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Optimisation of nitrogen fertiliser level for maximum colonisation of mycorrhizae on roots of coriander plants

F.S. ALI, G. ZAYED, O.A. SAAD & E. ABDUL-MOHSEN
Department of Agricultural Microbiology, Faculty of Agriculture, Minia University, Minia, Egypt

Abstract This work aimed to determine the optimum nitrogen fertilisation rate that lead to maximum benefits of vesicular-arbuscular mycorrhizae (VAM) inoculated to the root zone of coriander plants grown in clay or sandy soil. The lowest values of roots colonisation with mycorrhizal fungi, nitrogen uptake, phosphorous uptake, shoot dry weights, root dry weights and seed yield were obtained with the non-treated control plants in the clay and sandy soils. The inoculation with VAM-mycorrhizae without nitrogen fertilisation substantially increased these parameters as compared with the non treated control plants. In the non-fertilised control pots, mycorrhizal colonisation was higher at clay soil than at sandy soil. These pot experiments have already shown the existence of an optimum N fertiliser rate of 37.5-75 mg $(NH_4)\,NO_3$ kg^{-1} soil for maximum colonisation of mycorrhizae on coriander roots in clay soil and 75-112.5 mg $(NH_4)\,NO_3$ kg^{-1} soil for sandy soil. Increasing the nitrogen fertiliser level to more than these levels led to negative effects on the mycorrhizal colonisation and consequently on mycorrhizal benefits. The soil properties have been shown to modify the effects of fertilisation on mycorrhizal colonisation. Therefore, the benefit of N-fertilisation should be evaluated keeping secondary effects caused by changed mycorrhizal formations in mind.

Key words: Mycorrhizal, nitrogen, soil

Introduction

Beneficial microbial inoculants (biofertilisers) are presently attracting more attention in the context of sustainable agriculture. This is a consequence of the need to solve health and environmental problems caused by the excessive use of agrochemical compounds in traditional agriculture.

The use of vesicular-arbuscular mycorrhizae (VAM) as bio-fertilisers becomes an important factor to increase the availability of phosphorus and micronutrients to correct their deficiencies in many soils (Ghazi *et al.*, 2007). Mycorrhizal colonisation on plant roots is known to increase biomass production (Backhaus *et al.*, 1986). It can cause a two-fold increase in growth due to substantially higher P uptake (Jones *et al.*, 1991). Jones *et al.*, 1991. Fluxes of carbon and phosphorus between symbionts in willow ectomycorrhizas and their changes with time. *New Phytol.* 119, pp. 99–106. Full Text via CrossRef | View Record in Scopus | Cited By in Scopus (29)Positive effects of colonisation by mycorrhizal fungi on growth of different plant species are improved nutrition (Barea *et al.*, 1991) and enhanced protection against root pathogen fungi (Hooker *et al.*, 1994). Together with plant roots, hyphae of mycorrhizal fungi are important factors for the aggregation of soils (Bethlenfalvay *et al.*, 1997). The low rooting intensity of many plant species suggests that growth rates of plants may strongly respond to management practices that increase the populations of indigenous mycorrhizal fungi, in particular where these are highly effective but low in number (Haselwandter & Bowen, 1996).

Conflicting results have been reported on effect of nitrogen (N) fertilisers on number and activity of mycorrhizae in soils cultivated with different plants. On one hand significant positive effect on mycorrhizal growth and activity was obtained after fertilisation with high doses of nitrogen (Ghone & Abdel-Razik, 1999). After inoculation of maize with VAM in the presence of high dose of N fertiliser, the introduced mycorrhizal preparations were able to colonize actively the rhizosphere of maize (Saleh *et al.*, 1998). Jha *et al*. (2005) indicated that the application of mycorrhizae with full N dose significantly increased bulb yield of onion and quality. Among the mycorrhizae, *Endogne duseii* with N fertiliser resulted in the greatest number of leaves per plants, plant height and yield per plot.

On the other hand N-fertilisation has been found to cause changes both in mycorrhizal formation on fine roots and production of sporocarps of mycorrhizal fungi (Termorshuizen, 1993). Fertilisation can reduce the positive effect that mycorrhizae can have on plant growth (Bethlenfalvay *et al.*, 1997). Reduced mycorrhizal colonisation caused by N-fertilisation might reduce the benefit of N-fertilisation on biomass production of different plants.

Nitrogen fertilisers (ammonium or nitrate) positively affected dry weight of plants inoculated with *Paxillus involutus* and *Suillus bovines.* However the shoots and roots were less branched at higher N concentration and colonisation by *Pisolithus tinctorius* was higher at lower N concentration (Tingey *et al.*, 2005; Schalamuk *et al.*, 2006; Lestinigi *et al.*, 2007).

Since nitrogen availability is important for the rapid establishment of mycorrhizal growth, low N applications may improve growth and stimulate mycorrhizal growth until the grown mycorrhizae can provide adequate N for plant development. Fertiliser rates exceeding those that stimulate the mycorrhizal growth may, however, have a detrimental effect on mycorrhizal activity. The aim of this work was to determine the optimum nitrogen fertiliser level that lead to maximum benefits of VAM inoculated to the root zone of Coriander plants. Coriander (*Coriandrum sativum*) is a common condiment grown in many countries for its fresh herb and dry seeds. Coriander seeds are used for flavoring food products, pharmaceutics and perfumes. The essential oil of coriander stimulates the flow of digestive secretion and is useful in the treatment of intestinal disorders.

Materials and methods

Soils. Clay soil collected from the farm of Minia University and sandy soil collected from Shosha Agriculture Research Center, Minia University were used in this study. Soil samples were air dried ground to pass through a 2 mm sieve and stored for analyses. The most important properties of the used soils are shown in Table 1 as determined by the Service Laboratory for Soil, Plant and Water analysis.

Host plants. Seeds of the medicinal and aromatic plants Coriander (*Coriandrum sativum*) were supplied by the Horticulture Department of, Faculty of Agriculture, Minia University. Coriander seeds were sterilized in a 10% commercial bleach (3.5% sodium hypochlorite as active agent) solution for 30 min, then washed five times with sterile water to remove any trace of chemical that could interfere with seed germination. Surface sterilized seeds were sown in plastic pots each filled with 8.0 kg air dry soil. The watering regime consisted of weighing each pot once a day and adding water to the weight corresponding to 100% of water holding capacity.

Nitrogen fertilisation. Seedlings were thinned to four plants per pot after 30 days. Plants were fertilised with ammonium nitrate 33.5% at rates of 0.0, 37.5, 75.0, 112.5 and 150.0 mg kg^{-1} soil. Each amount of ammonium nitrate was divided into three equal doses to be added at three interval times i.e. 30, 45, 60 days after sowing. Pots were left without fertilisation for comparison.

Soil inoculation. The mycorrhizal inoculum used was a mixture of stock cultures *Glomus mosseae* and *Glamus fasiculatum* isolate. Mycorrhizal inoculation was carried out by adding 100 g per pot of a mycorrhizal inoculum from our stock culture collection. These thoroughly mixed rhizosphere samples contained spores, hyphae and mycorrhizal root fragments (80% root colonisation) were maintained in polyethylene bags at 4 °C for 3-6 months before being applied to the corresponding pots. Pots were left without inoculation for comparison.

Table 1. The main physical and chemical properties of the used soils.

Character	Clay soil	Sandy soil
Sand %	26.44	92.4
Silt %	31.17	4.04
Clay %	42.39	3.16
Texture grade	Clay loam	Sandy
pH	8.01	8.3
Total N %	0.09	0.02
Available P(ppm)	12.62	2.41
Organic matter %	1.69	0.06

Determination of the degree of mycorrhizal colonisation. Samples of coriander roots were mixed to yield approximately 1 g fresh weight, cleansed with tap water and stored in 70% alcohol prior to the examination of VAM colonisation. After storage, root samples were cut into approximately 1 cm segments, boiled in 15% KOH for 40 min, stained with aniline blue and fixed in 40% lactic acid (Philips & Hayman, 1970). The stained roots were suspended in few drops of lactic acid on glass slides and examined microscopically. The percentage of infection in 100 pieces were calculated as follows:

$$\% \text{ root colonisation} = \frac{\text{Number of VAM positive pieces x 100}}{\text{Number of pieces scored}}$$

Measurements. Plants were harvested after 120 days of growth. The plants were thoroughly hand-washed to remove soil particles from the roots. Shoot heights, root lengths and Seed yield were recorded. Shoot and root weights were recorded after drying at 70 °C to constant weight. N and P concentrations were measured after Kjeldahl digestion or molybdenum blue procedures, respectively (Lachica *et al.*, 1973).

Results and discussion

Growth of coriander. Data presented in Tables 2 and 3 indicate that the lowest values of shoot heights, root lengths, shoot dry weights and root dry weights were observed in un-fertilised un-inoculated plants. Addition of N fertiliser led to high increases in these vegetative growth parameters in the two tested soils.

The results showed that inoculation with VA-mycorrhizae with 0 N fertilisation was more benefit to the growing plants than fertilisation with 37.5 mg $(NH_4) NO_3$ kg^{-1} soil concerning its effect on the above mentioned growth parameters. The superior effect of VAM could be explained in the light of the great role played via mycorrhizal mycelia, which were found to be more effective than plant root hairs in absorbing nutrient elements including phosphorus, nitrogen, potassium, calcium and some micro-nutrients (Corkidi *et al.*, 2002). Mycorrhizae were found to increase rate of photosynthesis and phytohormones such as gibberellins, auxins and cytokinins, which significantly promote plant growth (Bhoopander *et al.*, 2005).

The results showed that increasing N fertiliser rate up to 150 mg $(NH_4) NO_3$ kg^{-1} soil increased shoot heights, root lengths, shoot dry weights and root dry weights of coriander plants. Aside the environmental problems of using excess chemical N fertiliser, nitrogen has an essential role in increasing plant meristimatic activities and constitution of numerous organic compounds (amino acids, protein and nucleic acid) needed in the formation of protoplasm (Kamal & Bilgrami, 2007).

Table 2. Effect of N fertilisation and mycorrhizal inoculation on coriander shoot heights and root lengths.

	Shoot height (cm)		Root length (cm)	
	Clay soil	Sandy soil	Clay soil	Sandy soil
Un-fertilised un-inoculated	22	16	8	6
Fertilised with 37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	28	20	12	9
Fertilised with 75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	34	28	16	11
Fertilised with 112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	37	32	19	14
Fertilised with 150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	42	40	23	18
Inoculated with VAM + 0.0 $(NH_4)\ NO_3$	31	24	16	12
Inoculated with VAM + 37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	38	31	22	14
Inoculated with VAM + 75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	51	32	29	17
Inoculated with VAM + 112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	41	39	25	21
Inoculated with VAM + 150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	31	27	19	17
LSD 5%	7.02	1.8	1.76	1.79
LSD 1%	9.61	2.48	2.42	2.45

Table 3. Effect of N fertilisation and mycorrhizal inoculation on dry weight of coriander shoots and roots.

	Shoot weight (g pot^{-1})		Root weight (g pot^{-1})	
	Clay soil	Sandy soil	Clay soil	Sandy soil
Un-fertilised un-inoculated	7	5	3	2
37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	9	6	9	6
75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	13	8	13	9
112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	15	11	15	11
150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	21	17	18	13
Inoculated with VAM + 0.0 $(NH_4)\ NO_3$	19	13	12	8
Inoculated with VAM + 37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	24	20	16	11
Inoculated with VAM + 75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	27	23	18	14
Inoculated with VAM + 112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	22	18	21	15
Inoculated with VAM + 150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	19	13	16	11
LSD 5%	1.81	6.35	1.45	1..89
LSD 1%	2.48	8.69	1.99	2.59

The results showed that inoculation of coriander plants together with nitrogen fertilisation with rates up to only 75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil for the clay soil and 112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil for the sandy soil gave the highest values of shoot heights, root lengths, shoot dry weights and root dry weights. Addition of N fertiliser at rates more than 75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil in clay soil or 112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil in sandy soil had negative effects on these parameters (Tables 2 and 3). Vázquez *et al.* (2002) reported that the effects of VAM on plant growth were greatest when no nitrogen was added to the soil. Increasing nitrogen concentrations reduced mycorrhizal benefits and nodule formation of *Medicago sativa* plants was drastically inhibited in mycorrhizal plants by the addition of 4 and 8 mmol N kg^{-1} soil. Coriander shoot and root weights were increased by mycorrhizal inoculation, particularly under the lowest nitrogen concentrations. Additions of N at high concentration negatively affected mycorrhizal growth on plant roots since shoot and root biomass decreased with increasing N concentrations (Table 3).

The results in Tables 2 and 3 showed that the effects of N-fertilisation on growth of coriander depend not only on the absolute quantity of N-application but also on soil type. Same amount of N fertiliser gave better results in the clay soil than sandy soil due to the high N and organic content in the clay soil compared to the sandy soil. Similar observation was reported by Alriksson *et al*. (1997).

N and P uptake. The results in Table 4 show that the lowest values of nitrogen and P uptake by coriander plants were obtained with the control un-fertilised un-inoculated treatment in the two soils. Application of N fertiliser with increasing rates up to 150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil led to substantial increase in values of nitrogen and P uptake. The N uptake increased by about 500% in clay soil and about 1000% in sandy soil while the P uptake increased by about 200% in both of the two soils (Table 4).

These results may be due to the positive effect of nitrogen on plant growth and consequently on the efficiency of the root in absorbing various nutrients. The present results are in agreement with those reported by Vazquez *et al.* (2002), and Lestingi *et al.* (2007)

The results of inoculation with VA- mycorrhizae however without any N fertilisation were very close to the results of N fertilisation with 150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil concerning N and P uptake by coriander plants. Furthermore inoculation with VA-mycorrhizae together with only 37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil gave much better results than fertilisation with the maximum dose of N fertiliser (150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil) without inoculation (Table 4). These results suggest that inoculation of coriander plants grown in clay or sandy soil with VAM may save 75% of the chemical N fertiliser regarding its effect on N and P uptake. The fact that fungal hyphae facilitate the uptake of N and P ions by mycorrhizal roots (Johansen *et al.*, 1994) may explain the increase of N-uptake and P-uptake by plants inoculated with VAM especially at the low N fertiliser levels. However, this is probably the result of an indirect effect of mycorrhizae on N ion uptake since the highest N content in mycorrhizal plants was observed at 0, 37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil and 75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil. These results are also in agreement with those obtained by Saleh *et al.* (1998). Vázquez *et al.* (2002) reported that although the P content was higher in P-fertilised, non-mycorrhizal *Medicago sativa* plants than in mycorrhizal ones VAM colonisation significantly improved P uptake.

Mycorrhizal colonisation and seed yield of coriander. Similar to the previous results the lowest values of coriander seed yield and root colonisation with VAM recorded in Table 5 were obtained with the control un-fertilised un-inoculated treatment in the two soils. Data indicated that the percentage of mycorrhizal colonisation as a result of N fertilisation increased from 14% at 0 nitrogen fertilisation to 37% at the maximum dose of N fertilisation (150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil) in clay soil. In sandy soil the percentage of mycorrhizal colonisation as a result of N fertilisation increased from 10% at 0 nitrogen fertilisation to 26% at the maximum dose of N fertilisation (150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil). Inoculation of coriander plants with VAM without N fertilisation substantially increased

Table 4. Effect of N fertilisation and mycorrhizal inoculation on N uptake and P uptake by coriander.

	N-uptake (mg/4 plants)		P-uptake (mg/4 plants)	
	Clay soil	Sandy soil	Clay soil	Sandy soil
Un-fertilized un-inoculated	111	43	100	88
37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	157	100	123	100
75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	245	169	187	130
112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	312	245	200	160
150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	590	389	234	190
Inoculated with VAM + 0.0 $(NH_4)\ NO_3$	400	290	200	165
Inoculated with VAM + 37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	689	500	267	192
Inoculated with VAM + 75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	987	567	440	220
Inoculated with VAM + 112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	900	680	400	297
Inoculated with VAM + 150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	734	400	312	197
LSD 5%	2.96	2.29	563.3	3.32
LSD 1%	4.06	3.13	771.7	4.55

Table 5. Effect of N fertilisation and mycorrhizal inoculation on mycorrhizal colonisation and seed yield of coriander.

	Colonization (%)		Seed yield (g/pot)	
	Clay soil	Sandy soil	Clay soil	Sandy soil
Un-fertilized un-inoculated	14	10	07	04
37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	16	13	10	08
75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	22	19	14	12
112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	32	22	18	14
150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	37	26	20	17
Inoculated with VAM + 0.0 $(NH_4)\ NO_3$	32	30	17	12
Inoculated with VAM + 37.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	65	55	18	15
Inoculated with VAM + 75 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	80	60	27	22
Inoculated with VAM + 112.5 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	34	75	16	20
Inoculated with VAM + 150 mg $(NH_4)\ NO_3\ kg^{-1}$ soil	08	43	11	10
LSD 5%	1.93	1.76	1.32	1.64
LSD 1%	2.65	2.42	1.81	2.24

the percentage of mycorrhizal colonisation and seed yield compared to the results on control un-fertilised un-inoculated treatment. With the two soils the fertiliser effect on mycorrhizal colonisation significantly ($P<0.05$) varied depending on the fertiliser rate. However the maximum percentage of mycorrhizal colonisation was achieved in plants inoculated with VAM and received low N fertiliser rates. Nevertheless, the highest added N concentration substantially reduced plant response to VAM colonisation. The highest N concentration (150 mg $(NH_4) NO_3$ kg^{-1} soil) severely reduced VAM colonisation and hence reduced the mycorrhizal; benefits concerning seed yield (Table 5). These results showed that similar amounts of fertiliser caused different responses in mycorrhizal formation by coriander plants under different site conditions. The sandy soil with a low soil N content (Table 1) the mycorrhizal colonisation reached its maximum value at fertilisation rate of 75-112.5 mg $(NH_4) NO_3$ kg^{-1} soil, however at clay soil with relatively high soil N only 37.5- 75 mg $(NH_4) NO_3$ kg^{-1} soil was needed to reach the maximum root colonisation. At the maximum N fertiliser dose (150 mg $(NH_4) NO_3$ kg^{-1} soil) the mycorrhizal colonisation was inhibited in both of the two tested soils. It is known that mycorrhizal effect on plant growth in general depends on the soil conditions especially soil texture, the organic matter content and the pH level (Baar, 1995). We suggest that the soil N content could be used as a parameter for the prediction of fertilisation effects on mycorrhizal responses in coriander plants. This hypothesis should be tested with similar boundary conditions. We claim that these pot experiments have already shown the existence of an optimum N fertiliser rate for the maximum colonisation of mycorrhizae on coriander roots. In general these results agree with those obtained by Grandcourt et al (2004); Gaber & Sarge (1998); Ghone & Abdel-Razik (1999) and Tingey *et al.* (2005).

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