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Effect of some botanicals for the management of plant-parasitic nematodes and soil-inhabiting fungi infesting chickpea

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Abstract: A field experiment was conducted during 2009–2011 at the University Agricultural Research Farm to evaluate the efficacious nature of some botanicals such as *Argemone mexicana*, *Calotropis procera*, *Solanum xanthocarpum*, and *Eichhornia echinulata* in combination with normal as well as deep ploughing against plant-parasitic nematodes and soil-inhabiting fungi infesting chickpea (*Cicer arietinum* L.) cultivar K-850 in relation to its growth characteristics. Significant reduction was observed in the multiplication of plant-parasitic nematodes *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae*, and *Helicotylenchus indicus* and in the frequency of parasitic fungi such as *Macrophomina phaseolina*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Phyllosticta phaseolina*, and *Sclerotium rolfsii* by the application of botanicals to soil. However, the frequency of saprophytic fungi *Aspergillus niger*, *Trichoderma viride*, and *Penicillium digitatum* was significantly increased. Much improvement was observed in growth parameters like plant weight, per cent pollen fertility, pod numbers, root nodulation, nitrate reductase activity, and chlorophyll content in leaves. Depth of ploughing also influenced the population of plant-parasitic nematodes and the frequency of soil-inhabiting fungi in chickpea crop.

Key words: Botanicals, chickpea, growth, organic management, soil-inhabiting fungi, plant-parasitic nematodes

Introduction

Pulses occupy an important position among food crops grown in India and contributed 10×10^6 to 13×10^6 t of grain annually from 22×10^6 to 23×10^6 ha of cultivable land in this country during the 1980s (Khanna and Gupta 1988). Since then, only marginal improvements in pulse production have been recorded. According to a recent report, the production of pulses was 15.12×10^6 t from an area of 23.86×10^6 ha, with an average productivity of 638 kg ha^{-1} (Tomar et al. 2010). The production of pulses remained stagnant at around $(14 \pm 2) \times 10^6$ t in India. This resulted in a drastic reduction in per capita pulse availability from 45 g in 1995 and 1996 to 35 g in 2006 and 2007 (FAI 2007). This has accentuated the problem of protein malnutrition in a country where the majority of the population is vegetarian (Sharma and Prasad 2009). Overall, 70% of plant proteins come from pulses. Hence, there is an urgent need to increase pulse production in the country. Besides their food value, pulse crops also increase soil fertility through symbiotic nitrogen-fixing bacteria. Additionally, leguminous crops can fix the atmospheric nitrogen (27–206 ha^{-1} $year^{-1}$) with the help of *Rhizobium* and thereby improve the soil fertility (Reddy and Reddi 2002), thus substantially

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reducing the need for additional nitrogen and saving money to be spent on these synthetic fertilisers.

Chickpea (*Cicer arietinum* L.) occupies an important production position, ranking third among the pulse crops (FAO 2008) and being a good source of many nutrients in the diet (Wood and Grusak 2007). Chickpea occupies an area of 10.72×10^6 ha with a total annual production of 9.31×10^6 t and an average productivity of 868 kg ha^{-1} (FAO 2008). Chickpea was grown under both rain-fed and irrigated conditions over an area of 7.97×10^6 ha with total production of 7.05×10^6 t and productivity of 885 kg ha^{-1} (FAO 2009). However, the productivity of chickpea in India is still far below that in Mexico, Sudan, China, Israel, Lebanon, Greece, Yemen, and Italy. The productivity of chickpea is low because of several constraints like biological limits, inadequate availability of quality seeds of improved varieties, and cultivation of pulses on poor and marginal lands under rain-fed conditions without recommended input application.

Among the biological constraints, plant-parasitic nematodes and soil-pathogenic fungi are causes of losses in the productivity of chickpea (Tiyagi and Alam 1990; Tiyagi 1991; Sharma et al. 1992). Sasser and Freckman (1987) estimated 13.7% worldwide yield losses in chickpea due to various plant-parasitic nematode infestations. Nene et al. (1984) observed that several soil-borne fungal pathogens cause root rot and wilt of chickpea and seriously affect productivity. Therefore, it is a matter of great concern to manage pathogens in order to produce more plant biomass and grains for increased quality. This management objective could be achieved with the help of appropriate pathogen management tactics. One of the commonly used tactics worldwide is chemical control because of its definitive and fast results.

The increased use of various chemicals under intensive cultivation has not only contaminated the ground and surface water but has also disturbed the harmony existing among the soil, plant, and microbial populations (Bahadur et al. 2006). There has been growing public concern about the negative impact of pesticides and inorganic fertilisers on the environment and on the safety and quality of food. Due to increasing awareness of pesticidal hazards and contamination of the biosphere, organic-based materials have created worldwide interest in pest control methods of plant origin, which are safe, ecofriendly, and biodegradable in nature. Organic matter can be used to promote the healthy population of beneficial organisms in the soil. A number of organic items of plant origin, including oil-seed cakes, chopped plant parts, and seed dressing with plant extracts, have been used as nematode control agents (Muller and Gooch 1982; Akhtar and Alam 1993; Tiyagi et al. 2009a, 2009b) and to suppress pathogenic fungi (Khan et al. 1974a, 1974b; Mahmood et al. 2005). Judicious use of organic matters may be effective not only in sustaining crop productivity and soil health but also in supplementing chemical fertilisers of the crop. The beneficial effect of organic amendments with respect to the suppression of plant-parasitic nematodes and pathogenic fungi has been recognised in recent years. Today there is an increasing interest in discovering nematostatic compounds from the plant or plant products (Chitwood 2002).

A preliminary soil survey conducted in the chickpea fields in and around the Aligarh district of northern India revealed the presence of plant-parasitic nematodes such as *Meloidogyne incognita*, *Rotylenchulus reniformis*, *Tylenchorhynchus brassicae*, and *Helicotylenchus indicus* and soil-inhabiting fungi such as *Fusarium oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Aspergillus niger*, and *Penicillium digitatum* associated with unthrifty crop growth. Thus, the aim of the present investigation was to evaluate the efficacy of some wildly grown botanicals like *Argemone mexicana*, *Calotropis procera*, *Solanum xanthocarpum*, and *Eichhornia echinulata* in combination with normal as well as deep ploughing against plant-parasitic nematodes and soil-inhabiting fungi infesting chickpea in field trials.

Materials and methods

Preparation of field

The experiment was conducted during 2009–2011 under normal ploughing (20 cm deep) as well as deep ploughing (30 cm deep) conditions. The experimental field was thoroughly ploughed and

small beds of 6 m^2 were prepared, leaving buffer zones of 0.5 m. These beds were separately treated with plant parts of *Argemone mexicana*, *Calotropis procera*, *Solanum xanthocarpum*, and *Eichhornia echinulata* with 110 kg N ha^{-1}. Inorganic fertilisers (urea at 110 kg N ha^{-1}, superphosphate at 55 kg P ha^{-1}, and muriate of potash at 55 kg K ha^{-1}) were applied before sowing the seeds. Untreated beds and beds treated with inorganic fertilisers alone served as controls. The treatments were randomised with 5 replications. The beds were watered immediately to assist the decomposition of plant parts of noxious weeds, and 15 days later, bacteria-inoculated seeds of chickpea (*Cicer arietinum* L.) cultivar K-850 were sown. During the 4-month growing period, weeding and watering were done as required.

Plant-growth parameters

Different growth parameters such as plant weight, per cent pollen fertility, number of pods, nitrate reductase activity in leaves, root nodulation, and chlorophyll content were recorded at the end of the experiment. After the harvest, weight of shoots, weight of roots, and the number of pods per plant were recorded. At the flowering stage, the pollen fertility (percentage) was estimated by the method of Brown (1949) using the stainability of pollen grains in 1% acetocarmine solution. The root nodule index (on a 0–5 scale) was estimated by visual observation, where 0 = no nodulation, 1 = very light nodulation, 2 = light nodulation, 3 = moderate nodulation, 4 = heavy nodulation, and 5 = very heavy nodulation. Nitrate reductase activity in leaves was determined by the process of Jaworski (1971) and chlorophyll content of leaves was determined by the method of Hiscox and Israelstam (1979).

Extraction of nematodes

The population of plant-parasitic nematodes for each bed were determined before treatment and after finalising the experiment by processing representative soil samples by Cobb's sieving and decanting method along with the Baermann-funnel technique (Southey 1986). The nematodes were counted and identified under a stereo-binocular microscope at magnifications ranging from 10× to 100× (Southey 1986). An average of 5 counts was taken in each case to determine the population density of each nematode species. The number of root galls caused by *Meloidogyne incognita* per plant root was also counted.

Frequency of soil fungi

The frequencies of parasitic as well as saprophytic fungi from the soil rhizosphere (on a dry weight basis) were also determined, before treating the soil and after the harvest at the end of the experiment, by the dilution plate methods of Dickinson and Pugh (1965). With a sterilised pipette, 7 mL of a 1:1000 soil dilution was transferred to sterilised petri plates and 10 mL of melted, cooled peptone dextrose agar medium (Martin 1950) was added. Twenty petri plates were used for each treatment. These prepared petri plates were incubated at 28 °C and the 1-week-old fungi were examined and identified. The frequency of fungi was calculated by the formula of McLean and Ivimey-Cook (1957): (Number of plates containing a particular fungus / Total plates poured) × 100.

Statistical analysis

The data of 2 years were pooled and analysed statistically according to the method of Panse and Sukhatme (1978). The least significant difference was calculated at P = 0.05 and Duncan's multiple range test was employed to test for significant differences between the treatments.

Results

Growth parameters

There were significant improvements for all growth parameters in all treatments as compared to the untreated controls (Figure 1). In normally ploughed beds treated with *A. mexicana*, maximum improvement was noticed for plant weight (58.15 g), pollen fertility (91.54%), number of pods (44.64), root nodulation (5.0), nitrate reductase activity (0.713), and chlorophyll content (2.917 mg g^{-1}) compared to the other botanicals and the untreated control. Deep-ploughed beds showed greater increases in growth parameters than normally ploughed beds.

Population of plant-parasitic nematodes

The population of plant-parasitic nematodes increased beyond the initial population in untreated and inorganic fertiliser-treated beds. *Meloidogyne incognita*, *R. reniformis*, and *T. brassicae* were the dominant species in all beds (Figure 2). In normally

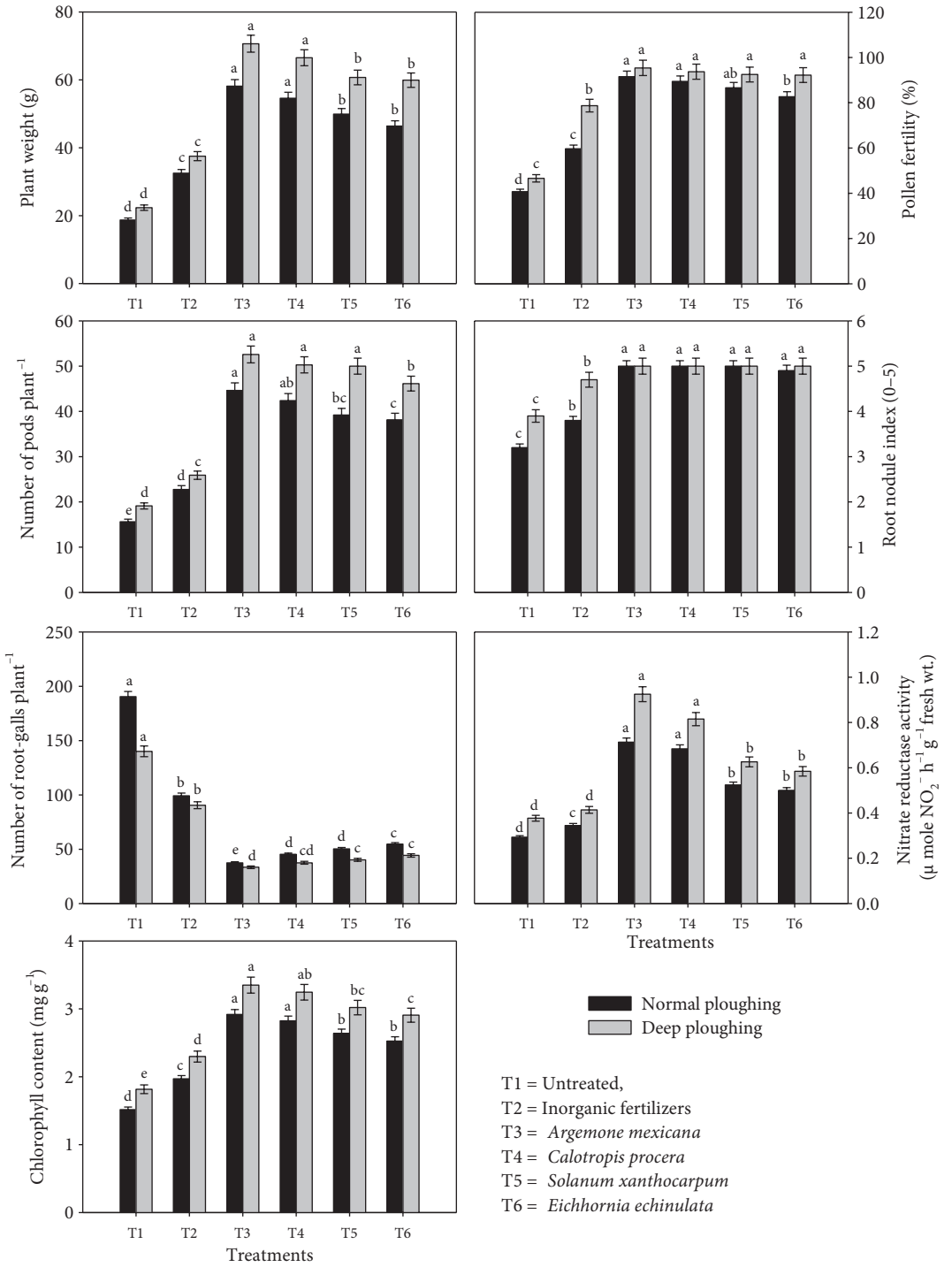
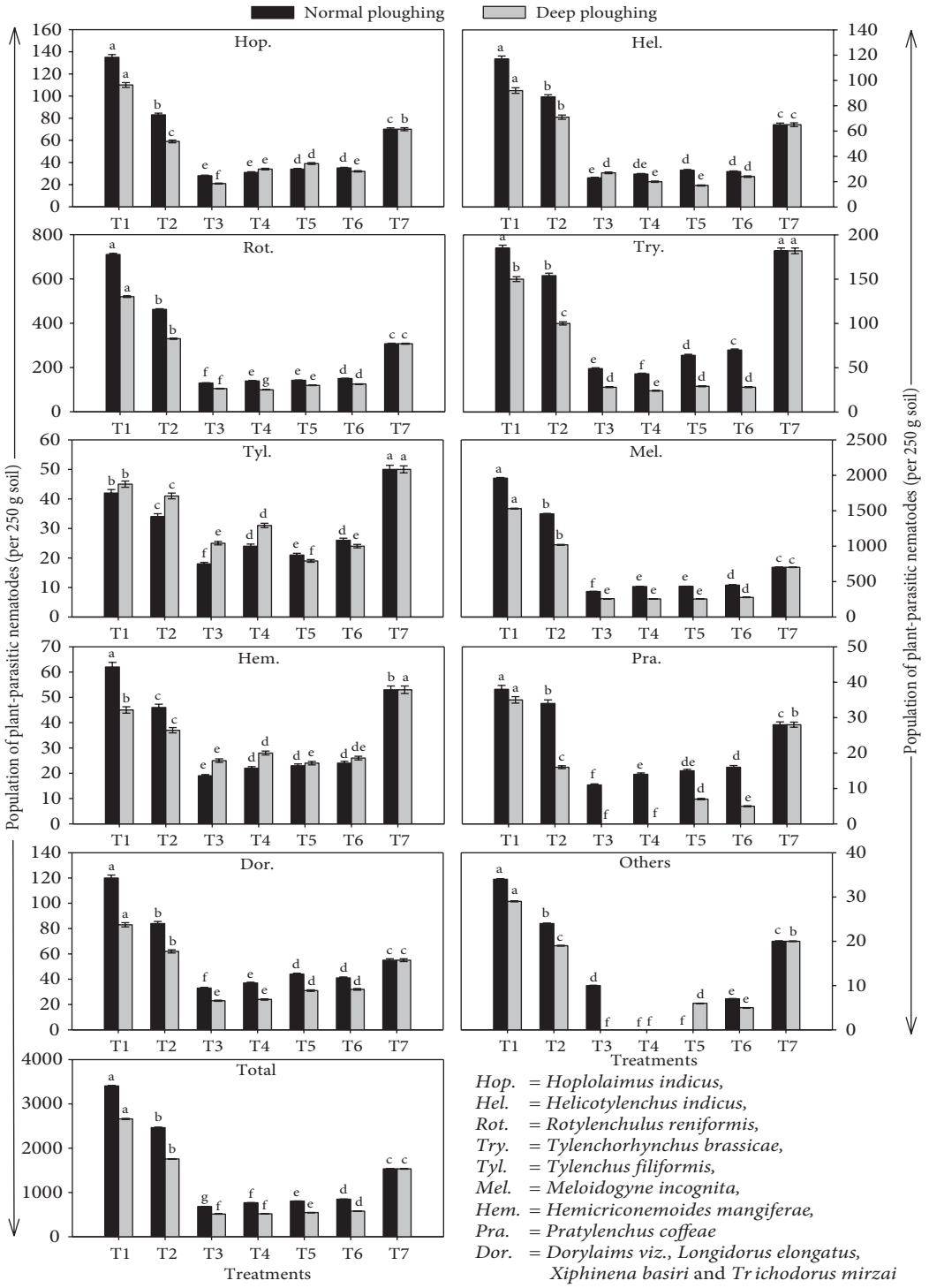


Figure 1. Effect of some botanicals in combination with normal and deep ploughing on different growth parameters of chickpea, *Cicer arietinum* 'K-850'. Values are means ± standard error. Data labelled by the same letters did not differ significantly at $P < 0.05$.



T1 = Untreated; T2 = Inorganic fertilizers; T3 = *Argemone mexicana* ; T4 = *Calotropis procera* ; T5 = *Solanum xanthocarpum*; T6 = *Eichhornia echinulata* ; T7 = Initial population

Figure 2. Effect of some botanicals in combination with normal and deep ploughing on the population of plant-parasitic nematodes associated with chickpea, *Cicer arietinum* 'K-850'. Values are means ± standard error. Data labelled by the same letters did not differ significantly at $P < 0.05$.

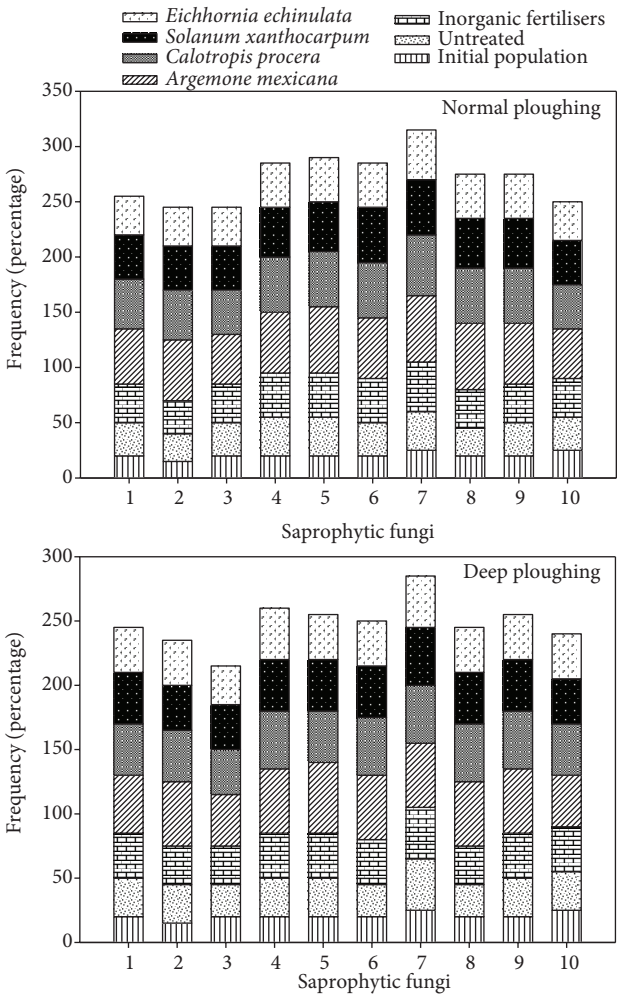
ploughed beds, the population of plant-parasitic nematodes increased from an initial level of 1531 250 g soil^{-1} to 3403 250 g soil^{-1} in untreated beds and 2463 250 g soil^{-1} in beds with inorganic fertilisers, whereas treatments with botanicals reduced the nematode population. The greatest reduction was observed in *A. mexicana* (681), followed by *C. procera* (766), *S. xanthocarpum* (804), and *E. echinulata* (847). Deep ploughing further reduced the population of these nematodes in all beds.

Root galling

Beds treated with botanicals sustained a great reduction in the number of root galls caused by *M. incognita*. The maximum reduction occurred in *A. mexicana*-treated beds (Figure 1). However, reduction in the number of root galls was also noted in other botanical-treated beds. Although the reduction in root galling was statistically significant for inorganic fertiliser beds, this reduction was not as great as in botanical-treated beds. Deep ploughing reduced the nematode populations more than normal ploughing.

Frequency of rhizosphere fungi

The frequency of saprophytic fungi *Aspergillus niger*, *A. flavipes*, *A. flavus*, and *Rhizopus oryzae* and of antagonistic fungi like *Trichoderma viride* and *Penicillium digitatum* increased in all the botanical-treated beds under normal ploughing. Deep-ploughed beds treated with these botanicals further supported the frequency of saprophytic fungi. Among the botanicals, *A. mexicana* proved most beneficial, followed by *C. procera*, *S. xanthocarpum*, and *E. echinulata*, which supported the frequency of saprophytic fungi (Figure 3). On the other hand, the frequency of most of the parasitic fungi, such as *M. phaseolina*, *R. solani*, *F. oxysporum*, and *Phyllosticta phaseolina*, was reduced after treatment with botanicals in normally as well as in deeply ploughed beds. Deep ploughing further reduced the frequency of parasitic fungi in all treatments. *A. mexicana* was found most efficacious among botanicals in decreasing the frequency of pathogenic fungi (Figure 4).

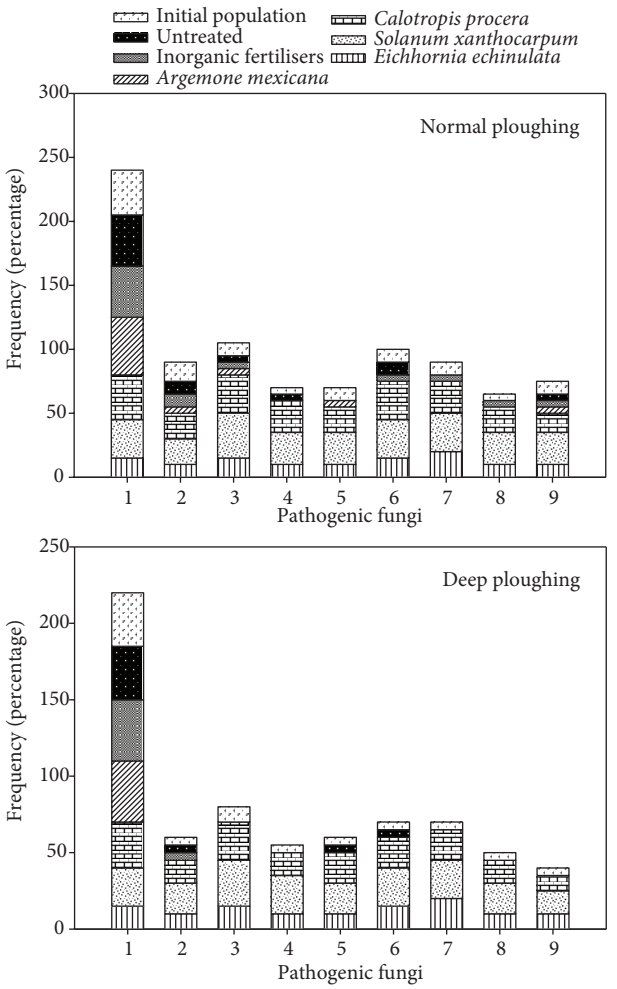


1 = *Aspergillus niger* 2 = *A. flavus* 3 = *A. fumigatus,* 4 = *A. flavipes*
5 = *Rhizopus oryzae,* 6 = *Ozonium taxanum,* 7 = *Mucor* spp.,
8 = *Trichoderma viride,* 9 = *Penicillium digitatum,* 10 = *Penicillium* spp.

Figure 3. Effect of some botanicals in combination with normal and deep ploughing on the frequency of saprophytic fungi in the rhizosphere of chickpea, *Cicer arietinum* 'K-850'. Values are means ± standard errors.

Discussion

In this study, soil amendments with botanicals such as *Argemone mexicana*, *Calotropis procera*, *Solanum xanthocarpum*, and *Eichhornia echinulata* significantly reduced the population of plant-parasitic nematodes and soil-inhabiting fungi and subsequently resulted in enhanced chickpea plant-growth parameters. Since equal amounts of nitrogen were added in all the beds, this was probably due to a reduction of nematode-induced disease in chickpea plant. An effect of nutrients other than nitrogen added with organic amendments on plant growth cannot be excluded. However, Rodriquez-Kabana et al. (1987) suggested that the effects of nematode control by organic additives depend on their chemical



1 = *Cunninghamella echinulata* 2 = *Alternaria tenuis,*
3 = *Fusarium oxysporum,* 4 = *Fusarium oxysporum f. ciceri,*
5 = *Rhizoctonia solani,* 6 = *Macrophomina phaseolina,*
7 = *Phyllosticta phaseolina,* 8 = *Sclerotium rolfsii,* 9 = *Curvularia tunata*

Figure 4. Effect of some botanicals in combination with normal and deep ploughing on the frequency of pathogenic fungi in the rhizosphere of chickpea, *Cicer arietinum* 'K-850'. Values are means ± standard errors.

composition and the type of microorganisms, which multiplied during degradation of such organic matter. These findings are in agreement with those of Mahmood et al. (2007). Application of organic matter would have helped the plant metabolism through the supply of many important micronutrients in the early growth phase. The effects of application of organic matter may be due to enhanced vegetative growth and photosynthesis, which led to the accumulation of more carbohydrates and other metabolites, resulting in more biomass. Our results are in conformity with those of Barani and Anbarani (2004) and Shukla et al. (2009). Incorporation of botanicals into soil increased microbial activity and is known to bring about increased conversion of N to nitrate form (Gunner 1963), which in turn appears to be responsible for stimulation of nitrate reductase activity. Their application provides an inducing substrate (nitrate) for the enzyme (nitrate reductase) to increase its activity, which results ultimately in increased metabolic activity of the plants and thereby the plant biomass. Similarly, chlorophyll content was also increased by amendments with these botanicals. Mahmood et al. (2007) also observed increased chlorophyll content due to application of organic matters. Root nodulation was also increased in soil amended with plant parts of these botanicals, which may be due to better growth of plants and subsequently the suppression of nematode and fungal populations. Supporting these results, Shukla and Tyagi (2009) also observed that incorporation of organic matter into the rhizosphere developed a more conducive environment for growth and nodulation of field-grown mung bean. This is attributed to the beneficial impact of organic matter on the rhizosphere.

The population of plant-parasitic nematodes increased as compared to the initial population in untreated and inorganic fertiliser-treated beds. Various scientists suggested that the action of decomposed organic additives leading to the control of plant-parasitic nematodes may be due to nematotoxic substances present in botanicals released after decomposition (Khan et al. 1974a), changes in physical and biological properties of soil (Ramesh et al. 2009), or toxicants released or produced during microbial decomposition. Southey (1978) observed that organic manures may suppress the population of nematodes and subsequently improve crop tolerance. Our results are in parallel with those of Khan et al. (2012), who found that farmyard manure alone and in various combinations with biofertilisers decreased the nematode population. Alam (1976) reported that ammonia, H_2S, fatty acids, aldehyde, formaldehyde, amino acids, and carbohydrates are released during decomposition of oil-seed cakes, and the same may also be produced after decomposition of the tested botanicals in this study. These chemicals have been found highly deleterious to plant-parasitic nematodes

in in vitro studies and may be effective against plant-parasitic nematodes in field conditions. The suppressive effect of some phytochemical compounds on nematode population has been well documented in several pathological systems (Chitwood 2002). These chemical compounds could be developed in the future for effective application as nematicides/ fungicides or could serve as model compounds for development of ecofriendly derivatives.

Beds treated with botanicals exhibited a great reduction in the number of root galls caused by *M. incognita*. A similar effect was also reported by Thomas (1978). Most probably the ecological set-up of nematodes is disturbed by deep ploughing because the soil is exposed to unfavourable environmental conditions like wind and sunlight, which affect their reproduction (Khan and Saxena 1980). Radwan et al. (2009) observed the nematicidal potential of oil-seed cakes in amended soil and found a reduced number of root galls caused by *M. incognita* on tomato.

The frequency of most of parasitic fungi, such as *M. phaseolina*, *R. solani*, *F. oxysporum*, and *Phyllosticta phaseolina*, was reduced after treatment with botanicals in normally as well as in deeply ploughed beds. According to Tiyagi and Alam (1995), the activity of trapping fungi was stimulated by soil application of organic additives against a number of parasitic organisms. Application of organic additives also releases nutrients, which enhance rapid root development and overall plant growth and thus help the plants against fungal attack. Our findings are in agreement with those of Tiyagi et al. (1991, 2001). Organic amendment has been effective in suppressing the soil population of pathogenic fungi such as *R. solani*, *Colletotrichum* spp., and *Fusarium* spp. in the rhizosphere of eggplant, okra, and tomato (Khan et al. 1974b); *F. oxysporum* f. *ciceri*, *M. phaseolina*, and *R. solani* on gram (Tiyagi and Alam 1995); and *M. phaseolina* and *R. solani* on chilli and tomato (Mahmood et al. 2005). Our findings are in accordance with the work of Srivastava and Yadav (2008). They reported that leaf extract of neem (*Azadirachta indica*) inhibited the mycelial growth of *F. oxysporum*. Singh et al. (2007) stated that some botanicals in the form of marigold leaf extract inhibited the growth of *Sclerotium rolfsii*. Bohra et al. (2006) reported that neem, a known botanical, has active components such as azadirachtin, nimbin, nimbidin, and azadiron, which are antifungal and antiinsecticidal in nature.

The inferences drawn from this study clearly revealed that soil application of these botanicals significantly enhanced the plant growth by reducing the population of nematodes and fungi. For economic evaluation, application of organic amendments like these botanicals, which are wildly and plentifully grown everywhere, would be much more beneficial when utilised properly. Moreover, the organic amendments have direct effect on improving the physicochemical properties and thereby maintaining soil health. Small and marginal farmers cannot afford to purchase costly chemical fertilisers for various purposes. Use of organic matter for disease management will serve the purpose of organic food production in this region, as the region is identified as a potential zone for organic food production. The concept of organic agriculture is receiving much attention and the organic food market is expanding rapidly in India. Such organic amendments could serve the purpose of alternative sources of nutrition supply in crop production, especially under organic farming. Finally, it is concluded that chemical fertilisers and pesticides can produce a good yield and fetch good remuneration at the cost of development of insecticidal resistance in the pests, environmental pollution, and health risks, but the application of organic matters like botanicals can also achieve the yield target and good return under better management practices while checking the multiplication of pathogenic agents like nematodes and fungi. These botanicals are locally available, ecofriendly, and help in sustaining soil health. Thus, the present findings strongly advocate the array of botanicals not only for nutrient requirements of the crop but also for pest management. Future studies are needed to investigate the active components in such botanicals that may be utilised as nematicides and fungicides.

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