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SOIL & CROP SCIENCES | RESEARCH ARTICLE

Potential role of bio-inoculants and organic matter for the management of root-knot nematode infesting chickpea

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Abstract: A pot experiment was conducted during 2013–14 to observe the potential role of some organics and bio-organics such as *Calotropis procera*, *Glomus fasciculatum*, and *Azotobacter chroococcum* on some growth attributes of chickpea and subsequently on the root-knot development caused by *Meloidogyne incognita*. Individual and conjoint treatments significantly enhanced the plant growth parameters as compared to unamended control. Physiological parameter such as chlorophyll content also exhibited significant improvement in all the treatments over non-amended control. The highest improvement in growth parameters of chickpea was observed in combined application of *G. fasciculatum* and *A. chroococcum* in pots amended with *C. procera*. Moreover, combined treatments of both bio-inoculants and *C. procera* markedly reduced the multiplication and reproduction rate of root-knot nematodes in terms of number of root galls and nematode population. Percent mycorrhization in terms of external and internal colonizations was increased significantly in plant amended with organic and bio-organics conjointly. The regression studies revealed significant relationship between number of galls and some plant growth variables. Present findings may promote organic-based farm products and eco-friendly management of *M. incognita* as this is a safer and cost-effective option.

Subjects: Bioscience; Earth Sciences; Environment & Agriculture

Keywords: *Azotobacter*; *Glomus fasciculatum*; root-knot nematode; mycorrhization; organic matter

1. Introduction

Chickpea (*Cicer arietinum*) is one of the most important pulse crops in India, occupying about 6.67×10^6 ha and producing 5.3×10^6 t annually (Singh, 2012). Its economic importance renders to

ABOUT THE AUTHORS

Nowadays, organic farming has been an important sector of sustainable agriculture. Aisha Sumbul and her group are working on organic agriculture. They are indulged to create the awareness among the large-scale farmers about the organic farming.

PUBLIC INTEREST STATEMENT

Chickpea is an important pulse crop being grown all over the world. It is highly economically important crop not only because of its high protein content, but also due to its ability to fix atmospheric nitrogen. Root-knot nematode, *M. incognita* poses major threat in the production of chickpea, both qualitatively as well as quantitatively. Chemical fertilizers, used to combat the disease, cause environmental hazard. Thus, the organic matter and bio-fertilizers, used as an alternative to inorganic fertilizers, can be proved to be beneficial for both crop production as well as for environment.

its high protein content as well as its ability to fix atmospheric nitrogen. Chickpea is a major source of human food and livestock because of high content of protein (Jiménez-Díaz, Castillo, Jiménez-Gasco, Landa, & Navas-Cortés, *in press*). Among all plant parasitic nematodes, *Meloidogyne* spp. are the most serious ones (Hussain, Mukhtar, & Kayani, 2011; Mukhtar et al., 2013). They are considered among the top five major plant enemies and ranked first among the ten economically important genera of phytonematodes globally (Jones et al., 2013; Kayani, Mukhtar, & Hussain, 2012). *Meloidogyne incognita*, one of the most economically important species of root-knot nematodes, adversely affects plant growth and yield causing an estimated \$100 billion loss per year (Mukhtar, Hussain, Kayani, & Aslam, 2014). *M. incognita* is found to be constantly associated with chickpea (Sumbul et al., 2015) and several other agricultural crops (Ansari & Khan, 2012a&b) and is of considerable importance, both in terms of qualitative as well as quantitative loss. Occurrence of root-knot in chickpea has been reported from various states in India (Jamal, 1976; Khan & Siddiqui, 2005). In order to meet the increasing requirement for chickpea, there is an urgent need to manage the constraints over its production (Ansari et al., 2015; Rizvi, Ansari, Zehra, & Mahmood, 2015; Rizvi, Singh, Ansari, Tiyagi, & Mahmood, 2015).

A variety of management strategies are being adopted to manage root-knot nematode, *Meloidogyne incognita*, one of the most difficult pests of agricultural crops. Although, the most effective and commonly used practice of combating this problem is use of chemical nematicides, they cause a huge damage to ecosystem. Indiscriminate use of chemical nematicides causes environmental degradation, which gives an impetus to search the alternative means (Collange, Navarrete, Peyre, Mateille, & Tchamitchian, 2011; Hallmann, Davies, & Sikora, 2009; Huang et al., 2009; Nico, Jiménez-Díaz, & Castillo, 2004; Siddiqui, Qureshi, & Akhtar, 2009).

Organic farming has emerged as an important priority area in view of the growing demand for safe and healthy food and long-term sustainability that concern on environmental pollution associated with indiscriminate use of agrochemicals. Amending soil with pesticides of botanical origin such as oil-cakes, chopped plant parts, and seed dressing with plant parts, which are safe, eco-friendly, and bio-degradable in nature, has now become the prime means to protect crops (Muller & Gooch, 1982; Tiyagi & Ajaz, 2004). *Calotropis procera*, a common weed is found in various parts of the country and is reported to possess some nematicidal properties (Hussain et al., 2011; Walia & Gupta, 1995). Plant parts of *C. procera*, have been reported to contain pesticidal properties that inhibit the larval penetration of *M. incognita* and consequently reduction in root-knot development (Mahmood, Tiyagi, & Azam, 2007; Tiyagi, Mahmood, Rizvi, & Dev, 2009). In addition, due to presence of some alkaloids, *C. procera* has been reported to cause juvenile mortality of *M. incognita* (Nelaballe & Mukkara, 2013).

Rhizosphere supports a large and active microbial population capable of exerting beneficial, neutral, and detrimental effects on plant growth. The micro-organisms, which can grow in the rhizosphere are ideal for use as biocontrol agents, since rhizosphere provides the initial barrier against pathogen attack of the root system (Weller, 1988). Biological nitrogen fixers help to enhance productivity by biological nitrogen fixation, producing hormones, vitamins, and other growth factors required for plant growth and development, solubilizing phosphorus, and suppression of growth of plant pathogens (Verma, Yadav, & Tiwari, 2010) on several crops. *Azotobacter* is a free-living nitrogen-fixing, plant growth promoting rhizobacteria (PGPR), that enhance emergence, colonize roots, and stimulate overall plant growth and can also suppress disease of plants. The manipulation of crop rhizosphere by inoculation with *Azotobacter* for bio-control of root-knot nematode has shown considerable promise (Siddiqui & Mahmood, 2001). Arbuscular Mycorrhizal (AM) fungi colonize the roots of many crop plants (Ozgonen, Bicici, & Erkilic, 1999) and are of immense value in enhancing the uptake of phosphorus, minor elements, and water, and thus improving the plant growth and yield (Allen, 1996; Rizvi, Singh, et al., 2015; Siddiqui & Mahmood, 1999). These fungi induce changes in the host root exudation pattern following host colonization with altered microbial microflora in the mycorrhizosphere (Akhtar & Siddiqui, 2008) and are also reported to reduce the severity of several plant diseases (Akkopru & Demir, 2005; Barea, Azcón, & Azcón-Aguilar, 2002; Khan et al., 2015;

Linderman, 2000; Rizvi, Singh, et al., 2015). They have also widely been reported as biocontrol agents against plant parasitic nematodes (Khan et al., 2008; Siddiqui & Mahmood, 1999; Tian, Yang, & Zhang, 2007).

Present study was conducted to monitor the effects of *A. chroococcum* and *G. fasciculatum* either solely or conjointly on some plant growth parameters and root-knot development on chickpea amended with or without *C. procera*.

2. Materials and methods

The root-knot nematode, *M. incognita* (Kofoid & White) Chitwood was selected as a test pathogen and chickpea (*Cicer arietinum* L.) var. Avrodhi as a test plant. PGPR, *A. chroococcum*, VAM fungi, *G. fasciculatum* and organic matter like chopped leaves of *C. procera* were used alone and in various combinations for the management of chickpea root-knot disease.

2.1. Preparation and sterilization of soil mixture

Appropriate amount of sandy loam soil was collected from the field of Department of Botany, A.M.U. Aligarh and allowed to pass through 20-mesh sieve. The soil, river sand, and organic manure was mixed in the ratio of 3:1:1, respectively, and the mixture were filled in the clay pots of 15 cm diameter at the rate of 1 kg soil per pot. Before transferring to the autoclave for sterilization at 20 lb pressure for 20 min, a little water was poured in each pot just to wet the soil. These sterilized pots were kept to cool down at room temperature before use for further experiments.

2.2. Growth and maintenance of test plant

Seeds of chickpea, var. Avrodhi were surface sterilized with 0.01% mercuric chloride (HgCl_2) for two minutes and washed three times with distilled water. Five seeds per pot were sown and thinning was done after germination to maintain one plant per pot. Pots were watered depending on the requirement. One week old, healthy, and well-established seedlings were used for experimental purpose.

2.3. Preparation of nematode inoculum

A number of egg masses were handpicked with the help of sterilized forceps from heavily infested eggplant roots on which pure culture of *M. incognita* was maintained. These egg masses, after washing thoroughly in distilled water were placed in a coarse sieve (10 cm diameter) mounted with cross double-layered tissue paper. The sieves were placed in Petri dishes containing water that were incubated at 25°C in the laboratory. Three days later, when the majority of the eggs hatched, the second stage juveniles (J_2) of the nematode were collected by rinsing the Petri dishes with distilled water. The identification of the species was confirmed by the morphology according to the perineal pattern (Hartman & Sasser, 1985). The concentration of second-stage juveniles of *M. incognita* in the water suspension was adjusted so that 2,000 freshly hatched juveniles could be added as initial inoculum level in 10 ml. of water suspension to each pot containing a chickpea seedling.

2.4. Preparation of organic matter

Leaves of *C. procera* were collected from the AMU campus and brought to laboratory, rinsed with distilled water, and chopped with a sharp sterile knife. These chopped leaves were incorporated in soil at the rate of 20 g/kg leaves per pot and the pots were immediately watered for decomposition to prepare the compost of *C. procera*.

2.5. Preparation of inoculum of biocontrol agents

Mycorrhizal spores were isolated by a modified wet sieving and decanting method (Gerdemann & Nicolson, 1963). For this aspect, a sample of 100 g dry soil was mixed in 1,000 ml water, and the heavier particles were allowed to settle for few seconds. The lumps were broken and heavier particles were removed. The suspension was poured through a coarse sieve to remove large pieces of organic matter. The liquid passed through this sieve was collected and again passed through sieves of 80, 100, 150, 250, and 400 meshes. Spores obtained on sieves were collected with water in

separate beakers and counted in 1 ml of the suspension in counting dish under the stereoscopic microscope. The final number of spores/100 g of soil was calculated accordingly for each treatment.

Charcoal-based commercial culture of free-living nitrogen-fixing bacteria *Azotobacter chroococcum* was obtained from Quarsi Agriculture Farm, Aligarh, India. For inoculation, 100 gram culture was mixed separately in 1,000 ml distilled water which means 10 ml (equivalent to 2 g culture) was added around each seedling. Commercially standardized culture of *A. chroococcum* having 1.2×10^8 cfu/g was used in the experiment.

2.6. Inoculation technique

For inoculation of *M. incognita*, *A. chroococcum*, and *G. fasciculatum*, soil around the root was carefully removed in order to avoid the damage. Inoculum suspensions of these micro-organisms were poured around the roots uniformly and soil was replaced to cover the roots. Equal amount of water was poured in the pot that served as control.

3. Experimental design

The 14 treatments each with 5 replicates were arranged in a completely randomized block design and maintained in a glasshouse with air temperature ranging from $22 \pm 3^\circ\text{C}$. All the plants were watered up to the soil capacity. Following combinations were used during the experimentation:

- Control (C)
- *Calotropis procera* (CP)
- *Meloidogyne incognita* (MI)
- *Azotobacter chroococcum* (Azo)
- *Glomus fasciculatum* (GF)
- CP + MI
- CP + AC
- CP + AC + MI
- CP + GF
- CP + GF + MI
- AC + MI
- GF + MI
- CP + AC + GF
- CP + AC + GF + MI

4. Observations

Percent pollen fertility was estimated at the flowering stage by the method suggested by Brown (1949), using 1% acetocarmine solution to stain the pollen grains. Plants were harvested 90 days after nematode inoculation. Root systems were gently washed with tap water taking care to avoid losses and injury during the entire operation. Data were recorded on plant length (cm), plant fresh weight (g), plant dry weight (g), chlorophyll content (mg g^{-1} fresh leaves), nematode population (both in soil and root), number of gall/root system, number of egg masses/root system, fecundity in terms of number of egg/egg masses.

4.1. Chlorophyll estimation

Chlorophyll content was estimated per gram of fresh leaf weight by the technique of Arnon (1949). 1 g finely cut fresh leaves was pulverized with the help of mortar and pestle using sufficient quantity of 80% acetone. The leaf homogenate thus obtained, was filtered through filter paper, and supernatant was collected in the volumetric flask. The final volume of the supernatant was made up to 10 ml

with 80% acetone. 5-ml chlorophyll solvent was transferred to a clean cuvette and the absorption values were noted at 645 and 663 nm in a spectrophotometer against the solvent (80% acetone) blank. The calculation of the total chlorophyll content per gram fresh leaves was done by the following formula:

$$\text{Total chlorophyll content (mg g}^{-1}\text{)} = 20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000} \times W$$

where A_{663} = solution absorbance at 663 nm, A_{645} = solution absorption at 645 nm, V = volume of solution (taken in cuvette), W = weight of leaf tissue used for extraction of pigments i.e. 1 g.

4.2. Extraction of nematode population

For extraction of nematodes from the soil, 250 g subsample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting method followed by Baermann funnel method (Southey, 1986). Nematode suspension was collected after 24 h and number of nematodes was counted in counting dish taking five replicates of 1 ml suspension from each sample. Mean of five such counting was obtained and a population of nematodes per kg soil was calculated. For estimation of larvae, eggs, and females inside the roots, each root system was cut into small pieces and mixed. One-gram root sample was taken and macerated for 30–40 s in Waring blender to recover nematode eggs, females, and larvae. Counting was done from the suspensions thus obtained. Total number of nematodes present in the roots was calculated by multiplying the number of nematodes present in one gram root with the weight of the root.

4.3. Assessment of root mycorrhizal colonization (Phillips & Hayman, 1970)

Roots were washed with tap water and cut into 1-cm long segments and then boiled in 10% KOH solution at 90°C for 45 min. KOH solution was then poured off and roots were rinsed well in a beaker until no brown color appeared in the rinsed water. Alkaline H_2O_2 which was used to bleach the roots was made by adding 3 ml of NH_4OH to 30 ml of 10% H_2O_2 and 567 ml of tap water. The roots were rinsed thoroughly at least three times using tap water to remove the H_2O_2 . Roots were then treated with 0.05% trypan blue (in lactophenol) and were kept for an hour. The specimens were then removed from trypan blue and kept for overnight in a destaining solution, prepared with acetic acid (laboratory grade)—875 ml, glycerine—63 ml and distilled water—63 ml. The cellular contents were removed by this method and the AM fungal structures stained dark blue. These stained root segments were used for determining the root colonization by AM fungi. The percentage/proportion of root colonization by AM fungi was determined by the grid line intersecting method (Giovannetti & Mosse, 1980). The root segments were selected at random from the stained sample and mounted on microscopic slides in group of 10. One hundred to one hundred and fifty root segments from each sample were used for the assessment. The presence or absence of colonization in each root segment was recorded and the percent root colonization (mycorrhizal infection in the roots) was calculated as follows:

$$\% \text{ Root colonization} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments screened}} \times 100$$

4.4. Statistical analysis

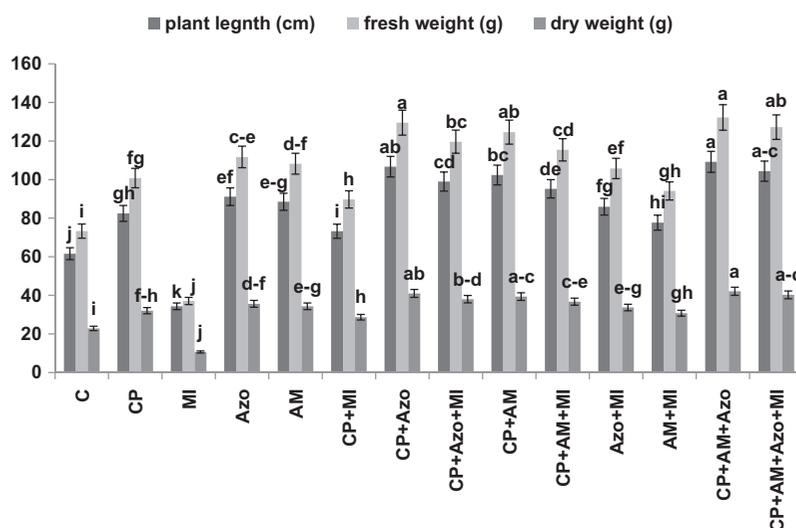
All data collected were analyzed statistically and least significant differences were calculated at $p \leq 0.05$. Duncan's multiple range test was employed to denote significant differences. Both the analyses were carried out using 'R' software (R i3863.2.3 version).

5. Results

The effects of individual as well as concomitant treatments were recorded in relation to different growth parameters of chickpea like plant length, fresh as well as dry weights, number of pods and branches per plant, percent pollen fertility, and chlorophyll content (Figure 1, Tables 1 and 2).

Plant treated with *C. procera*, *G. fasciculatum*, and *A. chroococcum* alone and in combination significantly increased plant dry weight over uninoculated control (Figure 2). *A. chroococcum* increased plant dry

Figure 1. Interactive effects of *Meloidogyne incognita*, *Glomus fasciculatum*, *Azotobacter*, in *Calotropis procera*-amended soil on the plant length, fresh, and dry weight of chickpea *Cicer arietinum*.



weight more than *G. fasciculatum*. Further enhancement in dry weight was observed when soil was amended with *C. procera*. Similar increase in dry weight was shown in plants treated with *C. procera* + *G. fasciculatum*, *C. procera* + *A. chroococcum* and those inoculated in combination with *C. procera* + *G. fasciculatum* + *A. chroococcum* (Figure 2). Inoculation of plants with *M. incognita* caused a significant reduction of plant dry weight compared to untreated control (Figure 2). Application of *C. procera*/*G. fasciculatum*/*A. chroococcum* alone or in combination caused a significant increase in plant dry weight even in pathogen-inoculated plants. Inoculation of *A. chroococcum*/*G. fasciculatum* resulted in a significantly greater increase in plant dry weight of pathogen inoculated plants than that caused by *C. procera*. Plants raised in *C. procera*-amended soil and inoculated with *A. chroococcum* and *G. fasciculatum* caused significant and similar improvement in plant dry weight even if inoculated with test pathogen (Figure 1).

Table 1. Interactive effects of *Meloidogyne incognita*, AM fungus, *Azotobacter*, in *Calotropis procera*-amended soil on the plant-growth parameters of chickpea *Cicer arietinum*

Treatments	No. of pods per plants	No. of branches per plant	Percent pollen fertility	Fresh weight of fruits (g)	Chlorophyll content (mg/g)
Control	24.33 ^h	7.56 ^d	79.62 ^e	50.24 ^g	2.24 ^f
CP alone	31.24 ^g	9.28 ^c	85.19 ^{de}	63.38 ^{ef}	2.81 ^e
MI alone	15.21 ⁱ	5.74 ^e	57.07 ^f	30.01 ^h	1.36 ^g
Azo alone	34.34 ^{d-f}	9.93 ^b	88.50 ^{b-d}	67.56 ^{b-f}	2.96 ^{cd}
GF alone	33.51 ^{d-f}	9.75 ^b	87.16 ^{c-e}	66.26 ^{c-f}	2.91 ^{de}
CP + MI	29.27 ^g	8.89 ^c	80.64 ^e	61.52 ^f	2.70 ^e
CP + Azo	38.78 ^{ab}	11.07 ^a	96.33 ^a	73.47 ^{ab}	3.27 ^{ab}
CP + Azo + MI	35.94 ^{b-d}	10.40 ^a	91.94 ^{a-d}	70.2 ^{a-d}	3.08 ^{a-c}
CP + GF	37.42 ^{a-d}	10.62 ^a	93.62 ^{a-c}	71.16 ^{a-c}	3.15 ^{a-c}
CP + GF + MI	35.23 ^{c-e}	10.19 ^b	89.90 ^{a-d}	68.9 ^{b-e}	3.04 ^{bc}
Azo + MI	32.34 ^{e-g}	9.51 ^b	86.73 ^{c-e}	65.09 ^{d-f}	2.86 ^e
GF + MI	30.45 ^g	9.07 ^c	84.77 ^{de}	62.65 ^f	2.73 ^e
CP + GF + Azo	39.82 ^a	11.43 ^a	97.52 ^a	75.18 ^a	3.34 ^a
CP + Azo + GF + MI	38.00 ^{a-c}	10.83 ^a	95.00 ^a	72.41 ^{a-c}	3.21 ^{a-c}
C.D ($p = 0.05$)	3.32	0.79	7.70	6.23	0.259

Notes: CP = *Calotropis procera*, MI = *Meloidogyne incognita*, Azo = *Azotobacter*, GF = *Glomus fasciculatum*. Letters are meant for comparison within columns and different letters depict values that are significantly different at $p = 0.05$.

*Each value is an average of 5 replicates.

Table 2. Interactive effects of *Meloidogyne incognita*, *Glomus fasciculatum*, *Azotobacter chroococcum* in *Calotropis procera* amended soil on the plant-growth parameters of chickpea *Cicer arietinum* in relation to mycorrhization, root-knot development, and nematode multiplication

Treatments	Root-galls	Nematode population	Rf = (Pf/Pi)	Root-nodule/plants	Mycorrhizal colonization (%)	
					External	Internal
Control	0.00	0.00	0.00	18.58 ^h	0.00	0.00
CP alone	0.00	0.00	0.00	24.08 ^{ga}	0.00	0.00
MI alone	158.50 ^a	14674 ^a	7.33 ^a	11.77 ⁱ	0.00	0.00
Azo alone	0.00	0.00	0.00	25.54 ^{c-f}	0.00	0.00
GF alone	0.00	0.00	0.00	25.19 ^{d-g}	40.19 ^c	45.80 ^d
CP + MI	120.20 ^b	11580 ^b	5.79 ^b	23.16 ^g	0.00	0.00
CP + Azo	0.00	0.00	0.00	27.90 ^{ab}	0.00	0.00
CP + Azo + MI	50.23 ^d	04612 ^f	2.30 ^e	26.67 ^{a-d}	0.00	0.00
CP + GF	0.00	0.00	0.00	27.06 ^{a-d}	46.48 ^b	49.62 ^c
CP + GF + MI	57.16 ^d	06257 ^e	3.12 ^{de}	26.03 ^{b-e}	24.00 ^d	33.93 ^e
Azo + MI	80.23 ^c	07586 ^d	3.79 ^d	24.82 ^{e-g}	0.00	0.00
GF + MI	110.73 ^b	09365 ^c	4.68 ^c	23.70 ^g	19.37 ^e	25.77 ^{fs}
CP + GF + Azo	0.00	0.00	0.00	28.45 ^a	56.62 ^a	63.56 ^a
CP + Azo + GF + MI	37.19 ^e	01264 ^g	0.63 ^f	27.59 ^{abc}	49.50 ^b	55.30 ^b
C.D (p = 0.05)	9.67	22.62	0.85	2.20	3.11	3.21

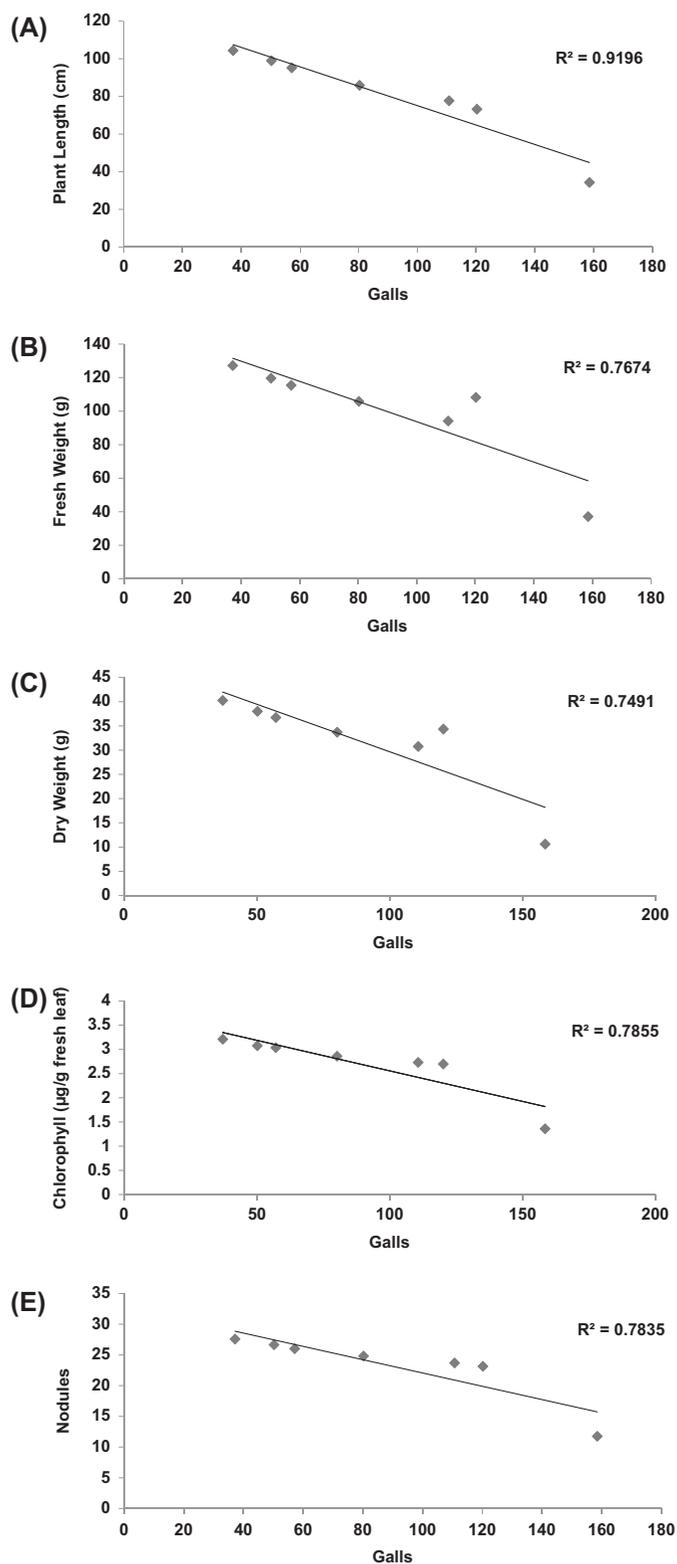
Notes: CP = *Calotropis procera*, MI = *Meloidogyne incognita*, Azo = *Azotobacter*, G F = *Glomus fasciculatum*. Letters are meant for comparison within columns and different letters depict values that are significantly different at $p = 0.05$.

*Each value is an average of five replicates.

The number of pods was significantly reduced in plants inoculated with *M. incognita* (Table 1). Application of *C. procera*/*G. fasciculatum*/*A. chroococcum* alone or in combination significantly increased the number of pods per plant both in pathogen inoculated and uninoculated plants. Highest improvement was found in case of plants treated in combination with *C. procera*, *G. fasciculatum*, and *A. chroococcum* in the absence of pathogen, and the inoculation with *M. incognita* resulted in not much significant change (Table 1).

Number of branches per plant decreased significantly in plants inoculated with *M. incognita* as compared to control. Treatment of plants with *C. procera*/*G. fasciculatum*/*A. chroococcum* alone or in combination significantly increased the number of branches per plant both in pathogen-inoculated and uninoculated plants. Highest and similar increase was found in plants treated with *C. procera* + *G. fasciculatum* + *A. chroococcum*, *C. procera* + *G. fasciculatum* + *A. chroococcum* + *M. incognita*, *C. procera* + *A. chroococcum*, *C. procera* + *G. fasciculatum*, and *C. procera* + *A. chroococcum* + *M. incognita* (Table 1). Pollen fertility of the plants was significantly lowered by the inoculation of plants with *M. incognita* as compared to control. Application of *C. procera*/*G. fasciculatum*/*A. chroococcum* alone and in combination significantly increased the pollen fertility in both pathogen-inoculated and uninoculated plants. Highest and similar increase in pollen fertility was observed in plants treated combinely with *C. procera* + *G. fasciculatum* + *A. chroococcum*, *C. procera* + *A. chroococcum*, and *C. procera* + *G. fasciculatum* + *A. chroococcum* + *M. incognita*. Increase in pollen fertility of pathogen-inoculated plants treated with *A. chroococcum* was similar to pathogen-uninoculated plants treated with only *G. fasciculatum*. Treatment of pathogen-inoculated plants with *C. procera*/*G. fasciculatum* gave similar increase in pollen fertility as that of control plants (Table 1). Inoculation of plants with *M. incognita* also caused a significant decrease in fresh weight of fruits as compared to control and again the application of *C. procera*/*G. fasciculatum*/*A. chroococcum* alone and in combination significantly increased the fresh weight of fruits. Highest increase was observed in pathogen-uninoculated

Figure 2. Regression analysis (plot) between galls vs. (A) plant length, (B) fresh weight (C), dry weight, (D) chlorophyll, and (E) nodules of chickpea, *Cicer arietinum*.



plants treated with combination of *C. procera*, *G. fasciculatum*, and *A. chroococcum* followed by those inoculated with *C. procera* and *A. chroococcum*. Application of both the inoculants along with *C. procera* in the presence of *M. incognita* increased fresh weight of fruits similarly as in case of those

treated with *C. procera* and *G. fasciculatum*. Chlorophyll content of the uninoculated plants increased greatly when *C. procera*, *G. fasciculatum*, and *A. chroococcum* were applied concomitantly (Table 1). Inoculation of plants with *M. incognita* significantly decreased the chlorophyll content. Combine application of *C. procera*, *G. fasciculatum*, and *A. chroococcum* caused a significant improvement in chlorophyll content of pathogen-inoculated plants. The increase was similar in case of inoculated plants treated with either *C. procera* plus *A. chroococcum* or *C. procera* plus *G. fasciculatum* (Table 1). Number of nodules per root system was significantly higher in plants treated with *C. procera*/*G. fasciculatum*/*A. chroococcum* alone or in combination. Combine application of *C. procera* and *A. chroococcum* was most effective in enhancing the root nodulation in both pathogen-inoculated and uninoculated plants (Table 2). External and internal colonization of root by *G. fasciculatum* was high when inoculated in the presence of *C. procera* and *A. chroococcum*, while presence of pathogen reduced its colonization. Highest root colonization was observed in plants combinely inoculated with *G. fasciculatum* and *A. chroococcum* in *C. procera*-amended soil in pathogen-free plants followed by pathogen-inoculated plants (Table 2).

The number of galls per root system and nematode multiplication was high in plants inoculated with *M. incognita* alone. Reduction in root galling was significantly higher and similar in plants treated with *C. procera* and *G. fasciculatum* followed by *A. chroococcum*. Amending soil with *C. procera* caused maximum reduction in nematode multiplication followed by *G. fasciculatum* and *A. chroococcum*. Combine inoculation of plants with *G. fasciculatum* plus *A. chroococcum* in the presence of *C. procera* caused highest reduction in galling and nematode multiplication than any other combination (Table 2). Similar trends of reduction were also recorded in reproduction factor (Table 2). Besides, regression analysis between galls and various growth parameters exhibited significant relationship. The regression values between number of galls and plant length ($R^2 = 0.919$; Figure 2(A)), between number of galls and fresh weight ($R^2 = 0.767$; Figure 2(B)), between number of galls and dry weight ($R^2 = 0.749$; Figure 2(C)), between number of galls and chlorophyll ($R^2 = 0.785$; Figure 2(D)), between number of galls and nodules ($R^2 = 0.783$; Figure 2(E)) which are presented in Figure 2.

6. Discussion

Application of biofertilizers in various combinations constantly improved the growth of plant in comparison to the control and those treated with *M. incognita* alone. Our results are in conformity with Singh, Kumar, and Rana (2000). Present study revealed that the application of *A. chroococcum*, *G. fasciculatum*, and *C. procera* concomitantly decreased the disease intensity in the nematode-infested chickpea. The detrimental effect of these biofertilizers against root-knot nematodes was also observed by various earlier researchers (Khan, Mohiddin, Ejaz, & Khan, 2012; Kumar & Gupta, 2010).

Effectiveness of AM fungi for increased plant growth has been reported by many workers (Jothi & Sundarababu, 2000; Oruru & Njeru, 2016; Rizvi et al., 2015; Shreenivasa, Krishnappa, & Ravichandra, 2007). Akhtar and Siddiqui (2008) observed the effect of *G. intraradices* on chickpea in relation to their plant growth parameters and found that it was surprisingly increased over control. The mechanisms to explain the antagonistic effects of AMF on nematode multiplication and their activities may be either physiological or physical in nature. *G. fasciculatum* can alter the physiology of the roots, including the root exudates responsible for chemotactic attraction of nematode (Mc Guidwin, Bird, & Safir, 1985). Yield loss normally caused by nematode is mitigated by enhancing the uptake of phosphorus and other nutrients due to AM fungi leading to improvement of plant vigor and growth (Hussey & Roncadori, 1982). Nematode development and reproduction might have retarded in mycorrhiza-treated plants due to decrease in available food (Saleh & Sikora, 1984). Physiological changes in root inoculated with AM fungi may result in development of resistance due to production of antagonistic substances (Suresh, Bagyaraj, & Reddy, 1985). Mycorrhizal fungi may alter the microbial activity in the rhizosphere affecting the survival of nematode and penetration of roots by nematode (Timothy & Robert, 1992). The above mechanisms may operate singly or in combination to make mycorrhizal plants resistant against the invasion of plant pathogens.

Similarly, inoculation of *Azotobacter* spp. significantly improved the plant growth parameters and nutrient status (Abdel-Monaim, Abdel-Gaid, & El-Morsi, 2012) resulting in reduced population of *M. incognita* and its pathogenicity. Khan et al. (2012) observed the effect of *A. chroococcum* in combination with organic matters on the population of plant parasitic nematodes on chili plants and found significant reduction in the multiplication of nematodes as well as number of root galls. Being soil bacteria, *Azotobacter* is reported to produce growth promoting substances as auxins, cytokinins, and gibberellins and these materials are the primary substances controlling the enhanced growth. These substances, which originate from the root surface, affect the growth of the closely associated higher plants (Wani, Chand, & Ali, 2013). The inoculation of *Azotobacter* along with oil cake increased the internal as well as external colonization of mycorrhiza which increased the phosphorus contents of the soil rhizosphere. This productiveness of the rhizosphere for AM fungi may be attributed to favorable impact exerted by root exudates (Bais, Weir, Perry, Gilroy, & Vivanco, 2006), which contain amino acids, carbohydrates, organic acids and growth-promoting substances, and phytohormones produced by *A. chroococcum*.

Organic matter acts as nutrient reservoir and it also provides suitable substrate for growth of micro-organisms. Thus, addition of oil seed cake of *C. procera* resulted in enhanced proliferation of bio-fertilizers, in turn improving the plant-growth parameters and reducing nematode multiplication. The rate of reproduction and multiplication got checked due to the presence of some nematicidal properties (Hussain et al., 2011). Chedekal (2013) also reported reduction in galling and fecundity of nematode larvae on chickpea over control when plant was provided with *C. procera*.

Chlorophyll contents were also increased in plants inoculated with *G. fasciculatum* and *A. chroococcum* individually as well as concomitantly in soil amended with *C. procera*. Similar results were recorded against root-rot disease of chickpea by Akhtar and Siddiqui (2008). The improvement in these parameters might be due to improvement in soil physical properties like porosity, water holding capacity, and tendency of soil toward neutral pH which in turn increased the microbial biomass pool in the soil rhizosphere. The increase in chlorophyll contents in leaves in the presence of decomposed organic wastes (Siddiqui & Akhtar, 2008), due to increase in N uptake by the addition of organic compounds resulted in increased photosynthetic efficiency, translocation of nutrients, and other metabolites toward formation of fruits. The chickpea plants also showed significant improvement in growth parameters with the addition of *G. fasciculatum* (Bagyaraj, Manjunath, & Patil, 1979).

7. Conclusion

The findings of our study showed that combined application of both bioagents along with the organic matter, *C. procera*, surrogate to chemical nematicides, may be helpful to the growers of chickpea suffering from root-knot disease. This approach will also minimize the environmental perturbations, preserve the biota, and keep the management module more economic.

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