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ARBUSCULAR MYCORRHIZAL FUNGAL-ASSOCIATED BACTERIA AFFECT MYCORRHIZAL COLONIZATION, ESSENTIAL OIL AND PLANT GROWTH OF *Murraya koenigii* L.

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ABSTRACT

Murraya koenigii L. (family: Rutaceae), commonly referred to as curry leaf, is a highly valued plant due to its aroma and medicinal features. Two dominant AM species *Glomus mosseae* and *Acaulospora laevis* were isolated from the rhizospheric soil of *M. koenigii.* A pot experiment was performed to verify the interactive potential of *G. mosseae* and *A. laevis* alone or in combination with *Pseudomonas fluorescens* on *M. koenigii*. Various morphological and biochemical parameters were measured after 120 days. Overall results suggest that all co-inoculation treatments showed beneficial effects on all the growth, physiological and oil content. The overall results demonstrate that the co-inoculation of bioinoculants, like *P. fluorescens* with AM fungi, promotes higher AM colonization and spore number enhancing the nutrient acquisition, especially phosphorus (P), improving the rhizospheric condition of soil.

Key words: *Glomus mosseae*, *Acaulospora laevis*, *M. koenigii*, growth, oil content

INTRODUCTION

AM fungi are essential propagules of rhizosphere microbial communities in ecosystems as well as they are used as biofertilizers. Horticultural crops inoculated with AMF are becoming common practice due to the reduction of indigenous AMF populations in the soil. The recognition of the status of AM association and its variation in horticultural crop is therefore, of particular concern to improve the growth and pharmaceutical substances.

Plant roots of vascular plants are colonized by AMF, belongs to the group Glomeromycota play an important role in improve soil fertility. AM fungi form a symbiotic association with roots of plants and supply nutrients especially Phosphorus and moisture to the host plant [Ferrol et al. 2004]. These AM fungi colonize root tissues and form an extensive network of extra radical mycelia thereby providing a direct contact between plant root and soil particle [Smith and Read 2010]. AM fungi are important component of the ecosystem and have been reported to induce biomass in plants [Scheublin et al. 2004]. Along with AMF, many rhizospheric bacteria like *Pseudomonas fluorescens*, produce substances that stimulate plant growth or could also stimulate mycorrhizal colonization [Vosatka and Gryndler 1999]. These potential microbial inoculants have been investigated in agricultural and horticultural systems where they must be compatible with the AM population [Barea and Jeffries 1995]. Apart from this, mycorrhizal colonization can also affect the mycorrhizal population through changes in

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root exudation through fungal exudates [Linderman 1992]. Qualitative and quantitative changes in microbial populations in the soil rhizosphere due to AM inoculation have been estimated through the study of soil enzyme activity. Acidic and alkaline phosphatase enzymatic activity mediated by AM colonization releases the inorganic phosphorus from organically bound phosphorus back into soil [Vazquez et al. 2000].

Although AM fungi are soil microbes that have been used as biofertilizers yet their potential to improve the nutritional value of spices has largely been overlooked [Smith et al. 2009]. AM fungi are probably the most under spread plant symbionts and are found with the roots of 90 percent of plant species. This includes numerous horticultural crops, ornamental and herbal plants like basil, thyme and rosemary [Newman and Reddell 1987]. This is consistent for spices as well. The plant requirement for phosphorus (P) for its growth and soil fertility status probably regulates the proliferation of AMF hyphae in soil. Studies on *Mentha arvensis* have shown a relation of percent AM colonization with increased growth, oil content and phosphorus uptake [Freitas et al. 2004]. Similar results were observed in *Foeniculum vulgare* and basil by Kapoor et al. [2004] and Sadaghiani et al. [2010] respectively.

Recently many studies have reported the significance of AM fungi for growth and quality of spices through different effects on the nutrient supply and changes in plant physiology and morphology of the host [Baum et al. 2015]. Another promising method and a useful approach for sustainable horticulture would be the co-inoculation with AMF and other microorganisms such as PGPB (Plant Growth Promoting Bacteria) [Colla et al. 2015]. The synergistic role of AMF inoculants and their compatible interaction that take place with other bacteria are important in understanding which factors limit the performance of these bioinoculants. These PGPB either decrease or eliminate deleterious effect of pathogenic organism or may provide the host plant with synthesize minerals such as phosphorus which enhances plant growth or synthesize some enzymes that modulate the plant growth and development [Gray and Smith 2005] AM fungi improve plant growth profoundly through increase uptake of water and mineral nutrients in the host plant in exchange for photosynthetically fixed carbon. The use of AM fungi in increasing plant growth and yield of horticultural crops has gained momentum in recent years because of higher cost and pernicious effects of heavy doses of chemical fertilizers. The extraradical hyphae of AM Fungi increase the absorptive surface area of the root system for greater nutrient acquisition through activation and excretion of various enzymes by AMF roots and hyphae [Smith and Read 1997].

Inoculation with phosphate solubilizing microorganism may help to solublize native soil phosphate as well as phosphate from the rock phosphate. Soluble phosphate released by the activity of phosphate solubilizing microorganisms can be actively taken up by mycorrhizal roots [Singh and Kapoor 1999].

M. koenigii commonly called as curry leaf or karri patta belonging to the family Rutaceae is a highly valued plant for aroma and medicinal value. Leaves of the plant have been used for the treatment of various diseases like rheumatism, inflammation, itching, blood disorders etc as plant have been reported to have rich quality of some vitamins, terpenoids, mineral contents like calcium, iron zinc which have great potential for antimicrobial properties making it attractive for clinical research. Plants have been reported to have anti-oxidative, antibacterial antihypertensive and cytotoxic activity [Saini et al. 2013]. Thus, there is a need to encourage the use of medicinal plants as potential source of drugs. The recognition of the status of mycorrhizal association and its variation in spices is therefore of particular concern to improve the quality of pharmaceutical substances.

The purpose of present investigation was to examine the influence of microbial inoculants such as *Pseudomonas fluorescens* on colonization of *Murraya* roots by different AM fungi and secondly the effect of both AM fungi and *Pseudomonas fluorescens* in different combinations on growth parameters of *Murraya* under polyhouse conditions. Keeping the above in view the above objectives of this study was to evaluate the effectiveness of AM inoculum alone or in combination on the physiology and growth of *Murraya koenigii*.

MATERIALS AND METHODS

Mass multiplication of Bioinoculants. The two dominant AM species *Glomus mosseae* and *Acaulo-*

spora laevis were used in this study. These two dominant AM fungi were isolated from the rhizospheric soil of *M. koenigii* grown in botanical garden of Botany Department, Kurukshetra University and Kurukshetra by using Wet Sieving and Decanting Technique of Gerdemann and Nicolson [1963] and identified using the key of Schenck and Perez [1990]. The starter inoculums for each species were propagated by "Funnel Technique" of Menge and Timmer [1982]. The AM species were propagated with maize as host for three months. *Pseudomonas fluorescens* (MTCC NO. 103) was procured from Institute of Microbial Technology (IMTECH), Chandigarh, India and cultured in a Nutrient Broth Medium incubated at 32°C for 48 hours to obtain a concentration of 1×10^9 colony forming units (cfu) mL^{-1}.

Experimental site and setup. The experiment was set up in the poly house, Botany Department, Kurukshetra University, Kurukshetra, Haryana during June to September, 2016. The soil used in the experiment consisted of Clay-3.78%, Silt-20.8%, Sand-74.5%, EC-0.26 dS/m, Organic Carbon-0.40%, total N = 0.042%, P = 7.30 kg^{-1} acre, K = 88 kg^{-1} acre and S = 14.80 ppm. The experiment was laid out in a randomized complete block design with five replicates per treatment. Top soil (0–30 cm) was collected and sieved through 2 mm sieve, mixed with sand, soil in the ratio 1 : 3 and sterilized in autoclave for 20 minutes at 121°C at 15 psi for two consecutive days. Earthen pots (25 × 25 cm) were selected having capacity of 2 kg soil. For AM treatment 10% (w/w) of soil of the selected AM inoculum having 845 AM spores approximately and 200 g of soil having chopped AM colonized pieces of trapped host barley with the infection level of about 90–95% were added. Before sowing, the roots of the seedlings were dipped in the nutrient broth having *P. fluorescens.* The experiment was set up with the following treatments:

Control (C)
Glomus mosseae (G)
Acaulospora laevis (A)
Pseudomonas fluorescens (P)
Glomus mosseae + *Acaulospora laevis* (G + A)
Acaulospora laevis + *Pseudomonas fluorescens* (A + P)
Glomus mosseae + *Pseudomonas fluorescens* (G + P)
Glomus mosseae + *Acaulospora laevis* + *Pseudomonas fluorescens* (G + A + P)

In the control set no inoculum was added. Plants were watered regularly to maintain humidity. Hoagland Nutrient solution without phosphorus (100 ml/pot) was added to each pot after regular interval of 20 days. Each treatment was replicated five times. After 120 days five plants from each treatment were analyzed for the various parameters.

Harvest and analysis

After 120 days, the plants were uprooted and analyzed for various morphological and physiological parameters. Shoot length and root length (cm), root and shoot fresh weight (g), leaf area in (cm^2), dry weight (g) were analyzed. Percentage root colonization was assessed by Rapid Clearing and Staining Technique of Phillips and Hayman [1970]. AM spores were isolated by Wet Sieving and Decanting Technique of Gerdemann and Nicolson [1963]. Alkaline and acidic phosphatase of fresh roots was estimated by Tabatabai and Bremner [1969]. Shoot and root phosphorus were determined by vanado-molybdo-phosphoric acid yellow colour method [Jackson 1973]. Chlorophyll *a*, chlorophyll *b*, carotenoids and total chlorophyll were estimated from the fresh samples taken from each replicate by Arnon [1949].

Essential oil

After harvesting, the leaves and shoots of *Murraya* were taken and essential oil was extracted by hydro-distillation of one-liter water in a Clevenger apparatus for eight hours [Rasooli and Mirmostafa 2003]. The essential oil was collected and stored in glass vials.

Statistical analysis

The experimental data was analyzed using analysis of variance (ANOVA), followed by post hoc test using the Statistical package for Social Sciences (ver. 16). Means were then ranked at ≤ 0.05 level of significance using Duncan's Multiple Range Test (DMRT) for comparison.

Results

Plant height. In Murraya plant height differed significantly among the different treatments, with maxi-

mum height recorded in GAP (35.66 cm) and lowest in *Pseudomonas fluorescens* at 20.90 cm. The height of the plants of the seedlings was in the order of GAP > AG > A > AP > G > GP > C. Data presented in Table 1 showed that mycorrhiza inoculated seedlings were about two times taller than the control ones. By analyzing the percentage root colonization and AM spore number as the important parameter of AM fungi alone or in combination with *Pseudomonas fluorescens* showed maximum colonization established in the treated plants in comparison to control after 120 days of growth (Tab. 2).

Biomass (fresh and dry root and shoot weight). The effect of different bioinoculants on shoot and root (fresh and dry weight) varied significantly (Tab. 1). In case of seedlings grown in different treatments, shoot dry weight (SDW) ranged among the mycorrhizal alone or in combination from 22.58 to 4.40 gm pot^{-1}. Similar trends for SDW was observed for root dry weight (RDW) with the order GAP > AG > A > AP > A > G > GP respectively.

Root colonization and spore number. The root systems were widely colonized by different bioinoculants alone or in combination but it was absent in *Pseudomonas fluorescens*. Root colonization was also different among the different treatments, with the maximum percentage of colonization by GAP followed by AG > A > G > AP > GP. The AM spore numbers of mycorrhizae present in the rhizosphere of the seedlings were analyzed after harvest and maximum spore number were 70 spores/ 20 gm of soil followed by GAP > AG > A > G > AP > GP > P > C (Tab. 2).

Plant nutrient status. Phosphorus is generally poorly available in the soil, due to insoluble iron, calcium and aluminium phosphate or fixation to clay surfaces and is required by plants in large amount for better biomass and yield. Regarding the plant phosphorus content, control seedling contained less phosphorus than the bioinoculants treated seedlings. The highest phosphorus was measured in GAP with the lowest in *P. fluorescens* treated plants. It was found that different treatments exhibited a varying response to uptake of

Table 1. Interaction of AM fungi and *P. fluorescens* on different growth parameters of *Murraya koenigii* L. after 120 days

Treatments	Plant height (cm)	Fresh shoot weight (gm)	Dry shoot weight (gm)	Fresh root weight (gm)	Dry root weight (gm)	Leaf area/plant	Shoot girth
Control	15^{h}	35.10^{f}	4.40^{h}	1.95^{g}	1.79^{f}	2.5^{e}	1.40^{e}
A. laevis	31^{g}	71.60^{c}	18.57^{d}	13.73^{b}	7.40^{c}	3.0^{d}	4.80^{b}
G. mosseae	28.60^{f}	62.40^{d}	16.66^{e}	10.45^{c}	7.14^{c}	3.5^{c}	3.10^{cd}
P. fluorescens	20.90^{e}	39.80^{e}	8.93^{g}	6.67^{d}	2.62^{f}	2.45^{e}	3.90^{bc}
A + G	32.00^{d}	89.20^{a}	25.88^{a}	15.88^{a}	9.42^{b}	4.52^{b}	4.20^{b}
G + P	23.98^{c}	77.0^{b}	13.02^{f}	4.52^{f}	3.74^{e}	3^{d}	3.24^{cd}
A + P	29.80^{b}	62.02^{d}	21.31^{c}	6.07^{e}	5.04^{d}	3.5^{c}	2.80^{d}
G + A + P	35.66^{a}	76.04^{b}	22.58^{b}	15.32^{a}	13.34^{a}	5^{a}	5.70^{a}
LSD ($P \leq 0.05$)	0.765	1.155	0.735	0.26	0.641	0.511	0.866
ANOVA ($F_{7,16}$)	640.436	2.169	801.138	2.190	1.538	942.47	19.181

G – *Glomus mosseae*, A – *Acaulospora laevis*, P – *Pseudomonas fluorescens*
± standard deviation,
*The mean difference is significant at 0.5 levels. Mean value followed by different alphabet/s within a column do not differ significantly over one other at $P < 0.05$ (Duncan's multiple range test)

Table 2. Effect of AM fungi and *P. fluorescens* on mycorrhization and oil content (%) of *Murraya koenigii* L. after 90 days

Treatments	AMF root colonization (%)	AM spore number per 20 gm of soil	Essential oil content (%)
Control	0^{e}	0^{e}	0.250^{a}
A. laevis	40^{b}	51^{b}	0.69^{a}
G. mosseae	30^{c}	42^{c}	0.56^{a}
P. fluorescens	0^{e}	0^{e}	0.39^{a}
A + G	45^{b}	58^{b}	0.83^{a}
G + P	22^{d}	30^{d}	0.48^{a}
A + P	27^{cd}	40^{c}	0.61^{a}
G + A + P	57^{a}	70^{a}	1.03^{a}
LSD ($P \leq 0.05$)	8.077	0.109	6.69
ANOVA ($F_{7,16}$)	82.560	82.560	0.910

G – *Glomus mosseae*, A – *Acaulospora laevis*, P – *Pseudomonas fluorescens*
± standard deviation
*The mean difference is significant at 0.5 levels. Mean value followed by different alphabet/s within a column do not differ significantly over one other at $P < 0.05$ (Duncan's multiple range test)

Table 3. Effect of AM fungi and *P. fluorescens* on different physiological parameters of *Murraya koenigii* L. after 120 days

Treatments	Chlorophyll content (mg/gm FW)			Carotenoid (mg/gm FW)	Phosphatase activity (IU/G FW)		Phosphorus content (P)	
	chlorophyll *a*	chlorophyll *b*	total chlorophyll		acidic phosphatase	alkaline phosphatase	shoot P	root P
Control	0.752^{f}	0.263^{c}	1.015^{d}	0.0018^{b}	0.370^{c}	0.306^{e}	0.0030^{d}	0.120^{d}
A. laevis	1.182^{d}	0.249^{c}	1.431^{c}	0.0023^{ab}	0.599^{ab}	1.262^{ab}	0.892^{a}	0.899^{a}
G. mosseae	1.419^{bc}	0.475^{ab}	1.894^{b}	0.0031^{ab}	0.596^{ab}	1.021^{c}	0.714^{b}	0.812^{a}
P. fluorescens	1.328^{c}	0.480^{ab}	1.808^{b}	0.0030^{ab}	0.500^{ab}	0.822^{d}	0.435^{c}	0.472^{c}
A + G	1.529^{ab}	0.547^{a}	2.076^{a}	0.0031^{ab}	0.610^{ab}	1.322^{a}	0.608^{b}	0.514^{bc}
G + P	1.10^{d}	0.400^{b}	1.501^{c}	0.0026^{ab}	0.534^{ab}	0.867^{d}	0.582^{b}	0.591^{bc}
A + P	0.942^{e}	0.429^{b}	1.371^{c}	0.0024^{ab}	0.558^{ab}	1.193^{b}	0.599^{b}	0.617^{b}
G + A + P	1.619^{a}	0.551^{a}	2.170^{a}	0.0033^{a}	0.641^{a}	1.354^{a}	0.891^{a}	0.938^{a}
LSD ($P \leq 0.05$)	0.111	0.0856	0.155	0.005	0.0912	0.097	0.138	0.127
ANOVA ($F_{7,16}$)	58.705	15.217	1.411	1.411	7.357	108.865	34.941	36.744

G – *Glomus mosseae*, A – *Acaulospora laevis*, P – *Pseudomonas fluorescens*
± Standard deviation
*The mean difference is significant at 0.5 levels. Mean value followed by different alphabet/s within a column do not differ significantly over one other at $P < 0.05$ (Duncan's multiple range test)

phosphorus. Although mycorrhizal inoculation either alone or in combination with other bioinoculants exerted better effect on *Murraya* phosphorus concentration, but the degree varies (Tab. 3). The control plant was having less phosphorus content in comparison to treated plants. Similarly acid and alkaline phosphatase activity was found maximum in mycorrhizal treated plant in comparison to control. GAP showed maximum increase in root and shoot acidic and alkaline phosphatase activity followed by AG > A > G > AP > GP > P > C.

Chlorophyll, carotenoid content. In general, plants inoculated with AM alone or in combination with other bioinoculants produced more chlorophyll and carotenoid in comparison to non inoculated plants (Tab. 3).

DISCUSSION

The present investigation clearly indicate the possibilities to induce *Murraya* plants to have more leaves (biomass) in mycorrhizal treated plants than in non-inoculated by using the AM inoculum individually or together with other bioinoculants. The present results are in agreement with the growth data of basil plants [Zheljazkov et al. 2008], who have reported an increase of leaf number as a result of AMF inoculation. The data obtained in the study on the increase of biomass of *Murraya* due to mycorrhizal symbiosis could be expounded by an increase or change in the architecture of roots provided by the action of extraradical and intraradical fungal hyphae [Liu et al. 2007]. The leaf biomass due to mycorrhizal treatment increased regardless of inoculum about two times the control plants, while the maximum fresh and dry shoot weight was observed by GAP treatment (Tab. 1). This was consistent with the data published by Faisal et al. [2000] and Jangra et al. [2018], where the leaf biomass of *Capsicum frutescens* was significantly high with mycorrhizal fungi.

In the present study, differences in the mean value of plant height, SDW, RDW, root colonization and phosphorus content among the different bioinoculants treatments were statistically significant (Tabs 2, 3). The results show that mycorrhizae inoculation increased fresh and dry shoot and root weight, which is in accordance with the findings of Ortas and Ustuner [2014]. The role of mycorrhizae symbiosis has been worked in *Oreganum* sp. where the increase of plant biomass was correlated positively with mycorrhizal colonization [Khaosaad et al. 2006]. The different species of AM fungi [Zubek et al. 2010] alone or in combination with other bioinoculants [Jangra et al. 2017] could be the main reason for the differences in plant growth and development of mycorrhizal plants. Many species of the same AM fungus have shown functional diversity in terms of the responsiveness [Tarraf et al. 2017] and at times varieties of plant may also influence host fungus (mycorrhizae) interactions [Gupta et al. 2014]. This diversity in AMF plant symbiosis could be affected by the successfulness of the fungus as symbionts and the development of plants in terms of growth and phosphorus uptake [Smith et al. 2003]. However, our results found a significant interaction between *Murraya* plant and mycorrhizae in terms of better plant growth, which confirms a living together relationship due to the functional activity of the symbiosis in the exchange nutrient among symbionts [Helgason and Fitter 2005]. In addition, the results of [Wu and Zou 2009] show that *G. mosseae* and *A. laevis*, an indigenous mycorrhiza inoculated indigenous plants significantly increased leaf P, K, Ca content of citrus plants compared to non-AMF control.

Inoculation with the three microorganisms (G + A + P) together had a positive impact on shoot biomass of *Murraya* in the present study. The triple association increased the root length for uptake of nutrient and proper establishment of *Murraya* plant which could indicate a synergistic effect when the three bioinoculants are present. It is well established fact that mycorrhizal roots have the potential to affect the non-mycorrhizal microorganisms during the process of initiation and formation of mycorrhizae [Johansson et al. 2004]. It seems that the presence of mycorrhizae (*G. mosseae* + *A. laevis*) did not inhibit *Pseudomonas fluorescens* population. In field conditions, synergistic relationship between *Rhizobium, Azospirullum* and AM fungi have been shown to increase biomass of legume and non-legume crops [Saini et al. 2014].

In the present study, acid and alkaline phosphatase activity was better in treated plants in comparison to control. According to Gianinazzi et al. [1979], acid phosphatase may be associated with the growth and development of fungus inside the host tissue as well as

with better acquisition of phosphorus from the rhizosphere. Our results are in accordance with Garcia-Gomez et al. [2002] who reported root acid phosphatase activity was higher with *Glomus claroideum* inoculated *Carica papaya* plants. Similarly alkaline phosphatase activity specific to AM fungi have been reported by Bertheau [1977]. In the present study, this enzyme activity can be linked to both plant growth and root colonization phase of *Murraya* plant. Alkaline phosphatase activity was clearly related to the level of fungal colonization in maize roots [Fries et al. 1998]. Our results are in agreement with the mark of Fries et al. [1998] who suggest that acid phosphatase activity was involved in increased uptake of phosphorus from the soil while alkaline phosphatase activity may be linked to active phosphate transport to mycorrhizal colonized roots.

The present data show that the phosphorus uptake in *Murraya* leaf tissues from mycorrhizal plants was better than non-mycorrhizal plants (Tab. 3). Nell et al. [2009] reported a positive effect of phosphorus leaf concentration in *Salvia* due to the inoculation with *G. mosseae*. The present study is in contrast to the observations of Copetta et al. [2006] on *Ocimum basilicum* who reported an absence of any increase of phosphorus due to AM fungi. This phenomenon in the present study in better uptake of phosphorus may be explained to the great capacity of AMF hyphae to explore more soil volume beyond the depletion zone [Marscher and Dell 1994] and thus trigger phosphorus transport from the soil to plant roots [George et al. 1992].

Our earlier studies on *Capsicum frutescens* have shown that different AM fungi alone or in combination had diverse effects of mycorrhizal plants [Jangra et al. 2017]. Mycorrhizal fungi alone or in combination, the chlorophyll and carotenoid content and fresh weight were found higher in mycorrhizal plants than control plants. Different species of AM fungi by improving the better uptake of nutrients [McArthur and Knowles 1993] can enhance chlorophyll content [Rachel et al. 1992]. The present results are in agreement with those previously reported by Mathur and Vyas [2000] who reported that AM root colonization increased chlorophyll synthesis. The associations of AM fungi with the roots of plants influence Mg, Zn, Mn, Cu and Fe uptake that have poor mobility rates and these micronutrients are essential component of chlorophyll molecule [Wiedenhoeft 2006]. Thus, the increase of chlorophyll and carotenoid pigments observed in the present study may be attributed to improved growth and better survivability of mycorrhizal treated *Murraya* plantlets.

CONCLUSIONS

In the present study, mycorrhizal treated plants showed increased essential oil yield of *Murraya* leaves. Our results are in agreement with those of Maffei and Mucciarelli [2003] who reported significantly higher amount of essential oil in mycorrhizal treated plants when compared to no-mycorrhizal plants. The positive response of AM fungi in increasing the production of essential oil has been reported by several workers in many aromatic plant species [Gupta et al. 2002, Kapoor et al. 2007, Chaudhary et al. 2008]. The same response was reported by Karagiannidis et al. [2011] while working with three AM fungi and observed increased growth, nutrient uptake and essential oil yield of Oregano and Mint. As AM fungi increase the absorption of phosphorus uptake may play a direct role in increasing the essential oil contents [Abu-Zeyad et al. 1999]. Kapoor et al. [2002] have indicated that inoculation with AM fungi increased the total essential oil content of *Coriandrum sativum*. In our results, total essential oil of *Murraya* was increased due to AM colonization.

The results of present study revealed a variation among the essential oils obtained from Murraya plant with regard to different bioinoculants treatments. The significant effect of the synergistically relationship on *Murraya* oil content may highly depend on the AM isolates used alone or in combination in the experiment and their compatibility with the plant. Finally scientists and industries need to join hand together to integrate this agricultural practice as an effective and low cost tool for improving yield of *Murraya* plant.

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REFERENCES

Abu-Zeyad, R., Khan, A.G., Khoo, C. (1999). Occurrence of arbuscular mycorrhiza in *Castanospermum australe* A. Cunn. & C. Fraser and effects on growth and production of castanospermine. Mycorhizza, 9(2), 111–117.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol., 24, 1–15.

Barea, J.M., Jeffries, P. (1995). Arbuscular mycorrhizas in sustainable soil-plant systems. In: Mycorrhiza, Varma, A., Hocl, B. (eds). Springer, Berlin−Heidelberg, 521–560.

Baum, C., El-Tohamy, W., Gruda, N. (2015). Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: a review. Sci. Hortic., 187, 131–141.

Bertheau, Y. (1977). Etudes des phosphatases solubles des endomycorhizes à vésicules et arbuscules. DEA thesis, Universite de Dijon, France.

Chaudhary, V., Kapoor, R., Bhatnagar, A.K. (2008). Effectiveness of two arbuscular mycorrhizal fungi on concentrations of essential oil and artemisinin in three accessions of *Artemisia annua* L. Appl. Soil Ecol., 40(1), 174–181.

Colla, G., Rouphael, Y., Di Mattia, E., El-nakhel, C., Cardarelli, M. 2015. Co-inoculation of *Glomus intraradices* and *Trichoderma atroviride* acts as a Biostimulant to promote growth, yield and nutrient uptake of vegetable crops. J. Sci. Food Agric., 95, 1706–1715.

Copetta, A., Lingua, G., Berta, G. (2006). Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. Mycorrhiza, 16(7), 485–494.

Faisal, E.A., Yagoub S.O. and Elsheikh E.A.E., 2000. Effects of mycorrhizal inoculation and phosphorus application on the nodulation, mycorrhizal infection and yield components of faba bean grown under two different watering regimes. Khartoum J. Agric. Sci., 1(1), 137–151.

Ferrol, N., Calvente, R., Cano, C., Barea, J.M., Azcón-Aguilar, C. (2004). Analysing arbuscular mycorrhizal fungal diversity in shrub-associated resource islands from a desertification-threatened semiarid Mediterranean ecosystem. Appl. Soil Ecol., 25(2), 123–133.

Freitas, M.S., Martins, M.A., Curcino, I., Vieira, I.J. (2004) Yield and quality of essential oils of *Mentha arvensis* in response to inoculation with arbuscular mycorrhizal fungi. Pesq. Agropec. Bras, 39, 887–89

Fries, L.L., Pacovsky, R.S., Safir, G.R., Kaminski, J. (1998). Phosphorus effect on phosphatase activity in endomycorrhizal maize. Physiol. Plant., 103(2), 162–171.

Garcia-Gomez, R., Chavez-Espinosa, J., Mejia-Chavez, A., Duran, B.C. (2002). Short term effect of *Glomus claroideum* and *Azospirillum brasilense* on growth and root acid phosphatase activity of *Carica papaya* L. under phosphorus stress. Rev. Latinoam. Microbiol., 44, 31–37.

George, E., Haussler, K., Vetterlein, D., Gorgus, E., Marschner, H. (1992). Water and nutrient translocation by hyphae of *Glomus mosseae*. Can. J. Bot., 70, 2130–2137.

Gerdemann, J.W., Nicolson, T.H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc., 46(2), 235–244.

Gianinazzi, S., Gianinazzi-Pearson, V., Dexheimer, J. (1979). Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. III. Ultrastructural localization of acid and alkaline phosphatase in onion roots infected by *Glomus mosseae* (Nicol. & Gerd.). New Phytol., 82(1), 127–132.

Gray, E.J., Smith, D.L. (2005). Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. Soil Biol. Biochem., 37(3), 395–412.

Gupta, D.K., Chatterjee, S., Datta, S., Veer, V., Walther, C. (2014). Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. Chemosphere, 108, 134–144.

Gupta, M.L., Prasad, A., Ram, M., Kumar, S. (2002). Effect of the vesicular–arbuscular mycorrhizal (VAM) fungus *Glomus fasciculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of menthol mint (*Mentha arvensis*) under field conditions. Biores. Technol., 81(1), 77–79.

Helgason, T., Fitter, A. (2005). The ecology and evolution of the arbuscular mycorrhizal fungi. Mycologist, 19(3), 96–101.

Jackson, M.L. (1973). Soil chemical analysis. Prentice Hall of India Pvt. Ltd., New Delhi, pp. 485.

Jangra, E., Yadav, K., Aggarwal, A. (2018). Potential role of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* on growth, physiological parameters, and yield of *Capsicum frutescens* L. Int. J. Veg. Sci., 24(2), 137–145.

Johansson, J.F., Paul, L.R., Finlay, R.D. (2004). Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol. Ecol., 48(1), 1–13.

Kapoor, R., Chaudhary, V., Bhatnagar, A.K. (2007). Effects of arbuscular mycorrhiza and phosphorus application on artemisinin concentration in *Artemisia annua* L. Mycorrhiza, 17(7), 581.

Kapoor, R., Giri, B., Mukerji, K.G. (2002). *Glomus macrocarpum*: a potential bioinoculant to improve essential oil quality and concentration in Dill (*Anethum graveolens* L.) and Carum (*Trachyspermum ammi* (Linn.) Sprague). World J. Microbiol. Biotechnol., 18(5), 459–463.

Kapoor, R, Giri, B, Mukerji, KG (2004). Improved growth and essential oil yield and quality in *Foeniculum vulgare* Mill on mycorrhizal inoculation supplemented with P-fertilizer. Biores. Technol., 93, 307–311.

Karagiannidis, N., Thomidis, T., Lazari, D., Panou-Filotheou, E., Karagiannidou, C. (2011). Effect of three Greek arbuscular mycorrhizal fungi in improving the growth, nutrient concentration, and production of essential oils of oregano and mint plants. Sci. Hortic., 129(2), 329–334.

Khaosaad, T., Vierheilig, H., Nell, M., Zitterl-Eglseer, K., Novak, J. (2006). Arbuscular mycorrhiza alter the concentration of essential oils in oregano (*Origanum* sp., Lamiaceae). Mycorrhiza, 16(6), 443–446.

Linderman, R.G. (1992). Vesicular-arbuscular mycorrhizae and soil microbial interactions. In: Mycorrhizae in suistainable agriculture, Bethlenfalvay, G.J., Linderman, R.G. (eds). ASA Special Publication 54, Madison, 45–70

Liu, J., Wu, L., Wei, S., Xiao, X., Su, C., Jiang, P., Yu, Z. (2007). Effects of arbuscular mycorrhizal fungi on the growth, nutrient uptake and glycyrrhizin production of licorice (*Glycyrrhiza uralensis* Fisch). Plant Growth Regul., 52(1), 29–39.

Maffei, M., Mucciarelli, M. (2003). Essential oil yield in peppermint/soybean strip intercropping. Field Crops Res., 84(3), 229–240.

Marschner, H., Dell, B. (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil, 159, 89–102

Mathur, N., Vyas, A. (2000). Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in *Ziziphus mauritiana* Lam. under water stress. J. Arid Env., 45(3), 191–195.

McArthur, D.A., Knowles, N.R. (1993). Influence of vesicular-arbuscular mycorrhizal fungi on the response of potato to phosphorus deficiency. Plant Physiol., 101(1), 147–160.

Menge, J.A., Timmer, L.W. (1982). Procedures for inoculation of plants with vesicular-arbuscular mycorrhizae in the laboratory, greenhouse, and field. In: Methods and principles of mycorrhizal research, Schenck, N.C. (ed.). Am. Phytopath. Soc., St. Paul, 59–68.

Nell, M., Voetsch, M., Vierheilig, H., Steinkellner, S., Zitterl-Eglseer, K., Franz, C., Novak, J. (2009). Effect of phosphorus uptake on growth and secondary metabolites of garden sage (*Salvia officinalis* L.). J. Sci. Food Agric., 89(6), 1090–1096.

Newman, E.I., Reddell, P. (1987). The distribution of mycorrhizas among families of vascular plants. New Phytol., 106(4), 745–751.

Ortas, I., Ustuner, O. (2014). Determination of different growth media and various mycorrhizae species on citrus growth and nutrient uptake. Sci. Hortic., 166, 84–90.

Phillips, J.M., Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55(1), 158–IN18.

Rachel, E.K., Reddy, S.R., Reddy, S.M. (1992). Seedling preinoculation within AM fungi on transplant survival and growth of sunflower. Proc. Natl. Acad. Sci. Technol, 2, 431–439.

Rasooli, I., Mirmostafa, S.A. (2003). Bacterial susceptibility to and chemical composition of essential oils from *Thymus kotschyanus* and *Thymus persicus*. J. Agric. Food Chem., 51(8), 2200–2205.

Sadaghiani, R.H., Hassani, A, Barin, M., Younes Rezaee Danesh, R.Y., Sefidkon, F. (2010) Effects of arbuscular mycorrhizal (AM) fungi on growth, essential oil production and nutrients uptake in basil. J. Med. Plants Res., 4(21), 2222–2228.

Saini, S.C., Reddy, G.B.S., Birari, P. (2013). Assessment of quality of curry leaves (*Murraya koenigii*). Int. J.Pharma. Sci. Invent., 2(10), 13–17.

Saini, V.K., Bhandari, S.C., Tarafdar, J.C. (2004). Comparison of crop yield, soil microbial C, N and P, N-fixation, nodulation and mycorrhizal infection in inoculated and non-inoculated sorghum and chickpea crops. Field Crop. Res., 89(1), 39–47.

Schenck, N.C., Perez, Y. (1990). Manual for the identification of VA mycorrhizal fungi.Synergistic Publications, Gainesville.

Scheublin, T.R., Ridgway, K.P., Young, J.P.W., Van Der Heijden, M.G. (2004). Nonlegumes, legumes, and root nodules harbor different arbuscular mycorrhizal fungal communities. Appl. Environ. Microbiol., 70(10), 6240–6246.

Singh, S., Kapoor, K.K. (1999). Inoculation with phosphate-solubilizing microorganisms and a vesicular-arbuscular mycorrhizal fungus improves dry matter yield and nutrient uptake by wheat grown in a sandy soil. Biol. Fertil. Soils, 28(2), 139–144.

Smith SE, Smith FA, Jakobsen I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. Plant Physiol., 133, 6–20.

Smith, S.E., Read, D.J. (1997). Mycorrhizal Symbiosis. Biol. Plant., 40(1), 154–156

Smith, F.A., Grace, E.J., Smith, S.E. (2009). More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbiosis. New Phytol., 182, 347–358.

Smith, S. E., Read, D. J. (2010). Mycorrhizal symbiosis. Academic Press, London, pp.769

Tabatabai, M.A., Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem., 1(4), 301–307.

Tarraf, W., Ruta, C., Tagarelli, A., De Cillis, F., De Mastro, G. (2017). Influence of arbuscular mycorrhizae on plant growth, essential oil production and phosphorus uptake of *Salvia officinalis* L. Ind. Crops Prod., 102, 144–153.

Vázquez, M.M., César, S., Azcón, R., Barea, J. M. (2000). Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum, Pseudomonas, Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. Appl. Soil Ecol., 15(3), 261–272.

Vosátka, M., Gryndler, M. (1999). Treatment with culture fractions from *Pseudomonas putida* modifies the development of *Glomus fistulosum* mycorrhiza and the response of potato and maize plants to inoculation. Appl. Soil Ecol., 11(2–3), 245–251.

Wiedenhoeft, A.C. (2006). Plant nutrition. Infobase Publishing, New York, pp. 136.

Wu, Q.S., Zou, Y.N. (2009). Mycorrhiza has a direct effect on reactive oxygen metabolism of drought-stressed citrus. Plant Soil Environ., 55(10), 436–442.

Zheljazkov, V.D., Cantrell, C.L., Evans, W.B., Ebelhar, M.W., Coker, C. (2008). Yield and composition of *Ocimum basilicum* L. and *Ocimum sanctum* L. grown at four locations. Hort. Sci., 43(3), 737–741.

Zubek, S., Stojakowska, A., Anielska, T., Turnau, K. (2010). Arbuscular mycorrhizal fungi alter thymol derivative contents of *Inula ensifolia* L. Mycorrhiza, 20(7), 497–504.