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Curative effect of mycorrhizal fungus and neemex against *Meloidogyne incognita* and on plant growth

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Abstract
A pot experiment was conducted to evaluate the curative effect of mycorrhizal fungi and neemex against *Meloidogyne incognita* and on plant growth and nutrient uptake by eggplant. Neemex significantly reduced the number of galls, egg masses, females, J_2/ root system and J_2/ cm^3 soil as compared to MF because delaying application of MF. Growth and nutrient uptake was recorded higher as compared to control.

Keywords: *Meloidogyne incognita*, mycorrhizal fungi, neemex, eggplant

Introduction
Root-knot nematodes (RKNs) are serious and economically important pest of many cultivated crops around the world. They are particularly damaging to vegetables in tropical and subtropical countries [16]. Among the various bio-control agents, arbuscular mycorrhizal fungi (AMFs) are being widely used in nursery seedling, as it enhances nutrient availability. The role of AMF in reducing harmful effect of root infection by many parasitic nematodes in crops is well recognized [15, 11].
Juveniles of *M. incognita* exposed to neem product show immobility and mortality. Neem products, particularly seed and neem cake are widely used as a soil amendment against nematodes. Neem products are absorbed by the plants and when nematodes come in contact for feeding they inhibit or delay their development [9, 12]. Present studies were designed to evaluate the curative effect of Mycorrhizal fungi and neem product against *Meloidogyne incognita* and plant growth [6].

Materials and Methods
Eggplant (*Solanum melongena* L.) seeds were surface sterilized and then rinsed thoroughly with distilled water. The seeds were placed on autoclaved filter papers soaked with sterile distilled water and incubated at 25 °C for 24 h. Four seedlings were planted in each pot and were thinned to one per pot after the emergence of the second leaf. Present studies were carried out during 2013-2014.
Mycorrhizal fungi were propagated on maize host plants in a growth chamber at 30 °C/18 °C with a 16 h/8 h light/dark regime and 50–75% relative humidity. The plants were harvested after growth for 4 months. Soils containing spores, external mycelium and roots of maize were used as inoculum.
Soil and sand mixture (1 kg) and 200 g mycorrhizal inoculum and 5 g neemex alone and in combination were placed in each pot 20 days before inoculation of nematodes. The inoculum was placed about 2 cm under the soil surface. Untreated pots received an equivalent amount of soil to provide a similar soil microbial community [3].
Meloidogyne incognita was isolated from heavily infected tomato roots and was multiplied on susceptible eggplant cultivar using single egg mass inoculation. The number of galls on the roots was recorded. Egg masses were stained in phloxine B for 20 min, rinsed in sterilized distilled water and then counted under a stereomicroscope, according to Hussey and Barker [8]. The number of female nematodes within the roots was counted using a dissecting microscope and staining by the NaOCl-acid fuchsin technique [2].

The plant samples of all the treatments were estimated for major nutrients uptake by eggplant after drying completely at 70 °C. Nitrogen (N) content was determined by Kjeldhal methods [13]. Phosphorus (P) estimation was done by spectrophotometer and potassium (K) by flame photometer after the digestion of plant samples [17].

Statistical analysis

To determine the significance level, complete randomized design (CRD) was applied, while to determine the significant differences, least significant difference (LSD) was applied as described by Steel *et al* [19].

Results

Effect of mycorrhizal fungi and neemex against *M. incognita.*

The maximum number of galls was recorded in *M. incognita* treatment (337) while minimum galls were observed in roots of plants treated with neemex (53) followed by application of MF and neemex together (62) and treated with MF alone (113) at $p<0.05$. In this experiment neemex show efficient results because mycorrhizal fungi were not effective as curative treatment because it takes time to colonization. Lower number of egg masses and females were recorded in neemex application (90 and 137) followed by MF and neemex together (47 and 87) and MF alone (90 and 137) as compared to control (Table 1).

Table 1: Curative effect of mycorrhizal fungi and neemex against *M. incognita* infectivity parameters

Treatment	No of galls	No of Egg masses	No of Females	J_2/root system	J_2/ 100 cm3 soil
Healthy	0 E	0 E	0 E	00 E	0 E
M. incognita	337 A	302 A	359 A	13284 A	1813 A
MF + Healthy	0 E	0 E	0 E	0 E	0 E
Neemex + Healthy	0 E	0 E	0 E	0 E	0 E
MF + Neemex + Healthy	0 E	0 E	0 E	0 E	0 E
MF + *M. incognita*	113 B	90 B	137 B	5063 B	690 B
Neemex + *M. incognita*	53 D	42 D	80 D	2640 D	360 D
MF + Neemex + *M. incognita*	62 C	47 C	87 C	2956 C	402 C
LSD at P<0.05t	4.1002	3.3910	3.8520	3.1885	3.0900

Means sharing similar letters are statistically non-significant at $P<0.05$

J_2/root system and J_2/ 100 cm^3 soil were estimated lower in neemex treatment (2640, 360), followed by MF and neemex together (2956, 402) and MF alone (5063, 690) as compared to check (13284, 1813).

Root weight was significantly higher in *M. incognita* inoculated plants (8.4 g) followed by MF and neemex together (7.1 g) and MF alone in the presence of *M. incognita* (6.8 g) as compared to other treatments at $p<0.05$ (Table 2).

Table 2: Curative effect of mycorrhizal fungi and neemex on plant growth parameters

Treatment	Root wt (g)	Root length (cm)	Shoot wt (g)	Shoot length (cm)
Healthy	5.6 BC	19.1300 AB	15.0900 CD	24.7800 B
M. incognita	8.4 A	10.0200 D	8.3900 E	16.6300 C
MF + Healthy	6.2 C	20.3700 A	21.1000 A	29.1300 A
Neemex + Healthy	4.2 BC	17.1000 BC	13.5700 D	19.9500 C
MF + Neemex + Healthy	7.1 AB	19.3400 AB	17.8700 BC	27.0400 AB
MF + *M. incognita*	6.8 AB	19.6700 AB	19.9100 AB	27.6000 AB
Neemex + *M. incognita*	4.5 C	15.7800 C	13.6900 D	19.0300 C
MF + Neemex + *M. incognita*	6.1 BC	19.5100 AB	17.7300 BC	26.4900 AB
LSD at P<0.05	2.2173	3.2381	3.1947	3.6775

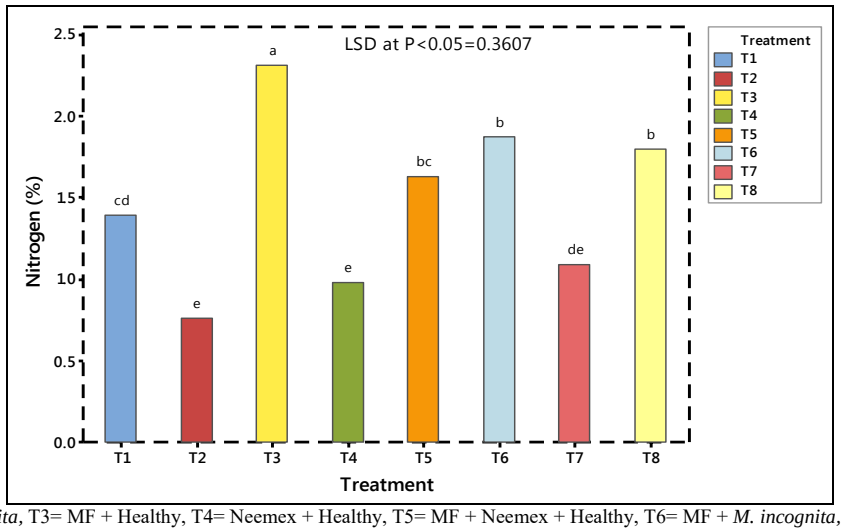
Means sharing similar letters are statistically non-significant at $P<0.05$

Significantly higher root length was recorded in plants treated with MF in absence of *M. incognita* (20.37 cm) as compared to control. Un-inoculated healthy, combined application of MF and neemex without *M. incognita*, MF alone and MF with neemex in the presence of *M. incognita* were statistically significant over control but non-significant with each other at $p<0.05$. Shoot weight and shoot length was significantly higher in mycorrhizal plants (21.10 g, 29.13 cm respectively) as compared to other treatments.

Curative effect of mycorrhizal fungi and neemex on nutrient uptake by eggplant

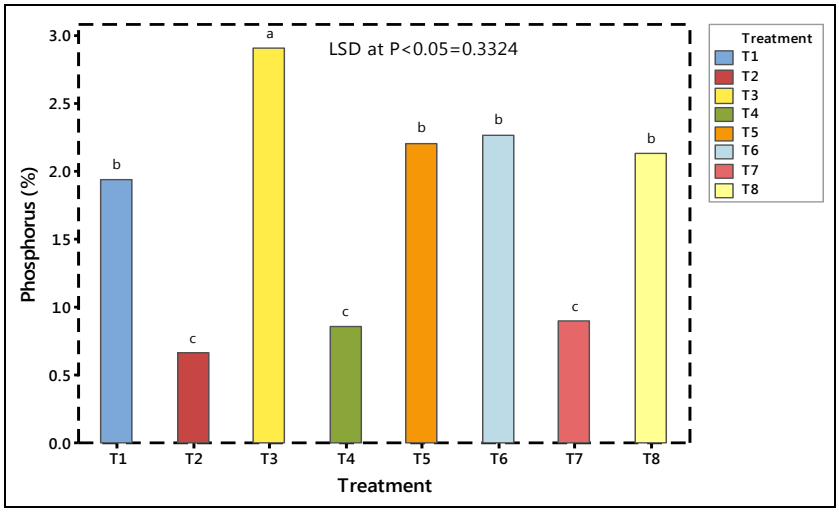
Nutrient uptake was increased by the application of mycorrhizal fungi but curative application was not effective as protective application because mycorrhizal fungi take time to colonize the roots. Amount of nitrogen was significantly higher in MF treatment without inoculation of *M. incognita* (2.31%) as compared to un-inoculated healthy (1.39%) and check (0.76%) at $p<0.05$. Nitrogen percentage in MF alone (1.87%) and in combination with neemex in the presence of *M. incognita* (1.8%) was also significantly higher than un-inoculated healthy and inoculated with *M. incognita* but non-significant with each other at $p<0.05$ (Fig 1)

Maximum phosphorus contents were recorded in plant tissues treated with MF alone (2.91%) followed by MF in the presence of *M. incognita* (2.27%), MF and neemex together in the absence (2.21%), and in the presence of *M. incognita* (2.13%) and un-inoculated healthy (1.94%) as compared to check (0.66%), neemex treated without (0.86%) and with *M. incognita* (0.9%) at $p<0.05$ (Fig 2).



T1= Healthy, T2= *M. incognita*, T3= MF + Healthy, T4= Neemex + Healthy, T5= MF + Neemex + Healthy, T6= MF + *M. incognita*, T7= Neemex + *M. incognita* and T8= MF + Neemex + *M. incognita*

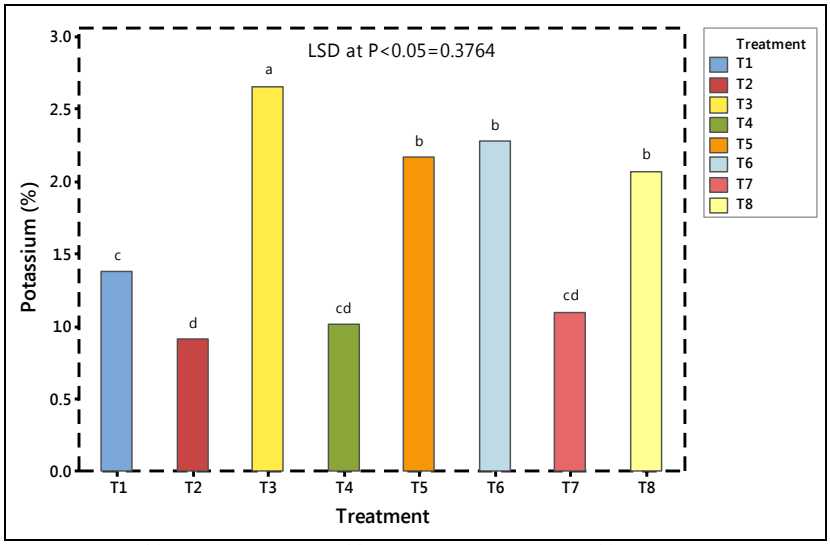
Fig 1: Curative effect of mycorrhizal fungi and neemex on nitrogen uptake



T1= Healthy, T2= *M. incognita*, T3= MF + Healthy, T4= Neemex + Healthy, T5= MF + Neemex + Healthy, T6= MF + *M. incognita*, T7= Neemex + *M. incognita* and T8= MF + Neemex + *M. incognita*

Fig 2: Curative effect of mycorrhizal fungi and neemex on phosphorus uptake

Potassium uptake was recorded significantly higher in MF treated plant tissues (2.66%) as compared to control (Fig 3).



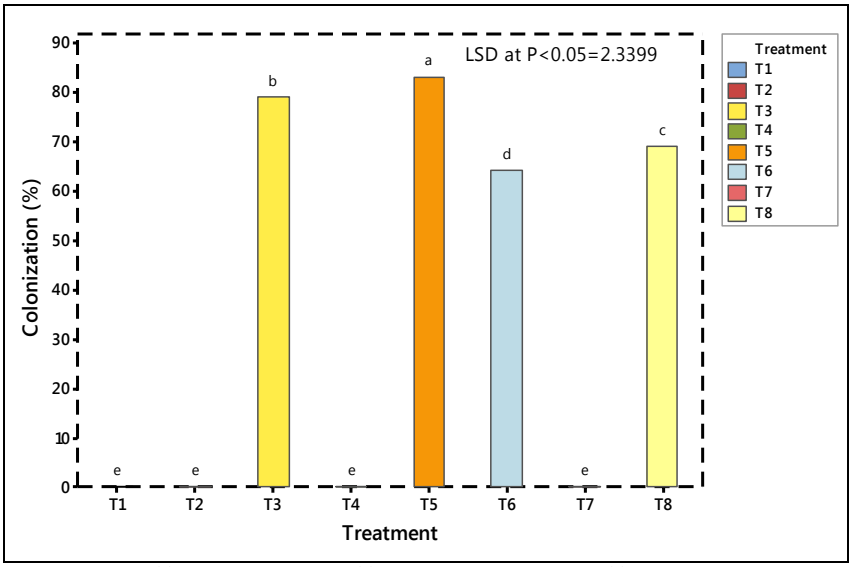
T1= Healthy, T2= *M. incognita*, T3= MF + Healthy, T4= Neemex + Healthy, T5= MF + Neemex + Healthy, T6= MF + *M. incognita*, T7= Neemex + *M. incognita* and T8= MF + Neemex + *M. incognita*

Fig 3: Curative effect of mycorrhizal fungi and neemex on potassium uptake

Amount of potassium in combined application of MF and neemex (2.17%), MF alone with *M. incognita* (2.28%) and MF and neemex together in the presence of *M. incognita* (2.07%) was also significantly higher than control but non-significant with each other at $p<0.05$. Potassium contents in un-inoculated healthy plant (1.38%) were significantly higher as compared to check (0.91%) which was inoculated by *M. incognita* only.

Curative effect of mycorrhizal fungi and neemex on colonization and spore abundance

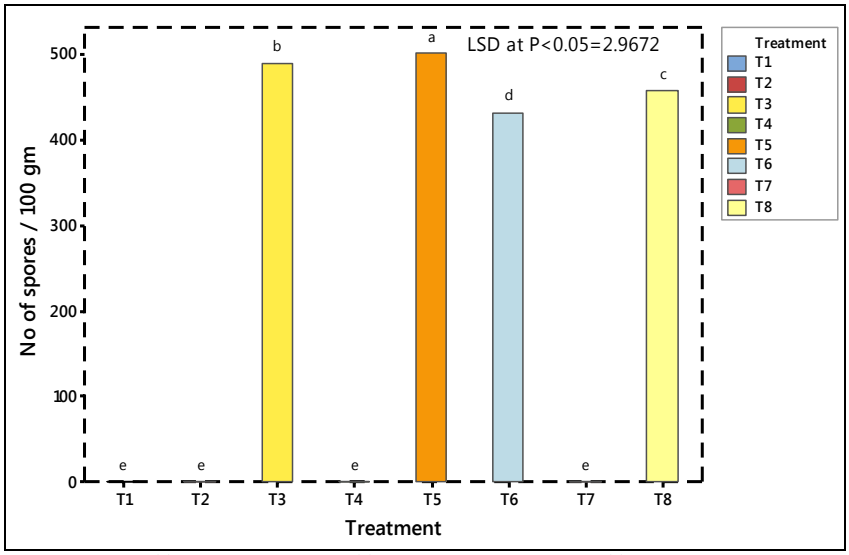
Root colonization was not observed in treatments where mycorrhizal fungi were not inoculated. Root colonization was recorded significantly higher in combined application of MF and neemex (83%) followed by MF alone (79%), MF and neemex together with *M. incognita* (69%) and MF alone (64%) at $p<0.05$ (Fig 4).



T1= Healthy, T2= *M. incognita,* T3= MF + Healthy, T4= Neemex + Healthy, T5= MF + Neemex + Healthy, T6= MF + *M. incognita,* T7= Neemex + *M. incognita* and T8= MF + Neemex + *M. incognita*

Fig 4: Curative effect of mycorrhizal fungi and neemex on colonization percentage

Number of mycorrhizal spores was significantly maximum in MF with neemex treatment without *M. incognita* (501) followed by MF alone on healthy (489), MF and neemex together with *M. incognita* (457) and MF alone in the presence of *M. incognita* (431.2) at $p<0.05$ (Fig 5).



T1= Healthy, T2= *M. incognita,* T3= MF + Healthy, T4= Neemex + Healthy, T5= MF + Neemex + Healthy, T6= MF + *M. incognita,* T7= Neemex + *M. incognita* and T8= MF + Neemex + *M. incognita*

Fig 5: Curative effect of mycorrhizal fungi and neemex on no. of spores

Mycorrhizal spores were not observed in treatments without inoculation of mycorrhizal fungi.

Discussion

Application of MF prior to nematode inoculation suppressed the nematodes to a greater degree than the application of MF after the nematodes. This might be related to the time interval necessary for the establishment of the mycorrhiza in the root cortex. It is well known that mycorrhiza establish in the root cortex in about 15 to 20 days, e.g. in tomato [18] and cotton [14].

The presence of mycorrhiza in the host can reduce attraction to roots and juvenile penetration and retard nematode development after penetration. Mycorrhizal spore population and mycorrhizal colonization were found to be lower when nematodes were inoculated before MF.

Delaying application of MF after the nematode inoculation resulted in increased root galling and nematode population in the soil and suppression of spore population and colonization by MF. Changes in MF colonization and spore population in the presence of nematodes have been observed previously [4] and were attributed mainly to competition between MF and *Meloidogyne* spp. for feeding sites and carbon substrates from host photosynthesis [7]. After *Meloidogyne* spp. invade the vascular cylinder, the root tissue around developing females usually proliferates to form knots or galls, which disrupt vessels and thus reduce the transport of water and nutrients through the altered roots [10]. This may interfere with the translocation of metabolites required by mycorrhizal fungi. The disease syndrome initiated by root knot nematodes very often includes the invasion of affected root tissue by secondary pathogens, which cause decay of root tissues [5] including the cortical tissue colonized by MF. Mycorrhizal development and growth of mycorrhizal and non-mycorrhizal plants were reported to be reduced in the presence of *M. arenaria* in grapes [1].

Conclusion

In conclusion, neemex effectively reduce the infection of *M. incognita* than mycorrhizal fungus. Mycorrhizae need some time to colonize the roots and make symbionts with the host plant. However, plant growth and nutrient uptake was observed more in mycorrhizal treated plants than all other treatments.

Acknowledgment

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