h photoperiod of fluorescent light). A tomato nursery (*cv.* Rio Grande) was raised in steam-sterilised sandy loam soil. About two-week-old tomato seedlings (one plant/pot) were transplanted into clay pots (15 cm mouth wide or 75 mm diameter) containing 1000 cm^3 (sand: clay loam, 2:1, v/v) that had been steam-heated at 100°C for 6 h (Naz *et al*., 2013a) to kill potential plant pathogens. For experimental treatments, the potting mixture was amended with powdered and homogenised plant material (in the form of stem, root, leaf, and whole plant powder) of *Fumaria parviflora* at 10, 20 and 30 g per kg of soil. Unamended soil and only inoculated soil served as controls. Each treatment was replicated ten times, and the pots were arranged on benches in a screen house in a completely randomised design (CRD) with factorial arrangement. The nematodes were obtained from pure culture on *cv.* Rio Grande tomato and eggs were extracted from the roots with 0.5% NaOCl (Naz *et al*., 2013b). A total of 4000 ± 10 eggs of *M. incognita* (contained in a 100 mL of H_2O) were applied at the root zone of a tomato seedling using a sterilised pipette three days after transplanting (Naz *et al*., 2013b) in the amended soil. Plants were watered daily with 50 mL tap water, and fertilised with slow-release fertilisers (14-14-14, N-P-K) (Sasser, 1990) at 5 g per kg of soil at the beginning of the experiment. The experiment was evaluated 60 days after inoculation. The shoot of each plant was cut off at the soil level, and the roots were washed with tap water.

Egg masses from the roots were extracted after exposure to 1% NaOCl for 5 min (Hussey and Barker, 1973). The severity of nematode galling of the root system was then assessed (Taylor and Sasser, 1978). The experiments were conducted twice during the spring and autumn of 2010, and data were recorded on number of galls per root system, galling index (GI), number of egg masses per g of the roots, number of adult females per g of root, shoot and root lengths (in cm), the fresh root weight (in g), fresh and the dry shoot weight (in g), number of flowers per plant, and number of branches per plant.

Field Experiments using Dry Powder Application of *Fumaria parviflora* in the Spring and Autumn of 2010 Growing Seasons

Field experiments were carried out during the spring and autumn of 2010 growing season in *M. incognita* naturally infested fields of Dargai, Khyber Pakhtunkhwa, Pakistan. Pre- and post treatment *Meloidogyne* spp., densities were calculated by taking 20 soil cores with the help of cylindrical sampling tube (10 cm diameter and 25 cm deep) from each plot and Baremann pan method (Southey, 1986). Initial and final densities were used to calculate the reproduction factor (*Rf*). Both experiments were performed in the same field of Dargai, using a susceptible tomato *cv.* Rio Grande. A nursery was raised in steam-sterilised soil (100°C for 6 h) in clay pots, and four-weeks-old tomato seedlings were transplanted into the naturally infested fields of Dargai. Five days after transplantation, dry powder of *F. parviflora* (consisting of stem, root, leaf, and whole plant powder) (at 10, 20 and 30 g per plant) were applied close to the tomato rhizosphere. The control plants were not treated with any of the plant amendments (0 g per plant). The experiments were laid out in a randomised complete block design (RCBD) with factorial arrangement. A total of ten replications were taken with plant-to-plant and row-to-row distance of 30 and 60 cm, respectively, whereas each replication consisted of two sub-rows, with 12 plants in each row. Normal agronomic practices used in the area (e.g irrigation, fertilisation, and earthing up near the root zone and hand weeding) were continued during the course of the experiments. Data were recorded on four randomly selected plants (two from each sub-row), and pooled as a means. The plants were carefully uprooted, separately labelled, and brought to the laboratory. The shoots were cut off at the soil line, the roots were washed gently with running tap water, and the data obtained were recorded on the following parameters: the number of galls per plant root system, GI, number of egg masses per g of root, number of adult females per g of root, reproduction factor (*Rf* = *Pf/Pi*; the final/initial nematode numbers); shoot and root lengths (in cm), fresh and dry shoot weight (in g), fresh root weight, number of flowers per plant, the number of branches per plant, number of fruits per plant, and fruit weight per plant (in kg), after three months. The roots were scored for GI, using a rating scale of 0-5 on the galling scale (where 0 = no gall on the roots; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = more than 100 galls per root) (Taylor and Sasser, 1978). Galled roots (1 g each) were stained for 15-20 min in an aqueous solution of phloxine B (15 mg per L). Egg masses were counted with the help of a stereomicroscope. Other agronomic parameters assessed were shoot and root lengths, number of flowers and branches per plant, and number and weight of fruit obtained (kg per plant). Chemical and physical properties of the field, namely the pH (7.3-7.9), the organic matter contents (9.9-11.0 g/kg of soil), the electrical conductivity (EC) (0.18-0.21 dSm^{-1}), the soil texture (29.9% sand, 23.0% clay, and 47.1% silt in 1 kg soil, at a depth of 30 cm), were all assessed in the Soil and Pesticide Chemistry Department (Agricultural Research Institute (ARI), Tarnab, Peshawar).

Statistics

Data of greenhouse and field trials were subjected to analysis of variance (ANOVA) using Statistix (NH Analytical Software, Roseville, MN, USA) (Campbell and Madden, 1990). Treatment means of the different parameters were compared using Fisher's protected least significant difference (LSD) test at P = 0.05 (Gomez and Gomez, 1984).

Results

Effect of Dry Amendments of *Fumaria parviflora* on *Meloidogyne incognita* on Tomato and Plant Growth Parameters in the Greenhouse Conditions

Results of the greenhouse trials for both growing seasons indicated that tomato plants, amended with different dry powder of *F. parviflora* at different application doses, significantly ($P < 0.05$) reduced the nematode parameters, and promoted the plant growth parameters. In the spring 2010 trial, the root powder at all application doses (10, 20 and 30 g per kg) effectively suppressed *M. incognita* on tomato plants (Table 1). The number of galls per plant (23.00), the GI (1.13), the egg masses per g of the root (17.75), and the number of adult females per g of root (8.00) were drastically reduced ($P < 0.05$), with the root amendments at 30 g per kg application dose, in comparison to the control. The stem powder, at all application doses, ranked second and reduced the number of nematode galls per plant, the GI, the number of egg masses per g of root, and the number of females per g of root by 61.20, 59.18, 61.63 and 72.41% respectively, over the untreated control (0.0 g per kg), at the highest dose of 30 g per kg. Plant growth parameters, namely the shoot length, the root length, the fresh shoot weight, the dry shoot weight, and the number of branches and the number of flowers per plant were significantly ($P < 0.05$) increased by 50.70, 69.49, 146.40, 102.00, 109.67 and 53.44% over the untreated control at 30

g per kg root dose application (Table 1). Plant growth parameters improved significantly with the application of dry amendments other than the roots (Table 1).

In the 2010 autumn trial, all the treated groups showed significant reduction ($P < 0.05$) in the nematode parameters, compared to the untreated control (Table 2). The number of nematode galls per plant (32.0), the GI (1.50), number of egg masses per g of the root (26.00), and the number of females per g of the root (8.70) were markedly reduced ($P < 0.05$), with the roots amendments applied at 30 g per kg dose rate (Table 2). A maximum increase in the disease severity and in the GI was observed in the control treatments at 0.0 g per kg dose rate. An increase in the dose rate of all amendments significantly reduced *M. incognita* on tomato plants at all application doses, compared to the respective control dose (0.0 g per kg) (Table 2). Results of the 2010 autumn trials on the plant growth parameters were similar to those observed in the 2010 spring trial. All the plant growth parameters, for example, the shoot and root lengths, the fresh and the dry shoot weight, the number of branches and flowers per plant showed differential results with an increase in the dose rate of the amended material, used as root, stem, leaf, or whole plant powder. Amongst the plant powder material applied, the dry root and stem powder showed very different results at the highest dose rate of 30 g per kg, and enhanced all the plant growth parameters studied under the 2010 autumn greenhouse conditions (Table 2).

Effect of Dry Amendments of *Fumaria parviflora* on *Meloidogyne incognita* on Tomato Plants and Plant Growth Parameters under Naturally Infested Field Conditions of Dargai

Data recorded under natural field conditions of Dargai in the spring of 2010 showed the nematicidal potential of all amendments (Table 3). All forms of *F. parviflora* preparations at the three different dose rates reduced ($P<0.05$) the number of galls, the GI, the number of egg masses per g of the root, the number of females per g of the root, and the *Rf* markedly, compared to the control treatments (Table 3). The fresh root weight was the highest in the control treatments. The highest dose of 30 g per plant resulted in outstanding reduction ($P < 0.05$) in gall numbers, in the GI, and in other nematode parameters, whereas the maximum increase in such plant growth parameters as the shoot and root lengths, the fresh and the dry shoot weight, the number of branches and flowers per plant, the number of fruits per plant and the fruit weight per plant was recorded (Table 3).

Results of the 2010 autumn field experiment showed significant ($P < 0.05$) efficacy of the dry amendments of *F. parviflora* (using the root, stem, leaf and whole plant powder) applied at 10, 20 and 30 g per plant dose rate (Table 4). At the 30 g per plant dose rate, the number of galls induced by *M. incognita* reduced drastically ($P < 0.05$) in all those treatments, where dry amendments were applied. Plants amended with the root powder of *F. parvilora* had a strong effect on the nematode invasion, and displayed maximum nematicidal activity. The number of galls per plant (31.0), the GI (1.25), the number of egg masses per g of root (45.25), and the number of females per g of root (40.25) were the lowest for the root amendments, whereas the dry stem powder ranked second at the highest dose rate (Table 4). The leaf and stem powder, at all application rates, markedly reduced the amount of nematode growth and reproduction that occurred in the tomato plant roots. The *Rf* was reduced to a minimum of 0.13 and 0.5 in the plants amended with the root and stem powder, as compared to the untreated controls, which gave *Rf* values of 1.9 and 2.0, respectively. Plants in the control treatments showed heavily galled roots and the highest fresh root weight. The shoot and root lengths (48.50 and 21.50 cm), the fresh and the dry shoot weight (55.00 and 27.00 g), and the number of branches and flowers per plant^{-1} (20.00 and 65.00) were the maximum in the treatments, where *F. parviflora* root preparations were applied at the highest dose rate. Compared to the control plants, the maximum number of fruits per plant and increase in fruit weight was recorded in treatments using any form of the *F. parviflora* preparations. The root powder showed an increase of 157.30 and 83.96% in the number of fruits per plant and in fruit weight, respectively, over the control plants (Table 4).

Discussion

The use of plants as nematicidal or nematostatic products has been regarded as effective, economical and eco-friendly by numerous researchers (Chitwood, 2002). A study that was conducted under greenhouse and naturally-infested field conditions in the spring and autumn of 2010 revealed the promising effect of *F. parviflora* against *M. incognita* on tomato plants. A variety of preparations (using the root, stem, leaf and whole plant powder) and doses (10, 20 and 30 g per kg) of *F. parviflora* effectively suppressed *M. incognita* on tomato plants, and promoted the plant growth parameters. The *in planta* study clearly demonstrated that the number of nematode galls on tomato roots, the GI, and the number of egg masses, and the number of adult females, per g of root were significantly reduced by means of the application of various preparations of *F. parviflora*. Nevertheless, the root doses gave very effective results, both in the greenhouse and in the field trials. Under natural field conditions, the dry root powder application of *F. parviflora* exhibited maximum reduction in the number of galls per plant (39.25 and 31.00), and in terms of the GI (1.87 and 1.13), in comparison to the corresponding controls. The stem powder ranked second in nematicidal effect at all doses. This study demonstrated that the variation in the reduction of the number of galls per plant, the GI, and the other parameters of parasitism was positively influenced by the different application doses used.

Table 1: Effect of dry powder of *Fumaria parviflora* on nematicidal properties of *Meloidogyne incognita* on tomato and plant growth parameters under green-house conditions (spring, 2010)[a]

Plant parts and doses (g kg^{-1})	Galls plant^{-1}	GI[b]	Egg masses g^{-1} of root	Adult females g^{-1} of root	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Number of branches plant^{-1}	Number of flowers plant^{-1}
Roots										
0	80 a	4.70 a	65.75 ab	41.25 c	26.25 j	5.72 f	19.33 gh	8.48 fg	7.75 e	29.00 ef
10	46.75 c	1.87 e	28.00 gh	19.00 fgh	48.75 b	13.38 b	24.88 ef	8.33 fg	9.25 cde	33.00 cde
20	36.75 de	1.63 ef	24.50 hi	17.75 gh	52.50 a	12.88 b	38.88 bc	14.55 b	12.00 bc	37.50 bc
30	23.0 f	1.13 f	17.75 i	8.00 i	53.25 a	18.75 a	47.63 a	17.13 a	16.25 a	44.50 a
Stem										
0	74.75 a	4.90 a	61.25 b	58.00 a	28.75 i	5.80 f	19.38 gh	7.70 g	10.00 cde	25.50 f
10	44.50 cd	2.85 d	36.00 def	25.50 def	37.75 ef	10.25 cd	20.50 gh	9.10 f	9.25 cde	33.00 cde
20	38.25 d	3.00 cd	30.50 fgh	26.25 de	41.50 c	11.50 bc	27.25 e	12.13 cd	11.50 bcd	38.00 b
30	29.00 ef	2.00 e	23.50 hi	16.00 h	38.75 de	12.38 b	32.00 d	12.50 c	13.25 b	39.00 b
Leaves										
0	79.25 a	4.82 a	69.00 a	56.50 ab	26.50 j	5.72 f	19.20 gh	7.50 g	8.75 de	26.75 f
10	62.50 b	3.85 b	44.00 c	31.25 d	35.50 h	7.75 ef	20.05 gh	9.30 f	9.00 de	28.50 ef
20	61.25 b	3.53 bc	41.25 cd	22.75 efgh	38.50 de	9.23 de	24.50 ef	10.50 e	9.500 cde	32.00 de
30	56.50 b	3.03 cd	36.00 def	22.75 efgh	39.00 d	11.70 bc	22.13 fg	11.25 de	8.75 de	36.50 bcd
Whole plant										
0	81.00 a	4.82 a	69.50 a	50.50 b	28.50 i	5.80 f	18.42 h	8.25 fg	7.25 e	25.00 f
10	60.25 b	3.47 bc	40.75 cd	27.00 de	35.75 gh	7.15 ef	35.50 c	12.50 c	7.75 e	29.25 ef
20	58.25 b	3.25 cd	38.75 cde	23.25 efg	36.75 fg	10.13 cd	37.75 c	14.63 b	9.75 cde	35.25 bcd
30	39.0 cd	3.03 cd	32.25 efg	17.00 gh	35.50 h	11.52 bc	41.20 b	17.75 a	10.00 cde	35.50 bcd
LSD values	3.92	0.28	3.76	3.4	0.62	1.04	1.69	0.57	1.39	2.36

[a]Data are means of 10 replicated plants per treatment. Plants were inoculated with 4000 ± 10 eggs + J2s (p_i) of *M. incognita* for each treatment. Means followed by the same letter do not differ significantly ($P \geq 0.05$) according to Fisher's protected LSD test. [b]GI = Galling index: 0 = no gall on roots; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = more than 100 galls per root system

Table 2: Effect of dry powder of *Fumaria parviflora* on nematicidal properties of *Meloidogyne incognita* on tomato and plant growth parameters under green-house conditions (autumn, 2010)[a]

Plant parts and doses (g kg^{-1})	Galls plant^{-1}	GI[b]	Egg masses g^{-1} of root	Adult females g^{-1} of root	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	Number of branches plant^{-1}	Number of flowers plant^{-1}
Roots										
0	99.25 a	4.87 a	98.00 a	70.75 ab	30.25 efgh	7.82 fgh	18.13 gh	7.47 gh	8.75 de	23.00 ef
10	63.5 de	3.00 ef	50.50 fg	20.75 gh	38.25 bc	9.52 de	23.40 ef	7.62 g	10.25 cde	26.25 def
20	50.75 f	2.50 f	45.00 g	16.75 h	41.50 b	15.63 b	34.75 c	14.95 c	14.25 b	37.75 b
30	31 g	1.50 g	26.00 h	8.750 i	52.50 a	20.00 a	55.70 a	19.13 a	17.25 a	48.75 a
Stem										
0	100.3 a	5.00 a	94.00 a	67.50 b	28.75 gh	8.00 fg	24.27 def	5.30 i	11.00 cd	21.75 f
10	78.75 b	3.62 bcd	62.00 de	34.25 e	29.25 efgh	9.22 def	20.75 efg	8.62 fg	10.25 cde	28.50 cde
20	63.75 cde	3.25 de	56.25 ef	26.50 f	31.25 defgh	12.00 c	21.63 efg	9.25 ef	12.25 bc	32.25 bc
30	48.75 f	2.50 f	44.00 g	19.75 h	34.00 cde	12.07 c	25.72 de	12.13 d	14.00 b	36.75 b
Leaves										
0	103.8 a	4.92 a	97.50 a	75.50 a	29.00 fgh	6.40 h	23.95 def	5.77 i	9.75 de	21.75 f
10	75.50 b	4.07 b	82.50 b	51.75 c	28.50 gh	8.00 fg	19.38 fg	6.10 hi	8.25 e	23.00 ef
20	71.50 bc	3.50 cde	74.75 bc	42.75 d	33.75 cdef	12.13 c	22.10 efg	8.75 fg	10.50 cde	28.50 cde
30	61.50 e	3.25 de	66.50 cd	33.00 e	26.75 h	12.32 c	23.55 ef	10.43 e	9.75 de	29.50 cd
Whole plant										
0	106.8 a	5.00 a	93.25 a	74.00 a	27.25 gh	7.00 gh	13.20 h	8.02 fg	8.25 e	23.50 ef
10	71.00 bcd	3.62 bcd	73.25 c	52.25 c	31.75 defg	8.40 efg	17.38 gh	10.63 de	8.75 de	27.25 cdef
20	71.25 bcd	4.00 bc	64.25 de	41.50 d	30.00 efgh	10.13 d	28.83 d	15.50 c	10.75 cd	25.50 def
30	66.75 cde	2.62 f	49.00 fg	26.00 fg	35.50 cd	12.75 c	42.20 b	17.50 b	10.75 cd	35.75 b
LSD values	3.92	0.52	4.5	2.8	2.41	0.74	2.55	0.76	1.2	2.77

[a]Data are means of 10 replicated plants per treatment. Plants were inoculated with 4000 ± 10 eggs + J2s (p_i) of *M. incognita* for each treatment. Means followed by the same letter do not differ significantly ($P \geq 0.05$) according to Fisher's protected LSD test. [b]GI = Galling index: 0 = no gall on roots; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = more than 100 galls per root system

The data for nematicidal activity of Fumariaceae agreed with the results of our previous studies, where the *n*-hexane and methanol root extracts of the plant significantly reduced the number of nematode galls, the GI, the number of egg masses, and the number of adult females in the root tissues at 3000 ppm concentration (Naz *et al*., 2013a). *Fumaria parviflora* quantity levels of amendments applied were lower than were the levels of other plants used in the biocontrol, as in the case of the dry powder of *Acoruscalamus* rhizome (Devi *et al*., 2011), *Datura stramonium* (Pariha *et al*., 2012), in the suppression of *Meloidogyne* spp. Similar studies were conducted by other researchers,

Table 3: Effect of dry powder of *Fumaria parviflora* on nematicidal properties of *Meloidogyne incognita* on tomato and plant growth parameters under natural field conditions of Dargai (spring, 2010)[a]

Plant parts and doses (g plant^{-1})	Galls plant^{-1}	GI[b]	Egg masses g^{-1} of root	Adult females g^{-1} of root	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	fresh root weight	Number of branches plant^{-1}	Number of flowers plant^{-1}	Fruits plant^{-1}	Fruit weight (Kg plant^{-1})	*Rf*[c]
Roots														
0	86.00 b	4.92 a	83.25 ab	59.00 b	30.00 i	11.25 f	34.25 d	16.00 f	28.50 a	10.25 gh	34.75 h	21.00 h	2.90 e	2.0 a
10	61.25 de	2.75 cdef	43.50 fg	28.50 f	48.00 b	19.50 bc	47.75 b	23.50 cd	17.75 de	18.75 bcd	61.25 bc	39.75 d	3.57 bcd	0.80 bcd
20	53.75 fg	2.37 efg	43.50 fg	19.25 g	50.50 b	24.50 a	51.00 ab	27.75 ab	18.50 de	19.75 b	65.50 ab	46.00 bc	3.95 b	0.733 cd
30	39.25 h	1.87 fg	31.00 h	18.50 g	55.00 a	26.75 a	53.00 a	29.25 a	20.75 bc	22.50 a	69.50 a	55.25 a	4.75 a	0.53 e
Stem														
0	89.25 ab	4.95 a	90.00 a	63.75 ab	29.50 i	10.25 f	27.00 e	14.75 f	29.25 a	10.00 h	30.00 i	21.75 h	2.87 e	2.1 a
10	63.25 de	3.12 bcde	52.25 de	44.00 cde	41.25 ef	14.75 e	42.25 c	20.25 de	16.50 ef	17.50 de	46.50 ef	34.00 f	3.30 cde	0.93 b
20	53.75 fg	2.62 def	49.75 ef	39.50 e	44.00 cd	17.25 cde	48.00 b	24.50 bc	18.75 cd	17.75 cde	50.50 e	39.00 de	3.70 bc	0.80 bcd
30	48.25 g	1.50 g	38.75 g	27.50 f	48.50 b	21.00 b	47.75 b	25.50 abc	19.25 bcd	19.25 bcd	56.75 d	46.00 bc	4.75 a	0.63 de
Leaves														
0	87.00 b	4.92 a	81.75 b	66.25 a	29.50 i	10.50 f	27.00 e	14.75 f	29.00 a	12.00 g	31.25 hi	21.00 h	2.90 e	2.0 a
10	70.75 c	3.75 b	69.50 c	50.00 c	33.75 h	15.25 de	39.25 c	17.75 ef	15.50 f	14.25 f	40.25 g	29.00 g	2.97 e	0.93 b
20	71.25 c	3.62 bc	57.50 d	43.50 cde	36.25 gh	17.25 cde	47.75 b	24.25 bcd	17.25 def	16.50 e	42.00 g	36.50 ef	3.65 bcd	0.83 bc
30	64.75 cd	3.12 bcde	49.75 ef	37.50 e	41.75 de	19.75 bc	49.25 ab	24.00 bcd	18.50 de	19.25 bcd	44.25 fg	43.25 c	3.90 b	0.73 cd
Whole plant														
0	96.50 a	4.95 a	85.50 ab	66.00 a	30.00 i	11.00 f	28.00 e	14.00 f	29.50 a	11.50 gh	30.50 hi	21.75 h	2.92 e	2.0 a
10	67.50 cd	3.00 bcde	57.50 d	48.75 cd	38.75 fg	17.25 cde	40.50 c	21.75 cde	17.25 def	16.25 e	48.50 ef	37.75 de	3.20 de	0.86 bc
20	56.50 ef	3.50 bcd	52.50 de	47.00 cd	42.00 cde	17.50 cd	48.75 ab	24.25 bcd	19.25 bcd	18.75 bcd	56.25 d	46.25 bc	3.70 bc	0.80 bcd
30	53.00 fg	2.25 efg	37.50 gh	43.00 de	44.50 c	21.50 b	51.50 ab	25.75 abc	21.00 b	19.50 bc	57.75 cd	48.75 b	4.00 b	0.73 cd
LSD values (P < 0.05)	3.63	0.44	3.49	3.47	1.35	1.31	2.13	2.1	1.07	0.99	2.2	1.56	0.24	0.17

[a]Data are means of 10 replicated plants per treatment. Data were recorded under natural field conditions in the spring, 2010. Means followed by the same letter do not differ significantly ($P \geq 0.05$) according to Fisher's protected LSD test. [b]GI = Galling index: 0 = no gall on roots; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = more than 100 galls per root system. [c]*Rf* = Reproduction factor = (*Pf*/*Pi*) = Final nematode population/initial nematode population

Table 4: Effect of dry powder of *Fumaria parviflora* on nematicidal properties of *Meloidogyne incognita* on tomato and plant growth parameters under natural field conditions of Dargai (autumn, 2010)[a]

Plant parts and doses (g plant^{-1})	Galls plant^{-1}	GI[b]	Egg masses g^{-1} of root	Adult females g^{-1} of root	Shoot length (cm)	Root length (cm)	Fresh shoot weight (g)	Dry shoot weight (g)	fresh root weight	Number of branches plant^{-1}	Number of flowers plant^{-1}	Fruits plant^{-1}	Fruit weight (Kg plant^{-1})	*Rf*[c]
Roots														
0	99.25 a	4.95 a	109.3 a	95.50 a	31.25 hi	14.75 cd	22.00 i	9.25 i	28.50 a	10.00 g	34.75 gh	22.25 i	2.62 gh	1.9 a
10	63.50 de	2.50 d	62.75 de	58.50 de	40.75 cde	15.25 cd	31.25 de	16.75 cd	18.00 cdef	15.00 bcde	48.00 cde	41.75 cd	3.47 cd	0.63 cd
20	50.75 f	2.07 de	58.75 ef	45.75 fg	46.00 ab	17.50 bc	36.75 c	19.00 bc	19.50 cde	17.50 ab	51.75 bcd	46.50 b	3.92 b	0.43 e
30	31.00 g	1.25 f	45.25 g	40.25 g	48.50 a	21.50 a	55.00 a	27.00 a	21.63 bc	20.00 a	65.00 a	57.25 a	4.82 a	0.13 f
Stem														
0	100.3 a	4.92 a	109.5 a	94.00 a	27.50 i	14.75 cd	23.00 hi	10.50 hi	28.75 a	11.00 fg	33.75 gh	22.75 i	2.50 h	2.0 a
10	78.75 b	3.23 c	72.75 bc	62.75 cd	38.25 def	14.25 cd	28.00 efg	14.20 ef	15.25 fg	13.50 def	42.25 ef	32.25 gh	3.07 ef	0.86 b
20	63.75 cde	2.50 d	69.50 bcd	60.00 d	43.75 bc	16.00 cd	30.00 def	15.00 def	18.00 cdef	13.00 ef	45.25 def	37.50 ef	3.47 cd	0.76 bc
30	48.75 f	1.82 e	54.50 f	51.75 ef	47.00 ab	20.25 ab	37.50 bc	17.75 bc	18.63 cdef	16.00 bcd	54.50 bc	45.75 bc	4.47 a	0.5 de
Leaves														
0	103.8 a	4.95 a	109.3 a	90.25 a	28.50 i	14.00 cd	23.50 hi	9.25 i	29.25 a	9.75 g	32.00 h	22.50 i	2.52 h	1.96 a
10	75.50 b	3.75 bc	73.75 b	74.00 b	36.75 efg	15.00 cd	25.50 ghi	11.63 gh	13.25 g	14.50 cde	39.75 efg	29.25 h	2.95 fg	0.86 b
20	71.50 bc	3.57 bc	72.25 bc	68.00 bc	41.75 cd	14.75 cd	26.50 fgh	12.88 fg	15.00 fg	12.75 ef	46.25 def	36.00 fg	3.20 def	0.76 bc
30	61.50 e	3.32 c	65.75 cde	66.25 bcd	44.00 bc	17.50 bc	27.00 fgh	13.75 efg	14.75 fg	15.75 bcd	51.00 bcd	41.75 cd	3.72 bc	0.63 cd
Whole plant														
0	106.8 a	4.97 a	105.3 a	92.25 a	28.50 i	13.50 de	22.00 i	9.50 hi	24.00 b	11.00 fg	31.50 h	22.00 i	2.70 gh	1.96 a
10	71.00 bcd	3.97 b	75.00 b	64.50 cd	33.25 gh	10.25 e	29.50 defg	14.38 ef	15.75 efg	15.25 cde	42.25 ef	34.75 fg	2.97 fg	0.86 b
20	71.25 bcd	3.45 bc	70.00 bcd	60.25 cd	34.25 fgh	16.50 cd	32.25 d	15.25 de	16.75 defg	15.00 bcde	54.25 bc	40.50 de	3.37 cde	0.83 b
30	66.75 cde	2.20 de	61.00 ef	51.50 ef	38.25 def	21.25 a	41.25 b	19.50 b	20.25 bcd	17.00 bc	56.00 b	45.00 bc	4.00 b	0.73 bc
LSD values (P < 0.05)	3.93	0.28	3.89	3.92	2.11	1.84	2.1	1.14	2.11	1.26	3.31	2.11	0.18	0.15

[a]Data are means of 10 replicated plants per treatment. Data were recorded under natural field conditions in the autumn, 2010. Means followed by the same letter do not differ significantly ($P \geq 0.05$) according to Fisher's protected LSD test. [b]GI = Galling index: 0 = no gall on roots; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = more than 100 galls per root system. [c]*Rf* = Reproduction factor = (*Pf*/*Pi*) = Final nematode population/initial nematode population

who found that the combination of poultry refuse and Furadan 5G (@ 3 t per ha and at 2 kg per ha, respectively) effectively suppressed *M. incognita* on tomato plants under natural field conditions (Faruk *et al*., 2011). These comparisons showed the great potential of *F. parviflora* for the control of PPNs, even at low and targeted application dose. The researcher reported that one way of reducing the large amount of material needed for the broadcast application of amendments was to make targeted applications only in the immediate vicinity of the plants, so that the seedlings developed in a soil environment that was very rich in the amendment (Thoden *et al*., 2011).

In the present study, the plant growth parameters of tomato plants were promoted significantly in all those

treatments, where *F. parviflora* amendments were applied at increasing doses both in the greenhouse and the field experiments. Even the low dose of 10 g per kg enhanced plant health, and increased the shoot and root lengths, the fresh shoot weight, the number of branches, and the number of flowers per plant in the spring and autumn experiments. The field study revealed that the number of fruits per plant increased with the amount of root powder (55.25 and 57.25) used at the highest application dose. An increase was also observed in other treatments that were amended with the stem, the whole plant, and the foliage powder. Likewise, the fruit weight of the tomato markedly increased with the root and stem amendments. These results agree with the work reported by other researchers, in which the use of amendments with nematicidal properties was found to enhance plant growth in tomato plants (Tariq *et al*., 2007; D'Addabbo *et al*., 2009; Pakeerathan *et al*., 2009). The combination of these amendments at lower doses could be combined with the use of nematicides, as in the case of Furadan. However, because of the inactivation of nematicides by means of high amounts of organic matter (Oka *et al*., 2013) these combinations should be used with caution, and after the conducting of preliminary studies. Additionally, the application of amendments in combination with biological control agents has been proven to be effective, as in the case of neem leaves (Khan *et al*., 2011). Biological control agents could be integrated with other control measures in order to achieve long-lasting and favourable results (McSorley, 2011).

The beneficial effect from *F. parviflora* on plant growth and yield could also be attributed to improvement in the physical, chemical and microbiological properties of the soil, after incorporation of the soil organic amendment. It has been shown that an increase in organic amendments can improve soil properties, and the decomposing plant materials can provide nitrogen and other nutrients that are needed by crops (Powers and McSorley, 2000). Several studies have revealed that microbial activities and biomass is higher in fields with organic amendments than they are in fields with conventional fertilisers (Drinkwater *et al*., 1995). Organic amendments stimulate a broad range of organisms in the soil food web, many of which act as potential predators or parasites of PPN (Oka, 2010). It has been suggested that increased crop yields observed with amendments are due to the activities of free-living nematodes, especially bacterivores (McSorley, 2011).

The present study suggests that the reduction in nematode parasitism could be due to the decomposition of plant material (in the stem, root, leaf and whole plant powder) in the potting mixtures, and the subsequent release of such secondary metabolites as alkaloids, saponins, tannins, glycosides, steroids, flavonoids and phenols, as demonstrated by the phytochemical screening of the stem and root of *F. parviflora* (Naz *et al*., 2013a, b). The low density /parasitism of *M. incognita* on tomato roots could also be due to the poor invasion of the nematode larvae into the roots, as affected by the application of dry plant amendments. In addition, the presence of other nematicidal compounds, namely nonacosane-10-ol and 23a-homostigmast-5-en-3β-ol (Naz *et al*., 2013b), and *cis*- and *trans*-protopinium, reported from the roots of *F. parviflora*, and the higher alkaloid (0.09 ± 0.04) and saponin (1.3 ± 0.07) contents could have synergistically contributed to the best nematicidal performance of the roots (Naz *et al*., 2013a, b). Likewise, the higher phenolic contents of the stem (16.75±0.07) could be attributed to the better nematicidal effect of stem, as had been evident in our previous findings (Naz *et al*., 2013a). The leaf powder of the plant also displayed promising results in this respect. Reports of the leaves of *F. parviflora* containing kaemferol and quercetine glycosides are available (Tandon *et al*., 2011), and the nematicidal activity of these compounds from pomegranate leaves has been demonstrated against *Ascaris lumbricoides* (Rahmatullah *et al*., 2010). These secondary metabolites are structurally highly diverse, and are produced in a varied ecological environment (Hawa *et al*., 2012).

The efficacies of *Fumaria* powder were decreased when the plant doses were gradually reduced, which might be due to the differences in the concentration of toxic substances that were present in the plant material (Naz *et al*., 2013a). The exact mechanism of the action of these phytochemicals is not known, with toxicity to nematodes and hatching having been shown in a previous study (Naz *et al*., 2013a). Additionally, these secondary compounds in the plant parts acted as a coating around the tomato roots, hence subsequently preventing the attack of the juveniles of *M. incognita* by creating an unfavourable environment for the nematode activity, or by means of indirectly effecting the acquisition of resistance or tolerance by the plants against the nematode attack. This was evident in our previous findings, in which the two compounds that were contained in the roots, for example the nonacosane-10-ol and the 23-homostigmast-5-en-3β-ol, effectively suppressed *M. incognita*, and reduced the population density in tomato root tissues in greenhouse trials (Naz *et al*., 2013b).

Conclusion

The use of dry preparations of the plant material from *F. parviflora* could make the nematode control more eco-friendly and practicable in the field with attendant yield increases. The assumption is made that the local application to roots, prior to them being planted in the field, could protect the roots at the beginning of the crop cycle. The beneficial potential of these amendments could further be strengthened by their economic convenience, due to the lower cost involved than with the use of chemicals, and also their easy availability. However, more studies should be done in order to test the viability of the seeds after the drying period, or the collection of the plant before seed production, in order to prevent *F. parviflora* as a weed in the field.

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