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EFFICACY OF ORGANIC MATTER AND SOME BIO-INOCULANTS FOR THE MANAGEMENT OF ROOT-KNOT NEMATODE INFESTING TOMATO

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Abstract

Efficiency of an organic matter like *Tagetes erecta* and bioinoculants *Azotobacter chroococcum* and *Glomus fasciculatum* was investigated in tomato cultivar 'Pusa Ruby' when inoculated individually as well as concomitantly for the management of the root-knot nematode, *Meloidogyne incognita* in terms of growth parameters such as plant length, fresh and dry weights, chlorophyll content, per cent pollen fertility and mycorrhization. Greatest reduction in the numbers of second-stage juveniles in soil, number of root-galls, egg-masses and nematode multiplication was recorded with combined application of *T. erecta* and bio-inoculants *A. chroococcum* and *G. fasciculatum* as compared to untreated control and other treatments. Similarly, the greatest improvement in the plant growth and biomass of tomato was noted in the same treatments. However, individual inoculation of these bio-inoculants and organic fertilizers also showed significant enhancement but was less as compared to combined treatment. *A. chroococcum* was found most effective against disease incidence followed by *G. fasciculatum* and *T. erecta*. Parameters like NP and K contents were significantly enhanced in those plants which received combined treatments of organic matter and bio-inoculants. *Azotobacter* was found more efficacious against nematodes than *Glomus fasciculatum*. Organic matter also influenced the activity of bio-inoculants, more with the *Azotobacter* than *G. fasciculatum*.

Keywords: Tomato, Tagetes erecta, Azotobacter chroococcum, Glomus fasciculatum Meloidogyne incognita

Introduction

Tomato (*Solanum lycopersicon* L) is an important vegetable crop grown worldwide for its multidimensional use in our daily life. In India, major tomato producing states are Andhra Pradesh, Bihar, Karnataka, Uttar Pradesh, Orissa, Maharashtra, Madhya Pradesh and West Bengal (NHB, 2012). Tomatoes are consumed throughout the world by diverge population and now-a-days it mainly used in food processed industries. The estimated world production of tomato is about 125.02 million tons and the total area under its cultivation is about 45.5 lakh ha. Indian contribution to the annual world production was 10.26 million tons with an area of 5.72 lakh ha in 2006 (Mane et al. 2010), however, production increased slightly about 17.50 million tons in the recent years (FAOSTAT, 2012). Productivity of this vegetable is far below of expectation particularly in India as compared to other countries which could be mainly of improper and inadequate supply of nutrients, disease incidence and lack of adoption of new improved production technologies. Root-knot nematode, *Meloidogyne incognita* remains to be one of the most important constraints in agricultural production worldwide (Khan and Pariari, 2013) and difficult crop pests to control it (Chitwood, 2002), because they have high reproduction rates (Ananhirunsalee, 1995). Yield losses in tomato due to root-knot nematode, *Meloidogyne* spp. ranges from 35-50% in India (Jain, 1991; Jonathan, 2001) and as high as 85% globally (Taylor and Sasser, 1978; Sasser, 1979).

In view of above facts it becomes imperative to make tomato production, a more cost effective enterprise by switching over to non-conventional sources of nutrients to meet the nutrient requirement of plants while at the same time maintain healthy edaphic environment and look for the lower cost of production. Conventional pest control means utilization of chemical pesticides in agricultural production system. Chemical nematicides though effective for management of nematodes, have received much attention in recent years but are hazardous to the natural ecological services. In contrast, microorganisms could be used and have either no harm or trivial to the environment and human health (Compant, 2010). Use of bio-inoculants alone and along with the chemical fertilizers have found to be beneficial for soil biota contributing towards the betterment and maintenance of healthy environment. The main aim is to find out a possible way which strikes on acceptance balance between production benefits and ecological conservation with reduced use of chemical fertilizers. The use of bio-inoculants will be of prime importance in the field of agricultural system. Considerable scientific information are available which shows that the sole and/ or combined application of organic matter and biofertilizers increase yield and influence quantity attributes in several vegetables (Worthington, 2001, Bahadur, 2003, 2006) by reducing the population of harmful microbes (Shama et al., 2014). A judicious combination strategy of using organic matter and bio-inoculants may be effective not only in sustaining crop productivity and harmony among themselves but also in supplementing a part of chemical fertilizers requirement of the crops. Thus, the evaluation of different bio-inoculants like *Azotobacter chroococum* and *Glomus fasciculatum* with combination and individual treatments of organic like *Tagetus erecta* is undertaken urgently and required in tomato for

improving fertilizer use efficiency, yield and quality under a cost effective sustainable production system.

Materials and Methods

A pot experiment was conducted in the experimental site of Department of Botany, Aligarh Muslim University, Aligarh to assess the effect of inoculation of biological nitrogen fixer, *Azotobacter chroococcum*, arbuscular vesicular mycorrhizae, *Glomus fasciculatum*, singly or in combination along with *Tagetes erecta* on growth, yield and quality parameters of tomato (*Solanum lycopersicon* L.). The seeds of tomato, *Solanum lycopersicon* L. var. 'Pusa Ruby' were procured from the Indian Vegetable Research Institute (IIVR), Shahenshahpur, Uttar Pradesh, India.

Plant culture

The seeds of tomato, *Solanum lycopersicon* var. 'Pusa Ruby' were surface sterilized in 0.01% mercuric chloride ($HgCl_2$) for 2 minutes and then rinsed 3 times with distilled water. Seeds were sown in the sterilized soil in 25 cm clay pots. Three week old seedlings were transplanted @ 1 seeding/pot containing 1 kg sterilized soil.

Composition of soil and treatment schedule

Soil was collected from the agricultural field of Botany Department, Aligarh Muslim University, Aligarh having the texture of sandy-loam. The soil was mixed with river sand and organic manure in the ratio of 3:1:1 (v/v/v) and 15 cm diameter pots were filled with 1 kg of soil. Water was poured into each pot to wet the soil before transferring to an autoclave chamber for sterilization at 138 kpa for 20 minutes. The treatments consisted of Control, TE + AC + GF, AC + GF, TE + AC, TE + GF, AC, GF, TE + AC +GF + MI, TE, AC + MI + GF, TE + AC + MI, TE + MI + GF, AC +MI, GF+ MI, TE + MI and MI. The experiment was laid out and kept on green house benches in complete randomized block design with five replications.

Preparation of nematode inoculum

Root sample of nematode-infected brinjal plants were collected from the infested field in and around Aligarh district situated in a Northern Province, Uttar Pradesh, India. Females of root-knot nematode were collected and identified as *Meloidogyne incognita* using perineal pattern technique. Pure culture of *M. incognita* was maintained after introducing single egg mass in microplots of greenhouse in the premises of the department on *Solanum melongena* L. Large number of egg masses of root-knot nematode was handpicked, using sterilized forceps from heavily infected eggplant roots. These egg masses were washed in distilled water to minimize the remains of undesired soil particles and then placed in 10 cm diameter 15 mesh sieves containing crossed layers of tissue paper and placed in Petri dishes containing water just deep enough to contact the egg masses. The newly hatched juveniles were collected every 24 hour and fresh water added to the Petri dishes. The number of second stage juveniles (J_2) of *M. incognita* in the water suspension was adjusted so that each milliliter contained 200 nematodes. Ten ml of this suspension containing 2000 freshly hatched juveniles (J_2) were added to each pot.

Inoculation technique of nematode

For inoculation of root-knot nematode, soil around the roots of the plant was carefully removed aside without damaging the roots. The inoculum suspension of 2000 J_2 was poured around the roots of tomato and the soil was placed on the roots again. The control treatment received distilled water of a volume equal to that of the nematode inoculum suspension. Five replicates of each treatment were taken. Necessary watering and weeding were done whenever required throughout the period of study.

AM fungus inoculum

Soil samples collected from tomato fields located at Agra road, Aligarh a known vegetable growing area for the isolation of fungal spores of *Glomus fasciculatum*. The mycorrhizal fungi were isolated and identified using synoptic key as described by Gerdeman and Nicolson (1963). The inocula were multiplied on tomato plants grown in sandy loam soil mixed with washed river sand and farmyard manure in the ratio 3:2:1 (v/v/v) respectively. Later on, the spores were isolated by sieving from soil maintained on tomato. Suspension obtained from sieve was poured on filter paper and spores of AM fungus were picked up with a fine camel hair brush under a dissecting microscope. Spores of *G. fasciculatum* were placed in 10 ml of distilled water and poured around the roots of tomato plant grown in the pots at the rate of 1200 spores/plant by the layering methods (Menge, 1977). Uninoculated plants served as control.

Application of organic matter (*Tagetes erecta*)

Leaves of *Tagetes erecta* were handpicked and rinsed with distilled water several times. After washing the leaves these were chopped with sharp sterile knife and incorporated in soil at the rate of 20g/kg soil (1% N per kg soil). These pots were immediately watered to prepare the compost of *T. erecta* leaves after decomposition process for 15 days.

Preparation of *Azotobacter chroococcum* inoculum and inoculation technique

Charcoal based commercial culture of nitrogen fixing bacteria *Azotobacter chroococcum* were obtained from Government owned Quarsi farm, Aligarh, India for the maintenance of this bioinoculant. Pure culture of *Azotobacter chroococcum* was centrifuged washed twice in sterile water and suspended in 0.015% phosphate buffer at pH 7.0. To inoculate bacterial culture, 100g culture was mixed separately in 1000 ml water which means 10 ml suspension contained 1g of *Azotobacter*. One gram culture of *Azotobacter chroococcum* had 4.6×10^6 CFU were added around each seedling as and when required.

Termination of experiment

Plant-growth parameters

The plants of each treatment were taken out from the pots after 70 days of nematode inoculations, and soil particles adhering to roots were removed by washing gently under tap water and properly labeled. The plants were cut with a knife just above the root emergence zone. Length of the roots and shoot was taken by measuring tape while fresh weight of the plants determined with the help of a physical balance. Then excess water was removed by using blotting paper before weighing shoots and roots separately. For dry weight

determination, shoots and roots were kept in labeled envelopes and dried in a hot air oven running at 60 °C for 24-48 h before weighing.

Pollen grains viability

The pollen grains were collected from the tomato flower, they were stained in acetocarmine solution and observed under microscope according to the method of Brown (1949). Pollen grains which took up the stain and are circular in outline are considered as viable/fertile, whereas those which remain unstained and become irregular are sterile. The pollen viability was calculated as;

$$\% \text{ Pollen viability} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains}} \times 100$$

Mycorrhization

The external as well as internal mycorrhizations were observed in percentage after harvesting the experiment. The root system were taken for colonization of roots by AM fungi along with the consideration of infection and subsequently the number of chlamydospores determined according to the clearing and staining methods as described by Phillips and Haymen (1970).

Estimation of Chlorophyll content

The green leaves were taken for chlorophyll estimation according to the method of Hiscox and Israelstam (1979). One hundred mg of tomato leaf pieces were kept in a vial containing 7ml of a chemical dimethyl sulfoxide (DMSO) and the chlorophyll content was extracted into the fluid by incubating for 60min. Then this extract was placed in a graduated tube and assayed immediately. A sample of 3ml chlorophyll extract was transferred in cuvette and the optical density (O.D.) value at 645 and 663nm were read in Spectronic-1001 Spectrophotometer against DMSO blank.

Extraction of nematode population

A 250g sub-sample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting method followed by Baermann's funnel technique to determine the final nematode population in soil (Southey 1986). Nematode suspensions were collected after 24 h, and the number of nematodes was counted in five aliquots of 1 ml of suspension from each sample. The means of the five counts used to calculate the population of nematodes per kg soil. To estimate the number of juveniles, eggs and females inside the roots, 1g sub-sample of root was macerated for 30-40 seconds in a waring blender and counts were made from the suspension thus obtained. The total number of nematodes present in the roots was calculated by multiplying the number of nematodes present in 1g of root by the total weight of root.

Number of root galls and egg-masses

On termination of the experiment, roots of harvested plants were washed under the tap water and examined for the presence of galls. Number of galls per root system was counted. For the assessment of egg masses, plant roots were immersed for 15 minutes in 0.015% Phloxine B, which specifically stains the gelatinous matrix of nematode egg masses

bright red and the egg masses per root system were counted according to Taylor & Sasser (1978).

Fecundity

The number of eggs per egg mass is known as fecundity. The eggs were extracted from the root of each treatment separately by Chlorox method (Hussey & Barker, 1973). Roots from each treatment were cut into 1-2 cm pieces. One gram of root pieces were shaken vigorously in 200 ml of 1.0% NaOCl solution for 1 to 4 min. Then NaOCl solution with root pieces was passed through a 200 mesh sieve, rested over a 500 mesh sieve to collect free eggs. After this 500 mesh sieve with eggs was placed under a stream of cold water to remove residual NaOCl (rinsed for several minutes). The rinsed roots pieces were put under water to remove additional eggs and collected by sieving process. The number of eggs was then counted in counting dish under stereoscopic microscope. The total number of eggs was calculated by multiplying the number with the fresh weight of the root in the treatment.

Statistical analysis

The entire data collected during this study was statistically analyzed by SPSS 17. Means were compared by using DMRT (Duncan's Multiple Range Test).

Results

The results presented in Table 1 and 2 clearly explained that the individual as well as combined inoculations of organic matter like *Tagetes erecta* and biofertilizers such as *Azotobacter chroococcum* and *Glomus fasciculatum* in various treatments brought about significant improvement in growth parameters, and agronomic parameters besides reduced the disease incidence caused by the *M. incognita* in terms of root-knot development and nematode multiplication.

Plant growth parameters

Soil application of *T. erecta* significantly improved the plant length, fresh weight as well as dry weights, number of fruits of flowers/plant, and per cent pollen fertility as compared to untreated control where it was recorded as 61.77 cm, 47.99 gm, 15.93 gm, 8.3 fruits/plant, 10.7 flowers/plant and 62.50%, respectively. Maximum improvement in plant growth parameters was observed in those plants which received the combined treatments of *Azotobacter chroococcum* in combination with *Tagetus erecta* and *Glomus fasciculatum*. In combined treatment, the maximum length was recorded as 113.36 cm which showed 83.51% improvement as compared to untreated control, however, 32.57% reduction was noted due to infection of *Meloidogyne incognita.* Similarly the fresh weight showed 88% improvement in combined treatment and 48.63% reduction observed in the treatment of nematode alone. Similarly dry weight of plant greatly improved as 93.53% in combined treatment of *Azotobacter*, *Glomus* and *Tagetes erecta* as compared to untreated control where it was only 30.83gm. Root-knot nematode inoculated plants, significant reduction in dry weight as 59.69%. Moreover, number of fruits and flowers also increased in combined treatment where the improvement was 224.09% and greatest reduction due to root knot nematode as 49.39% in number of fruits. Similar improvement was also noted in per cent pollen fertility. Maximum pollen fertility (98.03%) was recorded in plants receiving combined treatment of

Azotobacter chroococcum + G. fasciculatum + Tagetus erecta exhibited improvement as 56.84%, subsequently the reduction due to root-knot nematode was recorded as 10.88% over control. As far as individual application of bio-inoculants is concerned, *Azotobacter chroococcum* was found to be most effective and increased the plant length significantly followed by *G. fasciculatum* and *Tagetus erecta.* Further, biological nitrogen fixer exhibited more improvement in plant growth parameters when inoculated individually as well as concomitantly (Table 1).

Table 1. Interactive effects of *Meloidogyne incognita, Glomus fasciculatum* and *Azotobacter chroococcum* in *Tagetes erecta* amended soil on plant length, fresh and dry weights of tomato*

Treatments	Plant length (cm)	Plant fresh weight (g)	Plant dry weight (g)
Control	61.77j	47.99j	15.93j
TE+AC+GF	113.36a	90.30a	30.83a
AC+GF	107.02ab	85.09ab	29.40ab
TE+AC	102.13bc	83.46b	28.55abc
TE+GF	99.40cd	81.01bc	27.98bcd
AC	95.62cde	76.45cd	26.56cde
GF	92.85def	74.58de	26.02cdef
TE+AC+GF+MI	90.83efg	74.16de	25.72defg
TE	79.33hi	65.48gh	22.86hi
AC+MI+ GF	88.64efg	72.11def	25.01efgh
TE+AC+MI	85.65fgh	71.00defg	24.43efgh
TE+ MI+GF	83.28gh	68.97efgh	23.60fgh
AC +MI	80.61hi	66.51fgh	22.99ghi
GF+MI	78.97hi	64.88h	22.45hi
TE+ MI	73.20i	58.71i	20.58i
MI	41.65k	24.65k	6.42k

*Each value is an average of five replicates. Data labeled by the same letters did not differ significantly at $p<0.05$.

TE= *Tagetes erecta*, GF= *Glomus fasciculatum*, AC= *Azotobacter chroococcum* and MI= *Meloidogyne incognita*

Chlorophyll content

The results further explained that organic matters as well as bio-inoculants in different combinations have significantly improved the chlorophyll content of tomato. Maximum improvement (91.49%) was observed in those treatments that received the combined application of *Azotobacter chroococcum + G. fasciculatum + Tagetus erecta* followed by the plants that received the treatments of *Azotobacter chroococcum + G. fasciculatum.* Least amount of chlorophyll was measured from those plants that were treated

with *Tagetus erecta*. Maximum reduction (54.37%) was observed in chlorophyll content of tomato when plants inoculated with *Meloidogyne incognita* alone (Table 2).

Table 2. Interactive effects of *Meloidogyne incognita, Glomus fasciculatum* and *Azotobacter chroococcum* in *Tagetes erecta* amended soil on number of flowers, fruits, pollen fertility, fresh weight of fruits and chlorophyll content of tomato*

Treatments	No. of flowers/ plant	No. of fruits/ plant	Pollen fertility (%)	Fresh weight of fruits (g)	Chlorophyll content (mg/g)
Control	10.7k	8.3j	62.50i	135.3k	4.563l
TE+AC+GF	27.8a	26.9a	98.03a	280.5a	8.738a
AC+GF	26.9ab	26.3a	96.24a	266.0b	8.419b
TE+AC	26.4abc	25.4ab	93.29ab	250.1c	8.293bc
TE+GF	25.6bcd	24.5bc	89.78bc	239.4c	8.073c
AC	24.8cde	23.9bc	87.33bc	226.7d	7.744d
GF	24.1de	23.5cd	84.14cd	215.6d	7.604de
TE+AC+GF+MI	23.0ef	22.2de	83.74cd	196.3e	7.435ef
TE	17.7i	17.0gh	68.50ghi	160.0hi	6.380ij
AC+MI+ GF	21.9fg	21.2ef	79.97de	188.2ef	7.236fg
TE+AC+MI	21.5fg	20.8ef	76.56ef	177.8fg	7.060gh
TE+ MI+GF	20.6gh	19.7f	73.61fg	170.4gh	6.856h
AC +MI	18.9hi	18.0g	70.45fgh	162.5hi	6.542i
GF+MI	17.2ij	16.4h	67.48ghi	154.7ij	6.263jk
TE+ MI	15.5j	14.7i	64.37hi	144.6jk	6.031k
MI	5.3e	4.2k	55.70j	47.5l	2.082m

*Each value is an average of five replicates. Data labeled by the same letters did not differ significantly at $p<0.05$.

TE= *Tagetes erecta*, GF= *Glomus fasciculatum*, AC= *Azotobacter chroococcum* and MI= *Meloidogyne incognita*

Nematode related parameters

Soil Population

The results presented in Table 3 clearly revealed that the population of root-knot nematode *M. incognita* were obtained at varying extent when plants were treated with *Azotobacter chroococcum*, *Glomus fasciculatum* and *Tagetus erecta* either individually or in combinations. More population of nematodes were recovered from the plants that were inoculated with *M. incognita* alone as compared to the plants that were treated with the *Tagetus erecta* and *M. incognita* while their minimum population was obtained when plants were treated with *Azotobacter chroococcum* with *Tagetus erecta* and *Glomus fasciculatum* followed by the combination of *Azotobacter chroococcum*, *Glomus fasciculatum* with *M. incognita*. (Table 3).

Table 3. Interactive effects of *Meloidogyne incognita, Glomus fasciculatum* and *Azotobacter chroococcum* in *Tagetes erecta* amended soil on mycorrhization, root-knot development and nematode multiplication of tomato*

Treatments	No. of root gall/ plant	No. egg masses / plant	Nematode population	External colonization (%)	Internal colonization (%)	No. of chlamydospores (1kg soil)
Control	-	-	-	-	-	
TE+AC+GF	-	-	-	75.40a	89.77a	2074.32b
AC+GF	-	-	-	68.40b	83.93b	1845.50c
TE+AC	-	-	-	-	-	-
TE+GF	-	-	-	62.56c	77.35c	2256.10a
AC	-	-	-	-	-	-
GF	-	-	-	56.50d	73.40c	1865.50c
TE+AC+GF+MI	05.5h	11.0h	0472h	52.74d	67.35d	1782.44cd
TE	-	-	-	-	-	-
AC+MI+ GF	15.6g	27.8g	1024g	46.16e	63.72d	1692.20de
TE+AC+MI	21.4f	42.6f	3162f	-	-	-
TE+ MI+GF	30.2e	56.5e	4314e	40.79f	58.60e	1575.20e
AC +MI	40.2d	71.0d	5406d	-	-	-
GF+MI	47.4c	89.5c	6967c	37.94f	55.17e	1407.27f
TE+ MI	55.3b	101.4b	7732b	-	-	-
MI	78.7a	123.3a	8956a	-	-	-

*Each value is an average of five replicates. Data labeled by the same letters did not differ significantly at $p<0.05$.

TE= *Tagetes erecta*, GF= *Glomus fasciculatum*, AC= *Azotobacter chroococcum* and MI= *Meloidogyne incognita*

Number of root galls per plant

Significant reduction in the number of root galls per plant was observed in all the tomato plants treated with the various combinations of organic matter like *T. erecta* and bio-inoculants such as *A. chroococcum* and *G. fasciculatum.* The maximum reduction in root-galling was observed in plants that treated with the *A. chroococcum, G. fasciculatum* and *T. erecta* followed by the combined treatment of *A. chroococcum* and *G. fasciculatum* as compared to the treatment of *M. incognita* alone. Minimum reduction in root galling were

noticed with the treatment of *T. erecta* with *M. incognita* as compared the plants treated with *M. incognita* alone where they were counted as 78.7 (Table 3).

Number of egg masses per plant

Soil application of organic matters like *T. erecta* and bio-inoculants like *A. chroococcum* and *G. fasciculatum* brought about significant reduction in the number of egg masses of the tomato root system. Maximum reduction was observed with the application of *T. erecta* in combination with *A. chroococcum* and *G. fasciculatum* with the nematode followed by the combined treatment of *A. chroococcum* and *G. fasciculatum* with the *M. incognita.* Minimum reduction was observed with the treatment of *T. erecta* with *M. incognita* alone where they were counted as 123.3. Maximum number of egg masses produced in those plants treated with *M. incognita* alone, where they were determined as-123.3.

Mycorrhization parameters

In case of mycorrhization, maximum number of chlamydospores were recovered from the roots of tomato plants in soil amended with *T. erecta* + *G. fasciculatum* where they were counted as 2256.10, followed by *T. erecta* + *A. chroococcum* + *G. fasciculatum* (2074.32), *Glomus fasciculatum* alone (1865.50), *A. chroococcum* + *G. fasciculatum* (1845.50), *T. erecta* + *A. chroococcum* + *G. fasciculatum* + *M. incognita* (1782.44), *A. chroococcum* + *G. fasciculatum* + *Meloidogyne incognita* (1692.20), *T. erecta*+ *M. incognita* + *G. fasciculatum* (1575.20) and *G. fasciculatum* + *M. incognita* (1407.27). These two bio-inoculants minimize the adverse effects of *M. incognita* when inoculated in various combinations. Similar results were also recorded in percent internal and external colonizations, respectively (Table 3).

Discussion

The results presented in Table 1 and 2 clearly revealed that the application of organic matter like *T. erecta* and bio-inoculants such as *A. chroococcum,* and *G. fasciculatum* significantly increased the plant growth parameters such as plant length, fresh as well as dry weights, number of flowers, number of fruits, percent pollen fertility and chlorophyll content, and subsequently disease incidence caused by the nematodes in terms of their population, number of root-galls and number of egg masses per plant in the root of tomato. Mycorrhyzation parameters were also found on the tomato roots seem to be influenced due to the application of organic matter as well as *A. chroococcum*.

The application of organic and bio-organics 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 *et al.* (2000). Usefulness of AM fungi for increased plant growth has been reported by many workers (Jothi & Sundarababu, 2002; Shreenivasa *et al*., 2007). Present investigation reveals that the combined application of *A. chroococcum* + *G. fasciculatum* and *T. erecta* concomitantly decreased the disease intensity in the nematode infested plants. The detrimental effects of theses bio-inoculants against the root-knot nematodes have been reported by various researchers (Krishnaveni &

Subramanian, 2004; Khalil, 2009; Khalil 2012; Safiuddin, 2014). Talavera (2001) observed that colonization of tomato roots with *Glomus mosseae* compensated the reduction of plant growth caused by *M. incognita* infection while population density were reduced by 85% when tomato were colonized by mycorrhizae. Ploeg (1999) studied the effects of preplanted marigolds on tomato root galling and multiplication of *M. incognita, M. arenaria, M. javanica* and *M. hapla*. Marigold cultivars of *Tagetes erecta, T. patula* and *Tagetes* hybrids all significantly reduced the galling and numbers of second stage juveniles in the nematode infested plants. Efficacy of these treatments has been tested for the management of root-knot nematode and on the growth parameters. All of them help to improved growth of plants as compared to the control plants. These treatments also effectively reduced the nematode multiplication. A similar result was observed by Tiyagi (2012). They observed plants when inoculated with *A. chroococcum* and *A. brasilense* in various combinations. A number of mechanisms can be proposed to explain the antagonistic effect of AMF on nematode parasitism and their activities. These may be either physical or physiological in nature. *Glomus fasciculatum* can alter physiology of the roots including the exudates responsible for chemotactic attraction of nematodes (Mac Guidwin, 1985). Yield losses normally caused by nematodes are mitigated by enhancing the uptake of phosphorus and other nutrients due to AM fungi leading to improvement of plant vigour and growth (Hussey and Roncadori, 1982). Nematode development and reproduction might be affected in mycorrhizal treated plants due to non- availability of food and unfavorable condition (Saleh & Sikora, 1984). Physiological changes in roots inoculated with AM fungi may result in development of resistance due to production of antagonistic substances (Suresh, 1985). Mycorrhizal fungi may alter the microbial activities in the rhizosphere affecting the survival of nematodes (Timothy & Robert, 1992). The above mechanisms may operate singly or in combination to make mycorrhizal plants resistant against invasion of plant pathogens. Similarly, inoculation of *A. chroococcum* as bio-inoculants significantly improved the plant growth parameters and nutrient status, consequently reduced the population of *M. incognita* and its pathogenicity. Khan, (2012) observed the effect of *A. chroococcum* in combination with organic matter on the population of plant-parasitic nematodes on chilli plants. They found significant reduction in the multiplication of phytonematodes as well as number of root-galls infested with *M. incognita*. In the present study the individual as well as combined inoculations of both the bio-inoculants such as *G. fasciculatum* and *A. chroococcum* significantly improved the growth characteristics of tomato plants. The increase in growth parameters attributed to improve nutrient uptake and favorable environment prevalent in the rhizosphere that resulted to release the auxins, gibberellins and cytokinins under the influence of bio-inoculants based treatments. Similar studied have also been observed by Devidas and Rehberger (1992) & Verma (2000). Sharma et al. (2005) also found increased biological nitrogen fixation with the application of *A. chroococcum* resulted higher vegetative growth. Chlorophyll content was found increased in those plants inoculated with *G. fasciculatum* and *A. chroococcum* individually as well as concomitantly. The improvement in these parameters might be due to soil physical properties like porosity, water holding capacity and tendency of soil towards

neutral pH which in turn increased the microbial biomass pool in the soil rhizosphere. The increase in chlorophyll content due to increased N uptake by the addition of organic components which increased photosynthetic efficiency, translocation of nutrients and other metabolites towards the formation of fruits. The tomato plants also showed significant improvement in growth parameters with the addition of *G. fasciculatum*. The inoculation of AM fungi increased root colonization in the form of higher number of chlamydospores which accounted for increased available phosphorus content of the soil rhizosphere. In various treatments with addition of bioinoculants, mycorrhizal infection in combination with *A. chroococcum* stimulated biological nitrogen fixation thus increased the soil fertility. Increased availability of N due to application of bio-inoculants might be attributed towards greater multiplication of microbes which converted organically bound nitrogen to inorganic form (Bhardwaj & Omanwar, 1994) which were early observed by plant roots (Tiyagi, 2012). The research reveal that utilization of organics like *Tagetes erecta* and bioinoculants such as *Glomus fasciculatum* and *Azotobacter chroococcum* to meet the nutrient requirement of crop would be inevitable practice in the years to come for sustainable agriculture and maintain good soil health. These make it suitable for organic farming systems to keep nematode population always under the economic threshold and improved plant performance, if used regularly.

Conclusion

The inference drawn from the present study explained that soil application of organic matter like *Tagetes erecta* and bioinoculants such as *Azotobacter chroococcum* and *Glomus fasciculatum* has a significant and profound effect on growth, yield and agronomic parameters of tomato when inoculated individually as well as concomitantly besides influencing the multiplication and root-knot development of *Meloidogyne incognita* and mycorrhization. Integrated nutrient and pest management are essential to sustain or enhanced soil fertility and plant nutrient supply to maintain crop production by optimizing the benefits from all available nutrient sources. The combined effect of organic matter and bioinoculant like *A. chroococcum* and *G. fasciculatum* caused maximum improvement in growth and related parameters and reduced the activity of *M. incognita* bioinoculant are considered as low cost and eco-friendly components of organic farming which continuously supplying the nutrients as well as nematodes suppressing chemicals. Their presence in large numbers as indicative of better soil health and enhanced nutrient availability to plants and subsequently to reduce the disease incidence. Continuous application of all the components enhanced soil organic carbon and nutrient contents in plants which leads to sustaining crop production.

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