SUMMARY
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Arbuscular mycorrhizal fungi are obligate symbionts that are found associated with almost all crop plants throughout the world. The ability of AM fungi to influence growth has been reported in many plant species throughout the world. Plant growth promoting rhizomicroorganisms (PGPRs) when selectively combined with these AM fungi influence plant growth in a more positive manner by acting as biocontrol agents and also by producing plant growth hormones, vitamins etc., that are effectively utilized by the plants for their growth. However, reports on the presence of AM fungi and PGPRs in medicinal plant roots and their role in the improvement of plant growth are less documented. Hence, the present study was undertaken to survey the AM fungal association in three selected medicinal plants of Kanyakumari District, Tamil Nadu namely Eclipta prostrata, L. (Family-Asteraceae), Indigofera aspalathoides, Vahl ex DC. and I. tinctoria, L. (Family-Leguminosae; Subfamily-Papilionaceae / Fabaceae). The influence of native efficient AM fungi and PGPRs on the growth, biochemical and antimicrobial potential of the three medicinally important medicinal plants were also studied by subjecting them to pot culture study.

The significant findings of the present study are as follows:

- The three sites selected for the present study were in and around Marunduvalmalai hills, Munchirai hillocks and Veli hills of Kanyakumari district, Tamil Nadu. Three medicinally important plants viz., Eclipta
E. prostrata, L., Indigofera aspalathoides, Vahl ex DC. and I. tinctoria, L., were collected from the selected study sites, identified and assessed for the presence of arbuscular mycorrhizal association in their roots and rhizosphere soil.

➢ E. prostrata plants were collected from the bunds of tapioca and plantain fields in the foothills of Marunduvalmalai and Veli and plains of Munchirai region. The other two Indigofera species were collected from the Marunduvalmalai hills, Munchirai hillocks and Veli hills.

➢ E. prostrata, L. (syn. E. alba), belongs to the family Asteraceae and is used as a hepatoprotective agent and to treat viral jaundice. I. aspalathoides, Vahl. ex DC. and I. tinctoria, L. belong to the family Leguminosae and subfamily Papilionaceae (Fabaceae). The leaves, flowers and tender shoots of I. aspalathoides are used in the treatment of cancer, tuberculosis and leprosy while the whole plant extract of I. tinctoria is used in the treatment of epilepsy and other nervous disorders and the plant is also used as a natural dye.

➢ The physicochemical characteristics of soil samples collected from the three localities indicated that the soils were slightly acidic to neutral.

➢ The texture of the rhizosphere soil collected from Indigofera species in Veli hills and Marunduvalmalai region was sandy loam and it was red sandy loam in Munchirai hillocks. However the texture of the rhizosphere soil of E. prostrata was loamy in the foothills of Veli and sandy clay loam in the foothills of Marunduvalmalai and Munchirai plains.
The nutrient status of the rhizosphere soil of the three plants from different study areas was found to be low to moderate in nature. The macronutrients status of the soil collected from the three sites were 101.54-119.12 kg/acre nitrogen, 4.14-5.51 kg/acre phosphorus and 152.62 - 166.86 kg/acre potassium.

E. prostrata, I. aspalathoides and I. tinctoria were positive for AM fungal colonization although the species of AM fungi varied in the rhizosphere soils of the three plants. AM fungal spores were also isolated from the root zone soils of the three study sites.

The three plants showed good colonization in all the three study sites and higher colonization was recorded in the roots of I. aspalathoides (92.72%) followed by I. tinctoria (80.94%) and E. prostrata (72.95%). Among the three sites, Veli hills showed better AM fungal colonization than the other two study sites.

The AM population in the rhizosphere soil of the three medicinal plants differed in each locality. The AM spore number was higher in Veli hills in the rhizosphere of I. aspalathoides (566 spores/100 g soil) followed by Marunduvalmalai (532 spores) and Munchirai (482 spores) hillocks. It was found to be less in clay loam soils.

A total of 21 species belonging to four genera, Acaulospora, Gigaspora, Glomus and Scutellospora were identified. Glomus was found to be the dominant genus with 12 species.
I. aspalathoides recorded the maximum number of AM fungal species (18) in its root zone soil, followed by I. tinctoria (15) and E. prostrata (12).

Species richness was higher in Veli hills followed by Marunduvalmalai hills and Munchirai hillocks.

The diversity indices were found to be higher in Veli hills region followed by the other two sites.

Analysis of correlation between spore number and various physicochemical properties of rhizosphere soil of E. prostrata showed that all the combinations were positively correlated.

The mean spore number of I. aspalathoides was positively correlated with pH, N, P and Mn, whereas it was negatively correlated with ECSE, organic carbon, K, Cu and Fe.

Mean spore number of I. tinctoria was positively correlated with pH, Cu, Mn and Fe. However it was negatively correlated with ECSE, organic carbon, N, P, K and Zn.

In the trap cultures from field rhizosphere soils, three additional AM fungal species (A. morrowae, G. hoi and G. microcarpum) were identified in E. prostrata, one species (A. spinosa) was identified in I. aspalathoides and three additional species (A. spinosa, G. pakistanica and G. reticulatum) were identified in I. tinctoria.

Of the 23 species recorded from the native soils, A. delicata, G. aggregatum, G. fasciculatum, G. geosporum and G. mosseae were
dominant and showed 100 % frequency in the three sites in the field rhizosphere soils of the three selected medicinal plants. Hence these five dominant species were screened further for selection of efficient AM fungal species.

A pot culture study was conducted in *P. maximum* Jacq., with the five native dominant strains namely *A. delicata*, *G. aggregatum*, *G. fasciculatum*, *G. geosporum* and *G. mosseae*. Among the five different AM fungal species tested in *Panicum* plants, the AM spore number, infection percentage and other morphometric factors such as plant height, biomass and plant phosphorus content were higher in *G. aggregatum* inoculated *P. maximum* plants than the other native dominant AM fungi treated plants.

The three medicinally important plants viz. *E. prostrata*, *I. aspalathoides* and *I. tinctoria* surveyed for AM fungal association were raised in pots inoculated with the efficient AM fungus *G. aggregatum* and PGPRs namely *B. coagulans* and *T. viride*. The test plants were assessed for the influence of these bioinoculants on the growth, nutrition, biochemical constituents and antimicrobial potential.

The three different bioinoculants were applied as single and combined inoculations to the three test plants and uninoculated plants were maintained as control.

*G. aggregatum* and PGPRs inoculated *E. prostrata*, *I. aspalathoides* and *I. tinctoria* plants had showed higher percentage root colonization by AM
fungi and more spore number in the rhizosphere soil, which in turn had resulted in better growth and dry matter production in mycorrhiza inoculated medicinal plants over the uninoculated plants.

- Biomass of *E. prostrata* was found to be higher in dual inoculation of *G. aggregatum* and *B. coagulans*, while the plant height was higher in the triple inoculum treatment.

- Maximum amount of macronutrients namely N, P, K and micronutrient Cu were recorded in *E. prostrata* inoculated with *G. aggregatum* + *B. coagulans* but the micronutrients Zn and Fe were higher in triple inoculation.

- Proteins, amino acids, lipids and phenols were higher in plants inoculated with *G. aggregatum* and *B. coagulans* followed by plants with triple inoculation. Chlorophyll and carotenoid contents were higher in triple inoculation. The native efficient AM fungus *G. aggregatum* alone and in combination with PGPRs recorded significantly higher biochemical constituents than the uninoculated plants.

- Among the enzyme activities assayed, acid phosphatase and alkaline phosphatase were high in *G. aggregatum* + *B. coagulans* treatment and the activity of the other two enzymes, peroxidase and polyphenol oxidase were higher in plants with triple inoculation of *G. aggregatum*, *B. coagulans* and *T. viride*. Alkaloid, flavonoid and saponin contents were also higher in triple inoculation.
In *I. aspalathoides*, plant biomass and plant height were higher in triple inoculation of *G. aggregatum*, *B. coagulans* and *T. viride* followed by plants treated with *G. aggregatum* and *B. coagulans*. Protein, chlorophyll and carotenoid contents were higher in dual inoculation of *G. aggregatum* and *B. coagulans*, while amino acid, lipid and phenol contents were found to be higher in triple inoculation.

N, K, Cu and Zn levels were higher in triple inoculation of test plants whereas P and Fe were observed to be higher in *G. aggregatum* + *B. coagulans* treatment than the other treatments. However significant difference was not observed in Mn content between the mycorrhizal plants and the uninoculated plants.

Acid phosphatase activity of *I. aspalathoides* was higher in *G. aggregatum* + *B. coagulans* treatment followed by plants with triple inoculation of *G. aggregatum*, *B. coagulans* and *T. viride*. Alkaline phosphatase, peroxidase, polyphenol oxidase, alkaloids and flavonoids were higher in triple inoculation followed by plants with dual inoculation of *G. aggregatum* and *B. coagulans*. Saponins were higher in *G. aggregatum* + *B. coagulans* inoculated *I. aspalathoides*.

Dry matter content of *I. tinctoria* was higher in *G. aggregatum* + *B. coagulans* treatment whereas plant height, protein and amino acid contents were higher in *G. aggregatum* coinoculated with *B. coagulans* and *T. viride*. 

155
N and Fe were higher in plants with triple inoculation than the other treatments while P, K, Cu and Zn were higher in *G. aggregatum* coinoculated with *B. coagulans*. In *I. tinctoria* also, the Mn content of bioinoculant treated and uninoculated plants did not show significant difference.

Chlorophyll, carotenoid, lipid and phenol contents and phosphatase activities were higher in triple inoculation of *I. tinctoria* than the other treatments. Polyphenol oxidase activity was higher in plants with dual inoculation of *G. aggregatum* and *B. coagulans*. The inoculation of efficient AM fungi in combination with the two PGPRs had positively influenced the growth and biochemical constituents of *I. tinctoria* whereas *B. coagulans* and *T. viride* as individual inoculations did not show significant results.

In all the three medicinal plants, the combined inoculation of *G. aggregatum*, *B. coagulans* and *T. viride* proved to be better than the other treatments in enhancing the growth and biochemical parameters. Even though the dual inoculation of *G. aggregatum* and *B. coagulans* produced better results in some cases it was statistically on par with the triple inoculation. Hence the three medicinally important plants viz. *E. prostrata*, *I. aspalathoides* and *I. tinctoria* subjected to triple inoculation with the native efficient AM fungus *G. aggregatum* and PGPRs, *B. coagulans* and *T. viride*, were further subjected to phytochemical and antimicrobial analyses.
Thin layer chromatography was performed to elute phytochemical compounds from the test plants by using solvents of increasing polarity and in various combinations of mobile phases. More phytochemical compounds were detected in aqueous extract than acetone extract of the three test plants.

Qualitative analyses of phytochemical constituents of both mycorrhizal and non-mycorrhizal *E. prostrata*, *I. aspalathoides* and *I. tinctoria* showed that the aqueous extract revealed maximum number of compounds than the acetone extract. Solvent extracts of mycorrhizal and non-mycorrhizal plants did not show much variation in the phytochemical composition.

Infrared spectra of the three test plants did not show any variation in spectral bands between mycorrhiza inoculated and uninoculated plants. Changes in functional groups of the plants have not been observed due to the inoculation of AM fungi and PGPRs.

Antibacterial activity of various solvent extracts of mycorrhizal and non-mycorrhizal test plants was studied against two Gram positive bacteria (*S. aureus*, *S. pyogenes*) and three Gram negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*).

Aqueous extracts were found to exhibit more antimicrobial activity than acetone extracts. All the five pathogenic bacteria were sensitive to the aqueous extract of the three test plants as the zone of inhibition were above 12 mm. The activities of aqueous extracts of mycorrhiza inoculated and uninoculated *E. prostrata* against four of the dreadful pathogens tested in
the present study were on par with the standard antibiotic, thus indicating that they were highly sensitive to the extract.

- The aqueous extract of mycorrhiza inoculated and uninoculated *I. aspalathoides* pronounced maximum activity against *E. coli* which was on par with the standard antibiotic.

- The aqueous extract of mycorrhiza inoculated *I. tinctoria* produced maximum and significant activity against *S. aureus*.

- The comparative antimicrobial study of the three test plants showed that aqueous extracts of native efficient AM fungus, *G. aggregatum* and PGPRs (*B. coagulans* and *T. viride*) treated plants produced significantly higher activity against the Gram positive bacteria viz., *S. pyogenes* and *S. aureus* than the uninoculated plants; however extracts of mycorrhiza inoculated and uninoculated plants were equally effective against the Gram negative bacteria.