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EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULATION ON UPTAKE OF MINERAL NUTRITION IN *ARTEMISIA PALLENS* AT FIELD LEVEL CONDITION

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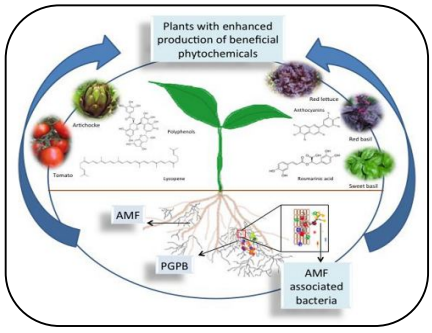
ABSTRACT:

Field experiment was conducted to investigate the Effect of Arbuscular Mycorrhizal Fungi inoculation On Uptake of Mineral Nutrition in Artemisia pallens at field level condition considering our objective of using environmental friendly biofertilizer instead of chemical fertilizer. Medicinal plant Artemisia pallens was grown with Arbuscular mycorrhiza and without mycorrhiza for 150 days. Macro and micronutrient was increased in arbuscular Mycorrhizal inoculated plant as compared to non-inoculated plant. Five replicates with 7 treatments were used for this field experiment.



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

INTRODUCTION: -

The term Mycorrhiza was discovered in 1885 by Albert Bernhard Frank and it describe symbiotic relationship between fungus and plant root. Mycorrhiza means Myco means fungus and rhiza means root. Mycorrhiza shows Mutualistic relationship between fungus and plant root. (Bagyaraj, 2014).

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:

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

i) Cultivation:

Cultivation of davana was done in winter season crop from November to March and ratoon crop increases up to April/may. Whitish, black or red soil was used for the cultivation

One year old seeds were used for cultivation but not more than one year because seed lose their viability after one year. Seven soil beds each of 30cm height and of dimension 1m × 1m was prepared. Firstly prepared the beds and it irrigated at 10 days before sowing the seeds. Then rock phosphate (500gm) and layer of ash was added in different beds then layer of soil was added on beds. Added 750 gm of AM fungi culture (mix and pure culture of AM fungi) was sprayed uniformly on separate beds before sowing the seeds of *Artemisia pallens* and it covers with soil. Seeds were sown in the soil and covered with soil.

The experiment was done on field in five fold replicate of each treatment.
Set-F1-Control(Without AMF and rock phosphate and ash)
Set-F2-Pure culture of AM fungi (*Glomus mosseae.*) with ash
Set-F3-Pure culture of AM fungi(*Glomus mosseae.*)
Set-F4-Pure culture of AM fungi(*Glomus mosseae*), rock phosphate and Ash
Set-F5-Mix culture of AM fungi and Ash
Set-F6-Mix culture of AM fungi
Set-F7-Mix culture of AM fungi, rock phosphate and Ash.

First week of sowing the beds watered regularly. . After 60 days AM inoculated seedlings of *Artemisia pallens* were transferred into field. it is reaches height 5 to 7 cm. The plots were irrigated near about transplanting. Seedlings were instantly watering after transplanting. Regularly the plots were irrigated at first ten days and later once in two days and finally 10-15 days before harvest the water supply to the plants was totally stopped. Davana grows extending upto 50-65cm in height.

ii) Harvesting:

The plants were harvested at the age of 30, 60, 90, 120 and 150 days after sowing.

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.

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

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 DISSCUSION:

In field cultivation experiment 95±1 (SD)% root colonization of *Artemisia pallens* was observed in Set-F7-Mix culture of AM fungi, rock phosphate and Ash, *as* followed by 90±1.41 observed in Set-F6-Mix culture of AMF and 88±1.58% root colonization was observed in Set-F-5-Mix culture of AMF+ ash, 85±1% was observed in Set- F4 containing *Glomus mosseae + rock phosphate +ash,* 82±1.22% was observed in Set-F2 containing *Glomus mosseae. + ash.* In set-F3 root colonization 81±1% inoculated with *Glomus mosseae.* Highest spore i.e 650 ±1.14 density was found in Set-F7- inoculated with Mix culture of AMF+ rock phosphate+ ash as compared all treatments. Similar observations were made by Belay *et. al*., (2013) in *Acacia*. Manimeghalai *et., al*. (2011) observed 98% root colonization in *Solanum Viarum* plant with inoculated *Glomus fasciculatum.*

Fig.1-Nutrient analysis by Atomic Absorbance spectrophotometer

Nitrogen, Phosphorus, potassium and zinc, copper, iron, manganese macro and micronutrient of *Artemisia pallens* highest quantity was recorded in set-F7 were the mix culture of mycorrhizal fungi supplemented with rock phosphate and ash as compared to set-F1 were in which plant not provided rock phosphate and ash. Similar observation were made by Wahid *et., al*.(2016) enhanced phosphorus and nitrogen of maize was increased with mix culture of arbuscular mycorrhizal fungi with rock phosphate. Sari *et. al.,* (2007) reported phosphorus was increased in Garlic. Bolduc and Hijri (2010) observed phosphorus uptake by mycorrhiza in all vascular plant. Mineral nutrient of *Artemisia annua* was increased with

inoculated *Glomus sp.* and phosphate reported by *Rapparini et.,al.,*(2007). Bagyaraj *et. al*., (2015) reported that phosphorus, copper and zinc was uptake by arbuscular mycorrhizal fungi.

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 field condition.

		Macronutrient (%)		
Sr. no	**Treatment**	**N**	**P**	**K**
1	F1	0.656±0.35	0.348±0.21	1.574±0.32
2	F2	1.522±0.21	0.512±0.11	3.692±0.17
3	F3	1.28±0.16	0.494±0.14	3.262±0.26
4	F4	1.658±0.19	0.834±0.13	4.572±0.18
5	F5	1.686±0.19	0.564±0.11	4.404±0.27
6	F6	1.524±0.22	0.484±0.12	3.408±0.27
7	F7	1.662±0.15	1.772±0.17	5.664±0.22

TABLE NO- 1 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 field condition.

		Micronutrient(ppm)			
Sr no	**Treatment**	**Cu**	**Fe**	**Mn**	**Zn**
1	F1	43.58±2.69	1023.61±2.92	129.936±3.21	14.766±2.71
2	F2	106±1.73	2564.14±1.77	243.656±2.54	28.602±2.39
3	F3	82.75±1.31	1994.234±2.38	207.114±2.77	23.934±1.65
4	F4	155.66±1.41	3033.946±2.23	354.948±2.66	36.45±1.59
5	F5	14152±1.48	2887.94±2.35	286.46±2.77	31.85±2.17
6	F6	93.86±2.28	2357.05±2.072	234.548±2.59	26.50±2.13
7	F7	172.18±1.64	3695.746±1.91	411.97±2.33	42.822±1.71

Mean ± SD,(Standard deviation with 5 replicates

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