EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON MACRO AND MICRO NUTRIENT OF *ARTEMISIA PALLENS* AT POT LEVEL CONDITION

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ABSTRACT

Arbuscular mycorrhizal fungi play an important role in mobilization of nutrients and enhancing plant growth. It maintain the intimate link between the plant roots and soil the present investigation deals with effect Effect of Arbuscular mycorrhizal fungi on Macro and micronutrient of Artemisia pallens at pot level experiment was done on pot in five fold replicate of each treatment.Set-i) Control (without AM fungi, rock phosphate and ash) Set-ii) UP100 (Pure culture of AM fungi (Glomus mosseae.) Set-iii)IP100(Pure culture of AM fungi (Glomus mosseae) rock phosphate100 (1gm) and ash). Set-iv)IP75 (Pure culture of AM fungi (Glomus mosseae.) rock phosphate75 (0.75gm) and ash). Set-v) DI (Dual inoculation of AM fungi i.e Glomus mosseae + Acaulospora laevis) Set-vi)DIP(Dual inoculation of AM fungi i.e Glomus mosseae + Acaulospora laevis with rock phosphate100(1gm), Ash). Macro and micro nutrient of Artemisia pallens was increased with dual inoculation of mycorrhiza Acaulospora levies and *Glomus mosseae* with addition rock phosphate and ash as compared to non-inoculated plant.

Keywords: Arbuscular Mycorrhiza, *Artemisia pallens,* Macro and micro nutrient.

1. INTRODUCTION

German Botanist Frank (1885) coined the term mycorrhizae for the first time to designate the symbiotic relationship between the fungi and plant roots. Since then scientists started exploiting them for the welfare of mankind. The term 'mycorrhiza' in its broadest sense is the non-pathogenic association of fungi and the roots of higher plants.

The importance of Arbuscular mycorrhizal fungi in natural and semi natural ecosystem is communally accepted by improved plant productivity and diversity as well as increased plant resistance against biotic and abiotic stresses.(Smith and Read,2008). Uptake of phosphorus, from soil solution is mediated by arbuscular mycorrhizal fungi in addition roots. Arbuscular mycorrhizal fungi greatly enhance plant growth. The improved growth mainly attributed diffusion limited nutrient such as P, Zn, Cu, etc from soil. (Bagyaraj *et. al*., 2015). Plant nutrient such as macronutrient P, Mg, Na and micronutrient Fe, Mn and Zn was increased by arbuscular mycorrhizal fungi.(Halder *et. al*., 2015).Use of arbuscular mycorrhizal fungi alone with rock phosphate and ash to enhance quantity and quality of plant production in agriculture is relatively recent technology.

Artemisia pallens wall is aromatic herb or shrub. It is commonly called as Davana. It is medicinal plant. In India 37 species of *Artemisia* are recorded. (Anonymous, 1985). Leaves and flower are used for the fragnant, floral decoration, religious offering. Oil is used for flavouring of bakery product and tobacco. It is conventially used Indian people for the treatment of immunomodelating, antipyretic, wound healing diabetes mellitus and antihelmentic. (Devare *et.al*., 2014). *Artemisia pallens* wall is offered to lord Jotiba. (Yadav and sardesai, 2002). It is called as wormwood in English, In Kannad, Marathi and hindi languages is called as Davana and in telagu Davannamu. (Suresh *et. al.,* 2011)

Davana is offered to God Shiva**.** Davana has been widely use in Iraqi and Indian medicine. The oil of davana is used for antispasmodic, antibacterial, antifungal and stimulant properties. (Devere *et.al*.,2014). It is used for treatment of diabetes mellitus, wound healing, antihelmentic, antipyretic, immunomodelating, stimulant and tonic (Suresh *et. al*., 2011). *Artemisia pallens* wall possesses anthalminitic, stomachic and anti-inflammatory properties (Nakhare and Garg,1991, Pravinkumar *et. al*., 2010). Oil is used for flavouring of cakes, pastries and tobacco. It is used for antipyretic, antihelmentic and tonic. (Ruikar, *et.al*., 2017).

2. MATERIALS AND METHODS

Pot Cultivation experiments (five replications):

1) Effect of pure culture, mix culture with addition rock phosphate and ash on *Artemisia pallens* Wall

Pot culture experiments was done by following methods suggested by Mulani and Andhale, (2008); Mulani and Contractor, (2008); Kavitha and Nelson., (2014), Seema and Garampalli, (2015), Plastic tray was selected for the experiments and It is filled with 2 kg sterilized soil mixture sand: garden soil: red soil (1:3:1) was filled in plastic tray. Rock phosphate and ash (as per treatment wise) were added over the tray mixture in one of the set mentioned below lastly 15gm of mix and pure AM Culture was added. Then layer of sterile soil was sprayed. Seeds were sown in the soil and covered with soil. Per treatment five replicates were used. After 60 days AM inoculated seedling of *Artemisia pallens* were transferred into pots containing 2 kg sterilized soil mixture sand: garden soil: red soil (1:3:1). Seedlings without mycorrhizal inoculum are considered as control.The experimental pot was maintained in the green house and watering was regularly. The plants were harvested at the age of 30, 60, 90, 120 and 150 days after sowing.

The experiment was done on pot in five fold replicate of each treatment.

Set-i) Control (without AM fungi, rock phosphate and ash)

Set-ii) UP100 (Pure culture of AM fungi (*Glomus mosseae*)

Set-iii) IP100 (Pure culture of AM fungi (*Glomus mosseae*) rock phosphate100 (1gm) and ash).

Set-iv) IP75 (Pure culture of AM fungi (*Glomus mosseae*.) rock phosphate75 (0.75gm) and ash).

Set-v) DI (Dual inoculation of AM fungi i.e *Glomus mosseae* + *Acaulospora laevis*)

Set-vi) DIP (Dual inoculation of AM fungi i.e *Glomus mosseae* + *Acaulospora laevis.* with rock phosphate100(1gm), Ash).

2) Isolation of spores from rhizospheric soil by using Wet-sieving-decanting method. (Gerdman and Nicolson; 1963).

Isolation of spores was done by using three sub steps such as wet-sieving, flotation, sedimentation**.** Mix 05 gm rhizospheric soil in 250 ml luck warm water in beaker and it stirred well by using glass rod. It allows the heavier particles and debris settle down. Then solution was decanted through series of sieves and the solution decanted though 710mm sieves for the removed of debris and roots.Then solution decanted through series of sieves i.e 710mm, 210mm, 150mm, 75mm, 45mm, and 25mm respectively. Highest number of spore density was found in sieves 75mm, 45mm, 25mm and large organic debris, unwanted stones and roots were found in the sieves i.e 710mm and 210mm were discarded. Then spores were taken from each sieve on glass slide with help of brush, capillary tube and it observed at microscope for spores and sporocarps.

3)Assessment of Mycorrhizal infection in root-(Percentage of root colonization)

Percentage of root colonization was done by using (Phillips and Hyman-1970) technique**.**

The roots of the plant were collected in polythene bags and collected roots were washed with tap water 2 to3 times for removing the soil and debris. Collected roots cut into 1cm segments and root segments were taken in test tube containing 10% KOH. Then autoclaved at 15 lbs for 1hrs and 10 drops of H_2O_2 was added after cooling for destaining the roots. After 15 minute 10% KOH was removed from test tube and it washed with water 2-3 times and for decolorisation of pigmented root deep in alkaline solution of Hydrogen peroxide until bleached. After washing 10ml 1N HCL was added in test tube and it kept for 5 minute for neutralization of root tissue. Then HCL was removed from test tube and root segments were washed with water 2 to 3 times. After 30 minute cotton blue with lacto phenol was added in test tube and it kept for 24 hours. After 24 hours stained root segments were mounted on glass slide with acetic acid and glycerol 1:1 respectively. Root segments were covered with cover slip by using DPX added on four corner of glass slide. It observed under compound microscope for root colonization and percentage of root colonization was calculated by using following formula.

$$\textbf{Percent of mycorrhizal colonization} = \frac{\textbf{Number of root segments colonized}}{\textbf{Total number of root segments examined}} \times 100$$

Sample preparation and analysis:.

Phosphorus

Plant phosphorus was estimated by vanadophosphomolybdate yellow colour method spectrophotometrically as given by Jackson (1973).The plant digest was used for determination of phosphorus by using vanadomolybdate solution.The intensity of yellow colour produced was measured on spectrophotometer at 420nm wavelength.

Potassium:
It was determined by the method of Jackson (1973) and acid extract was measured on flame photometer at 548nm wavelengths

Nitrogen:
The nitrogen content in plant was determined by Micro-kjeldahl's methods as described in A.O.A.C. (1975). 0.5 gm of plant sample was digested with 1 gm K2SO4. 0.5gm (CUSO4, H2O, and 25ml concentrated H2SO4 and then it was distilled with NaOH. The distillate was collected in a beaker containing four percent boric acid. The methyl red and bromocerol green mixed indicators were used. The contents were back titrated with 0.1 N sulphuric acid until light pink colour was obtained.

Total micronutrients (Cu, Fe, Mn, Zn)

It was determined by using aliqust obtained from plant digestion by HNO3 Aand HCLO4 and measurements were taken on atomic absorbtion spectrophotometer as described by Issac and Kerber,1971.

RESULTS AND DISCUSSION

In *Artemisia pallens* 92 % root colonization was observed in set-vi-DIP were mycorrhizal fungi containing *Glomus mosseae* and *Aculospora laevis* supplemented with rock phosphate and ash. In rhizospheric soil of *Artemisia pallens* was analysed and it showed 600 spores/100g of soil in set-vi were mycorrhizal fungi containing *Glomus mosseae* and *Aculospora* sp supplemented with rock phosphate and ash**.** Similar observation were made by Kavatagi and Lakshaman (2014) highest percentage of root colonization observed in *lycopersicon esculentum* with inoculated *Rhizophagus fasciculatius.* Mulani and waghmare(2012) observed 90 % root colonization in *Aloe vera.*

Macro and micronutrient was increased with provided Set-vi, DIP (Dual inoculation of AM fungi i.e *Glomus mosseae* + *Acaulospora laevis.* with addition rock phosphate 100(1gm) as followed by set-iii,IP100 (Pure culture of AM fungi(*Glomus mosseae*), rock phosphate100 (1gm) and ash), Set-iv, IP75 (Pure culture of AM fungi (*Glomus mosseae*), rock phosphate75 (0.75gm) and ash), then Set-v, DI (Dual inoculation of AM fungi i.e *Glomus mosseae* + *Acaulospora laevis* less quantity of macro and micronutrient was observed in Set-ii),UP100 (Pure culture of AM fungi without phosphate and ash as very less quantity was observed in Set-i) Control (without AM fungi, phosphate and ash).(Table No. 1 and 2).

Table no-1 Effect of various treatments of inoculation of AM fungi in addition of Phosphates and ash and on macronutrient of *Artemisia pallens* at time of harvesting in pot condition.

		Macronutrient (%)		
Sr. no	Treatment	N	P	K
1	Control	0.598±0.41	0.308±0.25	1.12±0.26
2	UP100	1.388±0.27	0.4028±0.14	3.61±0.14
3	IP100	1.644±0.17	0.646±0.16	4.504±0.21
4	IP75	1.552±0.17	0.576±0.15	4.276±0.14
5	DI	1.448±0.22	0.51±0.17	3.804±0.13
6	DIP	1.768±0.16	0.914±0.14	4.762±0.14

Table no-2 Effect of various treatments of inoculation of AM fungi in addition of Phosphates and ash and on micronutrient of *Artemisia pallens* at time of harvesting in pot condition.

		Micronutrient(ppm)			
Sr. no	Treatment	Cu	Fe	Mn	Zn
1	Control	47.78±2.69	845.79±2.72	92.76±2.88	13.25±2.92
2	UP100	114.29±1.98	1568.69±1.58	177.63±1.91	22.94±1.49
3	IP100	146.43±1.60	2444.24±1.63	277.73±1.86	32.12±1.51
4	IP75	136.33±1.32	2056.34±1.87	245.72±1.93	28.38±1.43
5	DI	125±1.81	1735.55±1.63	214.6±1.76	25.31±1.57
6	DIP	164.72±1.51	2808.72±1.53	346.46±1.62	37.25±1.69

Nitrogen, Phosphorus, potassium and zinc, copper, iron, manganese these macro and micronutrient of *Artemisia pallens* highest quantity was recorded in Set-vi were the mycorrhizal fungi containing *Glomus mosseae* and *Acaulospora laevis* supplemented with rock phosphate and ash followed by Set-iii in which the plants were provided with *Glomus mosseae* supplemented with rock phosphate and ash as compared to set-i in which plants were not supplemented by either fungi or phosphate an ash. Similar observation were made by Mohammed *et., al* (2014) reported Nitrogen phosphorus potassium, and sulphur was increased with inoculated *Glomus species* and *Gigaspora* sp. in maize and onion. Khan, *et., al*.,(2008) observed enhancement of nitrogen, phosphorus and potassium in *Madicago sativa* with inoculated *Glomus etunicatum* and *Glomus intraradiaces*. Nitrogen and phosphorus of *Capsicum annum* was increased with inoculated *Glomus mosseae and Acaulospora laevis* observation were made by Tanvar *et.,al.*(2013). Root and shoot phosphorus of *Chrysanthemum indicum* was enhanced with inoculated *Glomus mosseae, Acaulospora laevis* observed by Prasad *et.,al.*(2012). Wu and Zou, (2011) observed enhancement of mineral nutrient concentration in *Prunus persica.* Kanwal, *et. al*., (2015) reported macronutrient significantly increased in *Medicago sativa.* Kasliwal, *et. al*., (2016) macro and micronutrient observed in *Hibiscus rosa sinensis.* Macro and micro nutrient was increased with inoculated arbuscular mycorrhiza in chickpea reported by farzeneh et.al.,(2011).

REFERANCES

Anonymous. 1985. *The Wealth of India Raw materials*. volume-IA Publication and information director, CSIR New Delhi. 284-285.

A.O. A. C. 1975. Associated of official method of analysed ED. 12 Washington, D.C.

Bagyaraj, D. J Sharma, M. P and Maiti, D. 2015. Phosphorus nutrition of crops through arbuscular mycorrhizal fungi. Current science.(108):7.1288-1293.

Devare, S. M. Lavate, S. S. Shendhkar, C. D. Deshpande, N. R Salvekar, J. P. 2014. Quantification of phenolics and flavonoids by spectrophotometer from *Artemisia pallens* roots. *Journal of pharmacy research*. 8(3). 240-242.

Farzaneh, M. Vierheilig, H. Lossl, A, Kaul.P. 2011. Arbuscular mycorrhizal enhances nutrient uptake in chickpea. *Plant soil environment*. 57(10). 465-470.

Gerdmann, J. W. and Nicolson, T. H. 1963. Spores of mycorrhizal Endogone species extracted from the soil by wet sieving and decanting. *Trans.Br.Mycol.Soc*.46: 235-244.

Halder, H. Dhar P. P. Mujib, A. S. Khan, S. M, Joardar. J .C and Akhter S. 2015. Effect of arbuscular mycorrhizal fungi inoculation on growth and uptake of mineral nutrition in *ipomoea aquatic*. *Current world environment.* 10(1):67-75.

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

Kavitha, T and Nelson. R. 2014. Effect of arbuscular mycorrhizal fungi and growth and yield of sunflower (*Helianthus annus* L.) *Journal of Experimental Biology and Agricultural Sciences*, 2(2S). 226-228.

Kanwal, S Bano, A and Malik, R. N. 2016. Role of arbuscular mycorrhizal fungi in phytoremediation of heavy metals and effect on growth and biochemical activities of wheat (*triticum aestivum* L.) Plants in Zn contaminated soils. *African journal of microbiology*. 15(20)872-883.

Kasliwal, M and Srinivasamurthy. 2016.Influence of arbuscular mycorrhizae inoculation on growth and development of *Hibiscus rosa-sinesis. International journal of current microbiology and applied sciences.* 5(3). 659-666.

Khan, A.G. 1975.The effect of vesicular arbuscular mycorrhizal associations on growth of cereals. *An International Journal of the Abb*.80(1):27-36.

Mohamed, A. A. Eweda, E. E. Heggo, A. M. Hassan, E. A. 2014. Effect of dual inoculation with arbuscular mycorrhizal fungi and sulphur oxidising bacteria on onion (*Allium cepa*) and Maize (*Zea mays.L*) grown in sandy soil under green house condition. *Annals of agricultural sciences.* 59(1)109-118.

Mulani, R. M & Andhale, P. R 2008. Effect of inoculation arbuscular mycorrhizal fungi and *Rhizobium* on biomass and morphological parameters on *Pongamia glabra* at seedling level. *Journal of the University of Mumbai*. 58 (85):68-71.

Mulani, R. M. and Contractor, M. R. 2008. Comparative effect of *Piriformosporindica* arbuscular mycorrhizal fungi on chlorophyll contect of *Oryza sativa* var. Karjat-3. *Journal of the University Of Mumbai*. 58(85):63-67.

Nakhare, S and Garg, S. C. 1991. Anthelmintic activity of the essential oil of *Artemisia pallens* wall. *Ancient science of life*. 10(3):185-186.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc*. 55: 152-160.

Pravinkumar, A. Manojkumar, S. Chopra, H. Upadhyaya, K. 2010.Analgesic and Anti-inflammatory, properties of *Artemisia pallens*. *Pharmacology*. 1. 567-573.

Prasad, K. Aggarwal, A. Yadav, K and Tawar, A. 2012. Impact of different levels of superphosphate using arbuscular mycorrhizal fungi and *Pseudomonas fluorescence* on *Chrysanthemum indicum* L. *Journal of Soil Science and Plant Nutrition.*12(3).451-462.

Ruikar, A. D. Khatiwora. E, Ghyal, N. A, Misar, A. V. Mujumdar, A. M, Puranik, V. G and Deshpande, N. R 2017.studies on the aerial parts of *Artemisia pallens* wall for phenol, flavonoids and evalution of antioxidant activity. Journal of pharmacy and biollied sciences 3(2):302-305.

Seema. H. S and Rajkumar H. G. 2015. Effect of arbuscular mycorrhizal fungi on growth and biomass enhancement in *piper longum L.*(Piperaceae). *Int. J. Curr. Microbiol. App. Sci*. 4(1): 11-18.

Smith, S. E and Read, D. J. 2008. Mycorrhizal symbiosis, 3rd edition Academic press London.

Suresh, J. Mruthunjaya, K. Paramkrishnan, N. Naganandhini. M. N. 2011. Determination of artemisinin in *Artemisia arbotanum* and *Artemisia pallens* by LC/MS method. *Journal of current pharmaceatical research.* 3(1):49-52.

Tanvar, A. Aggarwal,a. Kadian. N and Gupta. A . 2013.Arbuscular mycorrhizal inoculation and superphosphate application influence plant growth and yield of *Capsicum annum.Journal of soil science and plant nutrition.*13(1)55-66.

Yadav, S. R and Sardesai, 2002. Flora of Kolhapur university.

Wu. Q. S, Li , G. H and Zou, Y. N. 2011. Roles of arbuscular mycorrhizal fungi on growth and nutrient acquisition of peach (prunus persica. L. Batsch)seedlings. The *Journal of African and plant sciences*. 21(4):746-750.