"Investigation of efficacy of endophytes for the disease management and growth enhancement of endangered medicinal plant *Withania somnifera*"

Thesis
Submitted to
Babasaheb Bhimrao Ambedkar University
Lucknow

BABASAHEB BHIMRAO AMBEDKER UNIVERSITY
•LUCKNOW•
प्रज्ञा शील करुणा
ESTABLISHED 1996

For the degree of

Doctor of Philosophy

In

Environmental Microbiology

Submitted By

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(Enrolment no. 385/11)

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2016

SUMMARY

Culture filtrates of isolates WRE-1 and WSEF-4 showed inhibitory effect on fungal pathogens thus their bioactive secondary metabolites were extracted and partially characterized using FTIR analysis. Ethyl acetate extracts of both the isolates showed maximum inhibitory effect on fungal pathogens. Metabolite produced by isolates WRE-1 was partially characterized as a derivative of phenazine and bioactive secondary metabolite of WSEF-4 was characterized as an aromatic compound. Effect of selected strains on seed germination and seedling vigor was also examined. Isolates WRE-1 and WSEF-4 significantly improved percent seed germination in comparison to control. Fungal pathogen *A. alternata* inoculated seeds showed pre-emergent seedling mortality and most seeds failed to germinate. *F. solani* treated seeds showed post-emergent seedling mortality and damping off of seedlings. Both the pathogens highly reduced germination percentage. Seeds treated with *A. alternata* and *F. solani* when co-inoculated with isolates WRE-1 and WSEF-4, showed great improvement in germination and seedling vigor index with no apparent seedling mortality or damping off. Endophytic nature and colonization ability of both the selected endophytes was determined and their plant growth promoting and biocontrol potential was investigated by pot experiments and field trials. Pot experiments and field trials (during October 2014 to January 2015; February to May 2015; October 2015 to February 2016) were conducted using endophyte-free seedlings of 'Poshita' variety of *W. somnifera*. Pot and field experiments showed promising results for growth enhancing and biocontrol potential of endophytes WRE-1 and WSEF-4. Plants treated with endophytes showed significantly improved shoot and root length, fresh and dry weight and improved content of chlorophyll, total phenolics, reducing sugar and total withanolides. Endophytes when co-inoculated with fungal pathogens were found able to control the disease development in treated plants and increased plant growth. Compatibility of both the endophyte isolates was also examined with an important rhizospheric

AM fungi *Glomus intraradices*. Isolate WRE-1 showed inhibitory effect on *G. intraradices* and reduced the root colonization. On the other hand, WSEF-4 showed great compatibility with *G. intraradices* and no inhibition was observed for root colonization. Co-inoculation of endophyte WSEF-4 with AM fungi *G. intraradices* also showed maximum improvement in plant growth. Disease development in WSEF-4+GI treated plants was completely suppressed and plants showed significantly higher yield and quality in comparison to control. Soil analysis also revealed significant improvement in soil properties after inoculation of endophytes WRE-1 and WSEF-4. *W. somnifera* itself is capable to improve the soil properties as observed during comparative analysis of soil from uncultivated land and rhizospheric soil after plantation of *W. somnifera*. All the microbial inoculants including WRE-1, WSEF-4 and G. *intraradices* improved nutrient level and significantly reduced the alkaline nature of soil. Maximum improvement of total organic C, available N, P and K was observed in WSEF-4+GI amended soil.

From above mentioned findings, it can be concluded that endophytes hold a great potential for their application as biofertilizers and biopesticides in the commercial cultivation of medicinal plants. Less cultivation of *W. somnifera* despite its high demand in market is due to low seed germination, high seedling mortality and high yield loss due to fungal pathogens. Moreover, use of synthetic agrochemicals is restricted and alternative techniques to promote plant yield and to control pests and fungal pathogens are costly and not easily accessible to farmers. Present study provided an initial assessment of the potential of endophytes associated with *W. somnifera*, in plant growth promotion and biocontrol of destructive fungal pathogens. Isolates WRE-1 and WSEF-4 were not only found capable of enhancing seed germination in *W. somnifera* but also effectively controlled seed-borne fungal infections and significantly reduced pre- and post-

emergent seedling mortality. Both the isolates were efficient colonizers of *W. somnifera* and showed plant growth promoting and biocontrol potential under controlled conditions in green house as well as under field conditions. Results obtained from our study suggest that endophytes can be used as alternative of synthetic agrochemicals for the cultivation of medicinal plants. Studies also suggest that endophytes possess high colonization efficiency and survivability under field conditions thus less prone to fluctuations in environmental conditions. Endophytes-based products can support the organic farming practices for medicinal plants and can be adopted for safe and eco-friendly sustainable agriculture.