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ASSOCIATION OF VESICULAR ARBUSCULAR MYCORRHIZAE WITH MEDICINAL PLANT

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Abstract: Twenty two different medicinal plants were investigated for vesicular arbuscularmycorrhizal association from Nagarjun Medicinal Plants Garden, Dr. P.D.K.V. Akola. The vesicle and arbuscules colonization varied from 86.60 to 61.00% and 48.30 to 16.54 % respectively. In *Ocimumtenuiflorum* and *Alpiniagalanga* were showed maximum percentage of vesicle colonization i.e 86.60 and 83.30 % vesicle colonization respectively. In *Boerhaviadiffusa* and *Psoraleacorylifolia*were recorded minimum percentage of colonization i.e. 61.00 and 63.30% respectively. Arbuscules colonization 21.50 and 23.30 % respectively.Number of mycorrhizal fungus spores ranged between 7 to 28 per 10 g air dried soil in different group respective rhizospheric soil. *Glomus*and *Gigaspora* two types of spores were recorded.
Keywords: Vesiculararbuscularmycorrhizal fungi, association, medicinal plants.

INTRODUCTION

Vesiculararbuscularmycorrhizal fungi are ubiquitous plant root symbionts that can be considered as "keystone mutualists" in terrestrial ecosystem forming a link between biotic and abiotic components via carbon and nutrient fluxes that pass between plant and fungi in the soil (Oneill*et al* 1991). The presence of arbuscularmycorrhizae are symbiotic fungi found associated with roots of a wide variety of plant species. In natural communities approximately 80% of higher plants are obligatorily dependent on fungal associates and 18% typically non mycorrhizal. Arbuscularmycorrhizal fungi role in symbiotic partnership is provided with a fine hyphal network capable of extending the range of root hairs. Thepresence of arbuscularmycorrhiza fungi has been observed in medicinal plants rhizomes (Iqbal and Nasim, 1986). These medicinal plants occur naturally and this herbal wealth is being used not only by developing countries but also by developed countries for their health care system. Enhanced plant growth is due to VAM inoculation to medicinal plants. Most of vesicular arbuscularmycorrhizal fungi increase in plant height, root and shoot dry weight, plant biomass, chlorophyll and nutrient contents (N and P) of *Piper longum* (Gogoi and

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Singh, 2011). In *Andrographispaniculata* improved growth, biomass yield, P nutrition and andrographoliede concentration by inoculating vesicular arbuscularmycorrhizal fungi (Arpana and Bagyaraj, 2007). Most of arbuscularmycorrhizal fungi inoculation resulted increase in plant dry biomass, percent of colonization and the barbaloin content of *Aloe vera*. (Pandey and Banik, 2009). Considering the "Association of Vesicular Arbuscular Mycorrhizae in Medicinal Plants" present investigation was taken. Vesicular arbuscularmycorrhiza used as biofertilizer for development and growth of plants. Vesicular arbuscularmycorrhizal fungi seems to be more appropriate as they are effective in overcoming the stress conditions like drought, disease incidences and deficiency of nutrients.

Material and Methods

The different genera of medicinal plants were selected for VAM association from Nagarjun Medicinal Plants Garden. The roots were collected by digging and tearing the roots up to the base of the main stem.

Collection of different genera of plants root samples thoroughly washed in tap water to remove soil particles. Selected and cleaned roots were fixed in formaldehyde/acetic acid solution. To observe AM fungus structures within the root it is necessary to clear cortical cells of cytoplasm and phenolic compounds which usually hide them and then to differentially stain the fungus tissue. Clearing procedures, which use chemical agents to remove cell contents and cell wall pigments, are routinely used to view internal features in plant tissues.

Staining of vesicular Arbuscularmycorrhiza

1. Roots were washed with tap water to remove the soil particles. Then clean roots free from soil particle were cut into 1cm in length by sterilized blade.
2. Root pieces were placed in a small beaker with 10 % KOH solution.
3. The beaker with root pieces in 10% KOH solution was autoclaved at 1.04 kg/cm^2
4. The KOH solution was decanted from the beaker with leaving roots behind.
5. Roots were rinsed with 20 ml distilled water
6. Twenty millilitre of 0.1N HCL was added in the beaker, swirl and left it for a minute.
7. HCL solution was decanted.
8. Then sufficient amount of cotton blue or tryphan blue solution was added to cover the roots generously
9. Then root bits were observed under microscope.

Counting the vesicular arbuscularmycorrhizal infection

1) Percentage of vesicles colonization

$$\frac{\text{Number of root pieces contain vesicles}}{\text{Total number of root pieces}} \times 100$$

2) Percentage of arbuscules colonization

$$\frac{\text{Number of root pieces contain arbuscules}}{\text{Total number of root pieces}} \times 100$$

Wet sieving and decanting method

1. First 10 g soil sample was taken and dissolved in 100 ml distilled water in conical flask.
2. Then conical flask was shaken for 30 min.
3. After that the conical flask was kept in undistributed condition for 30 min.
4. The heavier particles were allowed to settle down.
5. Suspension was decanted through a 710 µm sieve to remove organic matter and roots.
6. This suspension was decanted through 250 µm, 75 µm and 45 µm sieves consequently.
7. The entire residue was collected on 45 µm sieve.
8. After settlement residue was dissolved in distilled water and filtered through filter paper
9. This paper was spread in Petri dish and a residue present in filter paper was taken and mounted on a slide and was examined under microscope

Observations:

Observe the vesicular arbuscularmycorrhizal fungal spore under microscope and identification of spores by different monographs available on Endogonaceae. Results of the present investigations along with statistical analysis have been presented under the different headings and sub headings

Results and Discussion

Different medicinal plants sample and their rhizospheric soil samples were collected from Nagarjun Medicinal Plants Garden of different families like Liliaceae, Nyctaginaceae, Labiatae, Leguminosae, Compositae, Acanthaceae, Crassulaceae, Piperaceae, Zingiberaceae and Vitaceae which are herb, climbers and grassees in Kharif season (Table1).

Table1: Association of vesicular arbusclarmycorrhiza isolated from medicinal plants

Sr No	Scientific name	Local name	Family
1	*Cissusquandragularis*	Kandvel	Vitaceae
2	*Aloe barbadensis*	Korphad	Liliaceae
3	*Iphiginiastalluta*	Jangalilasun	Liliaceae
4	*Andrographispaniculata*	Bhunimb	Acanthaceae
5	*Alpiniagalanga*	Kulinjan	Zingiberaceae
6	*Menthaspp*	Pudina	Labiatae
7	*Psoraleacorylifolia*	Bawachi	Papilanaceae
8	*Alocasiaindica*	Kasalu	Araceae
9	*Boerhaviadiffusa*	Punarnava	Nyctaginaceae
10	*Mirabilis jalapa*	Gulbas	Nyctaginaceae
11	**Ocimumtenuiflorum*	Krishna tulas	Lamiaceae
12	**Canna indica*	Kardal	Cannaceae
13	*Piper longum*	Pipali	Piperaceae
14	**Withaniasomnifera*	Ashwagandha	Solanaceae
15	*Bryophyllumpinnatum*	Panfuti	Crassulaceae
16	**Abelmoschusmoschatus*	Kasturibhendi	Malvaceae
17	**Abrusprecatorius*	Gunj	Leguminosae
18	*Cymbopoganflexuosus*	Gavtichaha	Gramineae
19	*Cymbopogan martini*	Tikhadi	Gramineae
20	**Adhatodavasica*	Adulasa	Acanthaceae
21	*Spilanthuspaniculata*	Akkalkara	Compositae
22	*Urganiaindica*	Pankanda	Liliaceae

(*plants whose total root length not counted)

Association of vesicular arbusclarmycorrhiza in roots of herbaceous medicinal plants.

The percentage of vesicular arbuscularmycorrhizal colonization in herbaceous medicinal plants were highest in *Ocimumtenuiflorum*i.e 86.60% vesicles colonization, 48.70 % arbuscules colonization, number of vesicles per cm root length were 9.71, number of arbuscules per cm root length were 3.66 followed by *Alpiniagalanga*83.30, 42.00, 8.20 and 3.12 respectively and total root length of *Alpiniagalanga* plant was 178.02 cm, number of vesicles were 1459.76 per plant, number of arbuscules were 555.42 per plant, where as least

arbuscularmycorrhizal colonization i.e 21.50 was recorded in *Boerhaviadiffusa* with least number of vesicles per cm root length (1.29). rest of plants showed optimum vesicular and arbuscular colonization.(Table 2, plate1)

Similar results were also recorded by Gaur and Kaushik (2011) VAM associated with medicinal plants i.e*Catharanthusroseus*, *Ocimum* and *Asparagusracemosus*. The percentage of root colonization was highest in *Ocimum* (76.88 to 95%) than the others. The present findings corroborates with the findings of Sharma and Jha (2012).

In *Mirabilis jalapa, Aloe barbadensis*and *Urganiaindica* percentage of vesicular arbuscularmycorrhizal colonization observed almost similar to each other. In *Mirabilis jalapa* vesicle colonization was 74.50% , arbuscules colonization 28.90% , number of vesicles were 4.27 per cm root length, number of arbuscules were 1.82 per cm root length, total root length of plant 119.25 cm, number of vesicles were 509.24 per plant, number of arbuscules were 217.05 per plant followed by *Aloe barbadensis*73.30, 28.00, 3.92, 1.26, 109.30, 428.85, 137.71 respectively and *Urganiaindica*vesicle colonizatio*n* 73.30%, arbuscules colonization 28.38 and number of arbuscules per cm root length were 1.46 and total root length of plant was 102.5 cm, number of vesicles were 691.87 per plant, number of arbuscules were 149.65 per plant. But in *Urganiaindica* number of vesicles observed were 6.75 and maximum than *Mirabilis jalapa* and *Aloe barbadensis.* (Table-2, Fig 1, Plate 2, 3, 4). Sharma and Jha (2012) also reported that *Mirabilis jalapa* root length with hyphae, root length with arbuscules, root length with vesicles, total root colonization were 26.18%, 29.30%, 13.89% and 62.8% respectively.

Table 2: Association of vesicular arbusclarmycorrhiza in roots of herbaceous medicinal plants

Sr no	Plant name	Percentage of vesicles colonization	Number of vesicles per cm root length	Percentage of arbuscules	Number of arbuscules per cm root length	Total root length of plant(cm)	Number of vesicles per plant	Number of arbuscules per plant
1	*Iphiginiastalluta*	78.50	2.13	28.00	1.46	90.5	192.76	132.13
2	*Urganiaindica*	73.30	6.75	28.38	1.46	102.5	691.87	149.65
3	*AdhatodaVasica*	64.25	2.89	29.12	1.43	-	-	-
4	*Canna indica*	77.70	6.45	33.15	2.71	-	-	-
5	*Ocimumtenuiflorum*	86.60	9.71	48.70	3.66	-	-	-

6	*Mirabilis jalapa*	74.50	4.27	28.90	1.82	119.26	509.24	217.05
7	*Boerhaviadiffusa*	61.00	1.29	21.50	0.98	77.05	110.95	75.509
8	*Abelmoschusmoschatus*	72.50	2.00	28.01	1.05	-	-	-
9	*Alpiniagalanga*	83.30	8.2	42.00	3.12	178.02	1459.76	555.42
10	*Withaniasomnifera*	65.00	1.94	40.00	1.24	-	-	-
11	*Psoraleacorylifolia*	63.30	1.44	23.30	1.00	112.05	161.352	112.45
12	*Alocasiaindica*	75.50	2.25	28.30	1.05	95.5	214.87	100.275
13	*Aloe barbadensis*	73.30	3.92	28.00	1.26	109.30	428.45	137.71
14	*Andrographispaniculata*	77.70	2.24	30.89	1.00	146.34	327.80	146.34
15	*Menthaspp*	64.00	1.54	25.00	1.20	156.67	241.27	188.00
16	*Spilanthuspaniculata*	77.00	1.72	30.30	1.00	100.31	172.53	100.31
17	*Bryophyllumpinnatum*	78.00	6.75	31.33	2.13	160.20	1081.35	341.226

In *Bryophyllumpinnatum*, *Andrographispaniculata*, *Canna indica* observed vesicular arbuscularmycorrhizal colonization nearer to each other i.e in *Bryophyllumpinnatum* vesicle colonization was 78.00%, arbuscules colonization 31.33%, number of vesicles were 6.75 per cm root length, followed by *Andrographispaniculata* 77.70, 30.89, 2.24 , 1.00, 146.34, 327.80, 146.34 respectively and *Canna indica* 77.70, 33.15, 6.45 and 2.71 respectively and root length of plant not observed. But in *Canna indica*percent of arbscules and number of arbuscules observed maximum than *Bryophyllumpinnatum.*(Table-2, Plate-3, 5, 6).Zaman*et al*. (2008) also observed the percentage of root colonization was 75 in *Andrographispaniculata*(Acanthaceae). Sharma and Jha (2012) also showed association of twenty species of medicinal plants and aromatic plants with vesicular arbuscularmycorrhizal. Similar observations were also recorded by Yaseen*et al*. (2011).

Vesicular arbuscularmycorrhizal spores observed in rhizospheric soil of herbaceous medicinal plants

In herbaceous medicinal plants highest vesicular arbuscularmycorrhizal fungal spore were observed in *Mentha*spp 28 per 10 g soil. *Mirabilis jalapa*, *Ocimumtenuiflorum*, *Canna indica, IphiginiaStallutas*howed almost similar number of vesicular arbuscularmycorrhizal fungal spore count per 10 g soil i.e 22.30, 22.00, 20.30, 20.60 respectively with *Glomus* spp. In *Bryophyllumpinnatum, Withaniasomnifera and Alpiniagalanga*almost similar number of vesicular arbuscularmycorrhizal fungal spores were observed per 10g rhizospheric soil i.e 19.60, 19.10,19 respectively with *Glomus* sp. Tanvirburni*et al* 2011 observed that30-245 vesicular arbuscularmycorrhizal fungal spore per 10 gram soil in *Menthaarvensis*.Sharma and Jha (2012) also reported similar results.Kumar *et al*. (2010) reported that least or minimum number of spores per 10 g of soil may be due to seasonal variationon association of arbuscularmycorrhiza (Table 3).

Table 3. Vesicular arbuscularmycorrhizal spores observed in rhizospheric soil of herbaceous medicinal plants

Sr. No.	Scientific name	No.of spores per 10g rhizospheric soil	Type of spore
1	*Urganiaindica*	16.60	*Glomus*
2	*Adhatodavasica*	16.60	*Glomus*
3	*Canna indica*	20.30	*Glomus*
4	*Ocimumtenuiflorum*	22.00	*Glomus*
5	*Mirabilis jalapa*	22.30	*Glomus*
6	*Boerhaviadiffusa*	7.00	*Glomus*
7	*Abelmoschusmoschatus*	12.30	*Glomus*
8	*Alpinia galangal*	19.00	*Glomus*
9	*Iphiginiastalluta*	20.60	*Glomus*
10	*Aloe barbadensis*	15.00	*Glomus*
11	*Withaniasomnifera*	19.60	*Glomus*
12	*Psoraleacoryifolia*	9.00	*Glomus*
13	*Menthaspp*	28.00	*Glomus*
14	*Bryophyllumpinnatum*	19.10	*Glomus*
15	*Andrographispaniculata*	16.60	*Glomus*

16	*Spilanthuspaniculata*	11.00	*Glomus*
17	*Alocasiaindica*	16.60	*Glomus*
		9.00	*Gigaspora*

Vesicular arbuscularmycorrhizal spores observed in rhizospheric soil of Climber and grasses medicinal plants

The highest percentage ofvesicular arbuscularmycorrhizal colonization were observed in *Abrusprecatorius*with vesicle colonization 71.52%, arbuscules colonization of 27.32%, number of vesicles were 2.53 per cm root length, number of arbuscules were 1.53 per cm root length. Minimum colonization were observed in *Cissusquandragularis*i.e., vesicle colonization 55.21%, arbuscules colonization 16.54%, number of vesicles were 1.63 per cm root length, number of arbuscules were 0.82cm per rootlength, total root length of plant 107cm, number of vesicles were 174.41 per plant, number of arbusculeswere 87.74 per plant. Arbuscules were not observedin *Piper longum*whereasvesicles colonization 64% and number of vesicles were 2.52 per cm root length Total root length of plant 105 cm, number of vesicles were 264.6 per plant .The results are on similar line of Thenmozhi*et al* (2011) who observed 65 per cent vesicular arbuscularmycorrhizal fungi colonization in *Piper nigrum* with 3 numbers of vesicles and one number of arbuscules per cm. (Table 4, Plate 2 and 3)

Table 4:- Association of vesicular arbuscularmycorrhiza in climbers and grasses

Sr No	Plant name	Percentage of vesicles colonization	Number of vesicles per cm	Percentage of arbusculescolonizaion	Number of arbuscules per cm	Total root length of plant(cm)	Number of vesicles per plant	Number of arbuscules per plant
1	*Cissusquandragularis*	55.21	1.63	16.54	0.82	107.00	174.41	87.74
2	*Abrusprecatorius*	71.52	2.53	27.32	1.53	-	-	-
3	*Piper longum*	64.00	2.52	-	-	-	-	-
4	*Cymbopoganflexuosus*	77.90	3.00	34.00	1.18	156.00	468.00	184.08
5	*Cymbopogan martini*	78.00	3.18	36.01	1.24	147.00	467.46	182.28

Table 5: Vesicular arbuscularmycorrhizal spores observed in rhizospheric soil of climber and grasses medicinal plants

Sr. No	*Scientific name*	No.of spore per 10g rhizospheric soil	Type of spore
1	*Cissusquandragularis*	14.60	*Glomus*
2	*Abrusprecatorius*	24.60	*Glomus*
3	*Piper longum*	12.30	*Glomus*
		9.00	*Gigaspora*
4	*Cymbopoganflexuosus*	20.60	*Glomus*
5	*Cymbopoganmartinii*	18.93	*Glomus*

In grasses viz*Cymbopoganflexuosus and Cymbopoganmartinii f*amilyGramineae wereobserved similar percentage of colonization. In *Cymbopoganflexuosus* 78 percent of vesicle colonization, 36.01 per cent arbuscules colonization, 3.18 vesicles and 1.24 arbuscules per cm root length followed by *Cymbopoganmartinii* 77, 34, 3 and 1.18 respectively (Table 5).

Nassrulah*et al. (*2010) stated association of vesicular arbuscularmycorrhiza 32-68% in wheat and 24-60% in maize (Gramineae) was observed.

In climbers (Table 5) i.e. *Abrusprecatorius, Cissusquandragularis* and *Piper longum* highest vesicular arbuscularmycorrhizal fungal spores were observed in *Abrusprecatorius* 24.60 species *Glomu*s and lowest in *Piper longum* 12.30 and 9.00 species *Glomus, Gigaspora* respectively and In *Cissusquandragularis*14.60 spores per 10g rhizospheric soil was observed with *Glomus sp.* Similar findings were reported by Thenmozhi*et al* (2011).

The data presented in table 5 indicated that in grasses i.e., *Cymbopoganflexuosus* and *Cymbopoganmartinii* observed almost equal number of spores per 10 g soil i.e., 20.60 and 18.93 respectively and type of spores was *Glomussp*.

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Plate 1: Association of vesicular arbuscularmycorrhiza with herbaceous plants

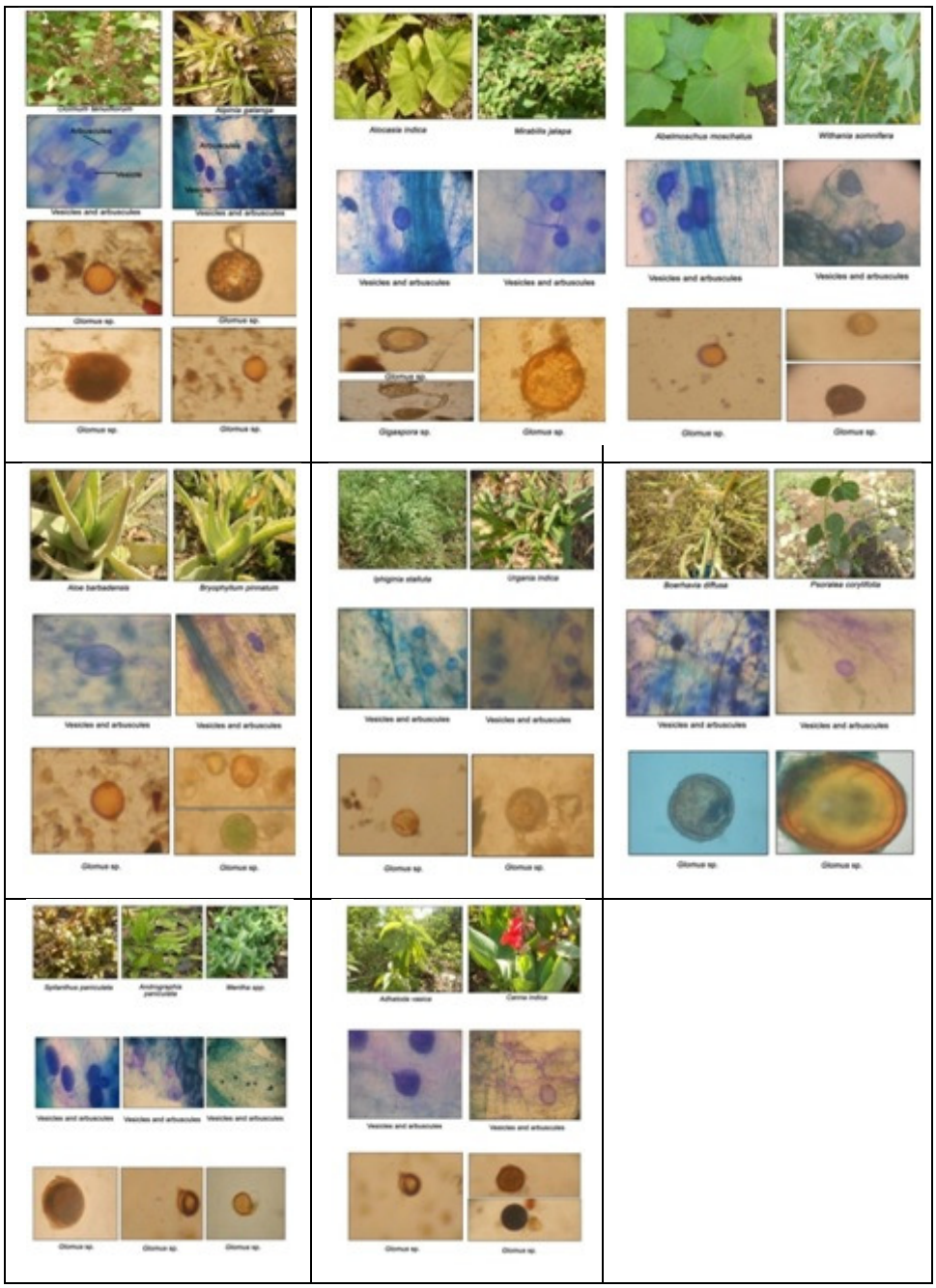

Plate 2: **Association of vesicular arbuscularmycorrhiza with Climbers**	**Plate 3**: **Association of vesicular arbuscularmycorrhiza with grasses**