SUMMARY AND CONCLUSION

G. sylvestre (Asclepiadaceae), a vulnerable species is a perennial, medicinal woody climber found in central and peninsular India. It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine. In addition, it possesses antimicrobial, antihypercholesterolemic, hepatoprotective and sweet suppressing activities. The leaves of G. sylvestre contain triterpene saponins belonging to oleanane and dammarene classes. Saponins considered as “antinutritional factors” but they have their limited usage due to bitter taste. Hence most of the earlier investigation on saponins processing targeted their removal to facilitate human consumption. But both food and non-food sources of saponins come into major focus in recent years due to mounting evidence of their health benefits such as cholesterol reducing and anticancer properties. Triterpenoid saponins are glycosides refers to the attachment of various sugar molecules to the triterpene units. These sugar molecules will be digested in the gut by gut microbes and allowing the aglycone (triterpene) to be absorbed. This property allows them insert in cell membrane and it modifies the membrane composition and influence the fluidity of membranes. Saponins can greatly impact the immune system due to their ability to act as adjuvant by stimulating immunological response against antigen and their oral administration facilitates the absorption of large complex molecules.

Due to their structural complexity and toxicity saponins have been limited their use in human vaccines, but the evolution of new processing and purification techniques yields different fractions with optimal immunological adjuvant activity and with minimal toxicity and haemolytic activity consequently there is a significant progress in the development of saponins as new generation vaccines. Many steroid and triterpenoid saponins have been demonstrated as potential anticancer agents Triterpenoid saponins refers to the attachement of various sugar molecules to the aglycone, triterpene units (C\textsubscript{30}H\textsubscript{48}). These sugar molecules will be cleaved off in the gut by gut microbes and allowing the aglycone (triterpene) to be absorbed. This allows them insert in cell membrane and changes the composition and impact the fluidity or plasticity of membrane and affecting signaling by many ligands. The
investigation made by various workers related to its medicinal uses, chemical constituents, pharmacological activities and other aspects considering this plant since years till date.

In the present study fungal endophytes were isolated from healthy leaf and stem segments of the *R.beddomei*, employed standard isolation methods. One hundred and twenty three of each leaf and stem segments were analysed. Isolated endophytic fungi were 8 sporulating strains and were identified based on their morphological characteristics and the other non-sporulating strains were grouped into seven morphotypes. The colony appearance, conidia and hyphal features of non-sporulating endophytic fungi (morphotypes) isolated from *R.beddomei* were considered. The sporulating isolates are *Alternaria alternate*, *Aspergillus aculeatus*, *Colletotrichum gloeosporioides*, *Pestalotiopsis maculans*, *Phyllosticta elongate*, *Xylaria*, *phomopsis* and *Penicillium corylophilum*. All the morphotypes isolated were then identified based on the 18S rRNA gene sequence analysis. The colonization and isolation frequency were greater on leaf tissue than on stem tissue this is may be due to change in their anatomical structure.

In the present study, A total of 15 fungal endophytes were screened, two fungal endophytes showed maximum production of phytoharmones when compared to others. The range of IAA production with or without tryptophan was found to be (25.2 μg/ml) in *Aspergillus japonicus*, while *Sordariomycetes* shows a significantly higher amount of IAA (27.8 μg/ml). When compared to control. Similarly in gibberellins *Sordariomycetes* (0.13 μg /25ml) shows significantly higher amount of gibberellic acid followed by *Aspergillus japonicus* and control.

The co-culture system is assumed to be a meaningful and effective tool to enhance in planta production of natural products. The present study was carried out to examine the effect of VAM (*Glomus mosseae*), endophytic fungi *Sordariomycetes*+*Aspergillus* on the growth, biomass physiology and yield of gymnemic acid in *G.Sylvestre* in pot experiments carried out in green house. Seven different treatments were established to study the effect of different combinations of bioinoculants on growth and yield of Gymnemic acid in *G.Sylvestre* plants the treatments were as T1: Without inoculation, T2:VAM , T3: Fungal Endophyte (*Sordariomycetes*), T4: Fungal Endophyte (*Aspergillus japonicus*), T5: VAM+
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Sordariomycetes, T₆: VAM+Aspergillus japonicus, T₇: VAM+Sordariomycetes+Aspergillus japonicus. The results revealed that inoculation of endophytic fungi Sordariomycetes sp as single inoculation enhanced the shoot length, number of leaves, leaf area, fresh and dry biomass of shoot and root and mineral nutrients in plants compared to control. The contents of chlorophyll, carbohydrates, proteins were also found increased over control. But the inoculation of G. Sylvestre plants with Endomycorrhizae + Sordariomycetes + Aspergillus japonicus as mixed inoculum helped in highest accumulation of shoot and root biomass and mineral nutrients like N, P, K, Ca, Mg, Fe, Mn and Zn in plant tissue than single inoculum application. The mixed inoculum application helped the plant in synthesizing more chlorophyll, carbohydrates and proteins than single inoculum due to the synergistic interaction which exist among the microorganisms in the rhizosphere.

The soil analysis revealed a significant difference in pH at 1% level in different treatments. Soil pH in the control (T₁) was ranged from 6.84 to 6.91 and in the T₂, T₃, T₄, T₅, T₆ and T₇ treatments, it ranged from 7.01 to 7.17. On 30th and 90th day, the highest percent of organic carbon was recorded in T₇ (0.87, 0.90) followed by T₆ plants (0.84, 0.87). The percent of organic carbon in T₅ plants was found almost similar to T₄ plants. On 60th day highest organic carbon was recorded in T₇ plants (0.90) when compared to all other treatments. The content of organic carbon in T₂, T₃, T₄ T₅ and T₆, treatments was significantly (0.01%) high compared to T₁ plants. The soil nitrogen content in T₇ treatment was found to be the higher compared to all other treatments. The amount of nitrogen on 30th, 60th and 90th day in T₇ was 12.57, 26.74 24.65 (g/kg) respectively. Similarly the content of Potassium, Phosphorus, Copper, Iron, Zinc, Manganese were also high in T₇ plants compared to control and dual inoculation. This suggest the application of VAM+Sordariomycetes+Aspergillus japonicus as mixed culture is more beneficial to enhance the yield and active principles of pharmaceutical importance and soil fertility.

In the present study the amount of gymnemic acid content in G. sylvestre treated with fungal endophyte and VAM in T₁, T₂, T₃, T₄, T₅, T₆ and T₇ extracts, were 1.3, 1.5, 1.6, 1.4, 1.9, 1.8 and 2.0 (% w/w) respectively. The recovery of gymnemic acid was 2.0%. HPLC analyses showed higher amounts of gymnemic acid in the triple inoculated methanol extracts of G. sylvestre. This is due to increase in the number of leaves, biomass per
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plant and its biosynthesis between consortium and untreated plants. The total Gene expression content of Glucosyltransferase were significantly higher in treatment seven (T7:VAM+Sordariomycetes+ Aspergillus japonicus) when compared to single and dual inoculation.

In conclusion Endophytic fungi are of highly diverse and intriguing group of microorganisms spend the whole or part of their lifecycle colonizing inter or intracellularly in the healthy tissues of the plant typically causing no apparent symptoms of disease. These are considered as alternative of plant secondary metabolites as they are producing bioactive compounds of pharmaceutical applications such as antibiotics, antioxidants, anticancer and antiviral activities. And hence there is a need for isolation of endophytes and their exploitation at industrial level for producing pharmaceutically important bioactive compounds. The present study can be explored for large scale production of Gymnemic acid by the cultivars to meet the phytopharmaceutical demand. It can be concluded that G. sylvestre leaves extract exhibit antidiabetic activity, so it is recommended to use it as a complementary medicine in diabetes mellitus. Hence, clinical studies on G. sylvestre leaves extract as a complementary medicine are needed.