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BIOLOGICAL INTERACTION STUDIES OF AM FUNGI (*GLOMUS MOSSEAE*) ON *RUTA GRAVEOLENS* L. AND IT'S MICROBIAL ACTIVITIES

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ABSTRACT: Large-scale productions of medicinal plants using modern cultivation technologies are being practiced across countries, to meet the high demand of medicinal plants. The pests and diseases of the plant are hampering the growth and quality of medicinal plants. In addition, the excessive use of pesticides may degrade the quality of medicinal plant products. Therefore, the development of innovative technologies for the cultivation of medicinal plants is required. In the present study was designed to show the effect of arbuscular mycorrhizal fungi (*Glomus mosseae*) on *Ruta graveolens* plants and further, the antibacterial and antifungal activity of plant extract has been studied. After different periods from transplanting, parameters like physical growth parameters and chlorophyll and carotenoids content of plants was measured.

KEYWORDS: Arbuscular mycorrhizal fungi, *Glomus mosseae, Ruta graveolens,* and Anti-microbial

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1. INTRODUCTION

Mycorrhizae are highly evolved mutualistic symbiosis or associations between plants roots and soil fungi. The partners in this relation are members of most vascular plants and the fungus kingdom (Ascomycetes, Basidiomycetes and Zygomycetes). The AM fungi are the most complex group of

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Mycorrhizae which forms arbuscules or may vesicles. The arbuscules are the most significant structures in AM fungi and are the site for the exchange of carbon and phosphate metabolite between the fungus and plant. The vesicle is sacked (swellings at the end of hyphae) formed in cortical cells which accumulate Lipid droplets and acts like storage. Vesicle appears mostly in the mid to late vegetative period and arbuscules are usually formed earlier. Some of the beneficial effects of the AM fungi include nutrient uptake, micronutrient uptake, nitrogen fixation, water stress tolerance, influencing soil structure, phytohormone accumulation, regulating natural ecosystems. Use of AM fungi is one of the promising sustainable technologies for the maintenance and conservation of medicinal plants [1]. For different medicinal plants, variations in plant growth and active principles in mycorrhizae-inoculated plants have been reported [2], [3]. The AM fungi inoculation and with or without other beneficial soil microorganisms significantly increase the production of biomass in different medicinal plants [4], [5]. *Glomus mosseae* is an important species of Endogonaceae (Phycomycetes) with the ability to form AM. Genus *Glomus* includes close to 110 described species and making this genus the most important of the order Glomerales [6].

Fig. 1: *Ruta graveolens*

Ruta graveolens (English: Rue) belongs to the family of Rutaceae and in Hindi commonly called Pismaram. Rue is a sub-shrub that flowers in the summer. It has strong smelling leaves that have a bitter taste and are 1.5 to 4 inches in length. The plant can reach a height of 12 to 30 inches. Rue is a traditionally used medicinal plant and is used in Indian traditional medicines (Ayurveda, Unani and Siddha) and in herbal medicine in many other countries, The plant has medicinal properties of antispasmodic, antibacterial, analgesic, anti-inflammatory, insecticidal activities and antidiabetic flatulence, colic, stomachic, irritant, cough and abortifacient and used externally for sciatica, arthritis, muscular chest pain, headache, bronchitis [7], [8], [9]. The plant extract is used to treat inflammation [10] and ulcers [11]. This plant extract exerts cytotoxic, antibacterial [12],

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anthelminthic and phytotoxic [13] properties. Plant extracts are also used in the treatment of reproductive disorders [14]. A decoction of *Ruta graveolens* is used to promote menstruation. The plant contains various volatile compounds and oils [15], [16]. Oils are used to treat nervous nightmare and essential oils are also reported for their insecticidal properties. Fresh leaves used to relieve headache. In homeopathy this plant is used for the treatment of muscular pain, injuries, sprains, eye strain and joint and bone pain. The main active flavonoids present in *R. graveolens* are quercetin and rutin with a high quantity of ketones, aliphatic acids and alcohols [17], [18].

2. MATERIALS AND METHODS

2.1. Collection of Plants and Inoculation:

45 days old *Ruta graveolens* saplings were collected from Dhanvantari Vana, Department of Indian System of Medicine and Homeopathy, Karnataka Forest Department. AM fungi (*Glomus mosseae*) were isolated using wet sieving and Decanting Technique [19]. *Glomus mosseae* (*Gm*) fungi spores identification was done base on different characteristics [20], [21]. *Gm* spores were isolated as per the spore morphology and characteristics. *Glomus mosseae* (*Gm*) fungi maintained in pot culture containing sterilized soil and sand (2:1) and using Rhodes grass (*Chloris gayana*) as a host [22] under lab condition for six weeks. The substrate along with Rhodes grass was air dried and served as mycorrhizal inoculum. The Soil for this experiment was loamy red soil with pH 6.7. The collected soil and sand were sieved to remove the debris and was sterilized and stored. Farmyard manure was used for the present study. Irrigation was given twice a week for first four weeks and subsequently at weekly intervals to maintain enough moisture for growth of plants. Plants protection measures were taken against insect and pest attack.

2.2. Study of Plant Growth Parameters:

After 30, 60 and 90 days from transplanting, plants were harvested and different physical growth parameters *viz.* shoot length, root length, stem girth, root girth, number of leaves, number of branches, fresh weight of shoot, root, dry weight of stem, root, leaves, root volume, leaf area and soil pH were measured. The chlorophyll and carotenoids content was estimated and recorded. The percent mycorrhizal root colonization of plants was determined to know the intensity of colonization [23]. The chlorophyll and carotenoids in the leaves of *Ruta graveolens* were estimated [24], [25].

2.3. Antimicrobial study:

The antibacterial activity of the plant extract was determined by the Kirby-Bauer agar diffusion method, the bacterial cultures (*Escherichia coli and Staphylococcus aureus*) were grown in nutrient

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broth. The crude extract was dissolved in DMSO **(**Dimethyl Sulfoxide). The crude extract of different concentration about [100, 150, 200, 250, 300, 350µg/ml] was filled into the wells of the agar plates were incubated at 37º±C for 24-48 hours. Tetracycline was used as a control for bacteria. After the period of incubation, the zone of inhibition was measured and tabulated. For antifungal bioassay, the pathogenic fungi *Fusarium oxysporum* and *Phomopsis azadirachtae* were grown on SDA (Sabouraud Dextrose Agar) under a sterile condition at 25±10C and incubated in dark and were used as test inoculum. The antifungal assay was accomplished by the agar dilution method or food poison method for determining the antifungal activity of plant extract at different concentrations.

3. RESULTS AND DISCUSSION

45 days old *Ruta graveolens* saplings were treated with the inoculums *Glomus mossae* and a separate set of saplings were maintained as control (uninoculated). After measuring the various physical parameters like length, stem girth, fresh weight, root volume, leaf area the results are presented (Table 1),

Table1: Physical parameter studies for 30, 60 and 90 Days

DAYS	TREATMENT	FPW	FRW	SL	RL	PH	LA	SG	NOL	NOB
30	control	5.2	0.58	26	5.2	32	0.3	1.3	97	20
	T1	7.5	1.3	33	9	45	0.5	1.4	303	43
60	control	8	3.5	37	15	46	0.8	2.2	780	45
	T1	15	6.8	50.4	17.5	73	3.2	2.9	1116	110
90	control	16	8	46.5	20	58	0.9	1.4	540	48
	T1	20	11.5	55	28	82	1.8	3.3	1580	72

NOTE: (1)FPW: Fresh plant weight (g), (2) FRW: fresh root weight(g) , (3) SL: shoot length(cm) , (4) RL: Root length(cm), (5) PH: plant height(cm), (6) LA: leaf area(cm2), (7) SG: Stem girth(cm) , (8) NOL: number of fresh leaves, (9) NOB: Number of branches , (10).(ii) Values are results of triplicates.

On comparing the (Table 1) which shows shoot length, root length, stem girth, the number of leaves, the number of branches, leaf area, fresh weight of the treated plants have increased significantly. When compared to 30 days and 60 days, the plants of 90 days showed increased in root length, number of leaves and plant weight.

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(A) **(B)**

Fig. 2: *Ruta graveolens* A: control plant and B: 90 DAT inoculation

Although other plants are responding well to other treatments as well, the plant that is showing a significant increase in biomass and number of leaves can be considered as the one that is responding better (because its leaves has more medicinal values compare to other parts of plant).

Table 2: Measurement of PH

	control	T1
30 Days	6.7	6.7
60 Days	6.9	6.9
90 Days	6.8	6.8

The pH is an important factor that affects soil – endophyte specificity. In addition, a number of studies have shown that changing the soil pH affects the activity of indigenous endophytes and of certain introduced ones. Some endophytes like *G. mosseae* show marked pH preferences. It has been found that the organism is very effective at pH 6 –7 (Table 2).

Table 3: Root colonization study

	Percentage of root colonization (%)			Number of Spore / 50g		
Treatment	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Control	0	0	0	0	0	0
T1	53	57	66	115	180	265

The biochemical constituents (Chlorophyll and Carotenoids) were estimated by spectrophotometric analysis (Table 4).

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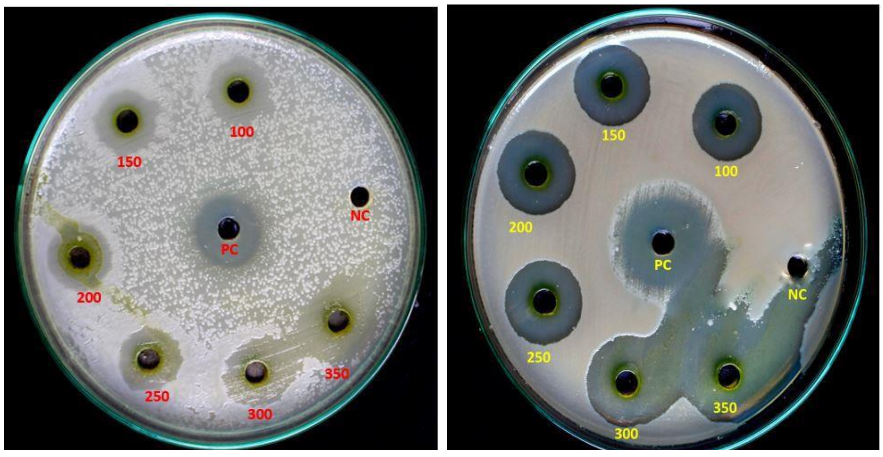
Table 4: Effect of AM treatments on the Chlorophyll and Carotenoids content of the *Ruta graveolens*

Treatment	Chlorophyll (mg/g)			Carotenoids (mg/g)
	Chlorophyll A	Chlorophyll B	Total chlorophyll	
Control	0.28	0.51	0.92	0.079
T1	0.89	1.46	2.14	0.099

Chlorophyll and carotenoids are photosynthetic pigments and their quantity in the plant will express its photosynthetic efficiency. Anti-bacterial activity of *Ruta graveolens* was tested against *Escherichia coli* and *Staphylococcus aureus.* Experiment was performed with chloroform extract (concentration: 100, 150, 200, 250, 300, 350µg/ml]. The zone of inhibition produced by extract was noted (Table 5).

Table 5: Results for antibacterial activity of chloroform extract of *Ruta graveolens*

	100µg/ml	150µg/ml	200µg/ml	250µg/ml	300µg/ml	350µg/ml	PC
Escherichia coli	4.03±0.3	3.43±0.5	3.3±0.5	2.63±0.43	2.90±0.6	2.50±0.4	3.9±0.3
Staphylococcus aureus	2.0±0.41	1.46±0.5	1.73±0.4	1.60±0.3	0.7±0.3	1.3±0.3	2.9±0.3



(A) **(B)**

Fig. 3: Result of antibacterial activity A: *E.coli*, B: *S.aureus*

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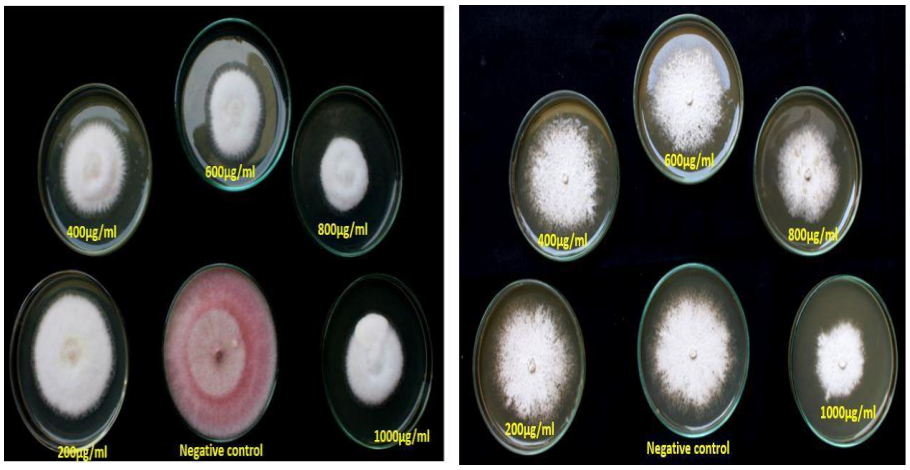
Anti-fungal activity of *Ruta graveolens* was tested against *Fusarium oxysporum* and *Phomopsis azardichtae*. The experiment was performed with chloroform extract (Concentration – 200, 400, 600, 800, 1000µg/ml). The zone of inhibition produced by extract was noted (Table 6).

Table 6: Results for antifungal activity of chloroform extract of *Ruta graveolens*

	200 µg/ml	400 µg/ml	600 µg/ml	800 µg/ml	1000 µg/ml	NC
Fusarium oxysporum	34.61±0.05	41.32±0.12	53.54±0.03	58.42±0.01	73.46±0.01	100
Phomopsis azadirachtae	31.88±0.05	53.54±0.03	60.62±0.05	69.72±0.05	84.72±0.03	100



(A) **(B)**

Fig. 4: Antifungal activity of *Ruta graveoens* against plant pathogenic fungi A: *Fusarium oxysporum* and B: *Phomopsis azardichtae*

4. CONCLUSION

In the present study inoculation of *Glomus mosseae* showed a positive influence on fresh plant weight, fresh root weight, shoot length, root length, plant height, leaf area, Stem girth, number of fresh leaves and Number of branches. Mycorrhization of *Annona squamosa* has been reported to have a marked effect on the height of the plant and fresh and dry weight of the roots and shoots compared to their respective controls [26]. It has been reported that the plant height of *Coleus forskohlii* significantly increased due to the inoculated *G. fasciculatum* of AM fungi [27]. The estimation of chlorophyll and Carotenoids contents reveals an increase in the production of these two pigments [28], [29]. The anti-bacterial activity of *Ruta graveolens* extract showed better

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respond to *Escherichia coli* compare to *Staphylococcus aureus* in different concentration. Application of bio-fertilizer is a technology which is very accessible to farmers in all countries. The inoculation of Arbuscular mycorrhizae is a green technology to increase the quantity and quality of the medicinal plants. However, choosing and inoculating most specific and efficient species AM fungi for a particular plant are essential for the cultivation of medicinal plants [30].

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

1. Karthikeyan B, Joe MM, Jaleel CA. Response of some medicinal plants to vesicular arbuscular mycorrhizal inoculants. J. Sci. Res. 2009; 1:381–386
2. Copetta A, Lingua G, Berta G. Effects of three AM fungi on growth, distribution of glandular hairs and essential oil production of *Ocimum basilicum* L. var. Genovese. Mycorrhiza. 2006; 16:485–494
3. Sailo GL, Bagyaraj DJ. Influence of different AM-fungi on the growth, nutrition and forskolin content of *Coleus forskohlii*. Mycol. Res. 2005; 109:795–798
4. Kothari SK, Sing S, Sing VB, Kumar S. Response of bergamot mint (Meuthacitrata) to vesicular arbuscular mycorrhizal fungi and phosphorus supply. J. Med. Aromat. Plant Sci.1999; 21: 990–995
5. Sena MK, Das PK. Influence of microbial inoculants on quality of turmeric. Indian Cocoa Arecanut Species J. 1998; 21:31–33
6. Schubler A, Schwarzott D, Walker C. A new fungal phylum, the Glomeromycota: Phylogeny and evolution. Mycological Res. 2001; 105 (12): 1413 -1421.
7. Fadlalla K, Watson A, Yehualaeshet T, Turner T, Samuel T. *Ruta graveolens* Extract Induces DNA Damage Pathways and Blocks Akt Activation to Inhibit Cancer Cell Proliferation and Survival. Anticancer Res. 2011; 31: 233-242.
8. Ahmed OM, Moneim AA, Yazid IA, Mahmoud AM. Antihyperglycemic, Antihyperlipidemic and antioxidant effects and the probable mechanisms of action of *Ruta graveolens* infusion and rutin in nicotinamide-streptozotocin-induced diabetic rats. Diabetologia Croatica. 2010; 39(1): 15-35.

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9. Asad MHHB, Murtaza G, Siraj S, Khan SA, Azhar S, Hussain MS et al. Enlisting the scientifically unnoticed medicinal plants of Pakistan as a source of novel therapeutic agents showing anti-venom activity. Afr. J. Pharma. Pharmacol. 2011; 5(20): 2292-2305.
10. Ratheesh M, Shyni GL, Sindhu G, Helen A. Protective Effects of Isolated Polyphenolic and Alkaloid Fractions of *Ruta graveolens* L. on Acute and Chronic Models of Inflammation. Inflammation. 2009; 9(24): 4983-6.
11. Raghav SK, Gupta B, Agrawal C, Gosami K, Das H. Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cell. J. Ethnopharmacol. 2006; 104: 234-239.
12. Haddouchi F, Mohammed CT, Zaouali Y, Ksouri R, Attou A, Benmansour A. Chemical composition and antimicrobial activity of the essential oils from four Ruta species growing in Algeria. J. Food Chem. 2013; 141: 253–258.
13. Asgarpanah J, Khoshkam R. Phytochemistry and pharmacological properties of *Ruta graveolens* L. J. Med. Plants Res. 2012; 6: 3942-3949.
14. Browner, CH. Plants used for reproductive health in Oaxaca, Mexico. Econ. Bot. 1985; 39:482-50.
15. Kostova I, Ivanova A, Mikhova B, Klaiber I. Alkaloids and coumarins from *Ruta graveolens*. Monatsh Chem.1999; 130: 703-707
16. Hilawie M, Unnithan CR, Muuz M, Gebremedhin D, Reddy DN, Birhan A et al. Extraction, isolation and chemical composition of the essential oil of *Ruta Graveolens* L of mekelle, northern Ethiopia. Inter. J. Pharmacother. 2015; 5(1): 05-07.
17. Saeed P, Mohammad RR, Abbas D, Seyed VR, Ali AH. Inhibitory effects of *Ruta graveolens* L. extract on guinea Pig Liver and bovine Milk Xanthine oxidase. Iranian J Pharm. Sci. 2009; 5(3): 163 – 170.
18. Soares AM, Ticli FK, Marcussi S, Miriam VL, Ana HJ, Suely VS et al. Medicinal Plants with Inhibitory Properties Against Snake Venoms. Curr. Med. Chem. 2005; 12: 2625 – 2641.
19. Gerdemann JW, Nicolson TH. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 1963; 46: 235-244.
20. Trappe JM. Synoptic key to the genera and species of zygomycetous mycorrhizal fungi. Phytopathol. 1982; 72: 1102-1108.
21. Schenck NC, Perez Y. Manual for the Identification of VA Mycorrhizal Fungi.3rd Edn. INVAM Publications, University of Florida, Gainesville. 1990; 245p.

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22. Sreenivasa MN, Bagyaraj DJ. (Rhodes grass) A better host for mass production of inoculum. Plant and Soil. 1988; 289-290.
23. Phillips JM, Heyman DS. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungus for rapid assessment of infection, Trans. Br. Mycol. Soc. 1970; 55: 158-161.
24. Arnon D1. Copper enzymes in isolated chloroplasts. Poly-phenoloxidase in Beta vulgaris. Plant Physiol. 1949; 24: 1-15.
25. Kirk JTO, Allen RL. Dependence of chloroplast pigments synthesis on protein synthetic effects on actilione. Biochem. Biophysics Res. J. Canada. 1965; 27: 523-530.
26. Ojha S, Chakkraborty MR, Dutta S, Chatter-Jee NC. Influence of VAM on nutrient uptake and growth of custared-apple. Asian J. Exp. Sci. 2008; 22:221–224.
27. Sakthivel U, Karthikeyan B. Effect of plant growth promoting rhizobacteria for the growth and yield of *Coleus forskohlii*. Int. J. Curr. Adv. Res. 2012; 1:39–43.
28. Nezhad HR, Kasliwal S, Srinivasamurthy K.M. Effect of Arbuscular Mycorrhiza (*Glomus fasciculatum*) and some plant growth promoting rhizomicroorganisms on growth and development of *Solanum viarum*. J. Int. Acad. Res. Multidisciplinary. 2017; 5(8): 44-52
29. Kasliwal S, Nezhad HR, Srinivasamurthy KM. Studies on effects of inoculation of *Glomus mosseae* and plant growth promoting rhizomicroorganisms (PGPR) on *Plectranthus amboinicus* (Lour.) Spreng. Int. J. Curr. Res. Biosci. Plant Biol. 2016; 3(7): 127-137.
30. Nezhad HR, Srinivasamurthy KM. Studies on Influence of Arbuscular Mycorrhiza and Some Plant Growth Promoting Rhizomicroorganisms on *Rauvolfia Serpentina*, Int. J. Technol. Management & Humanities. 2016; 2(3): 1-8.

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