EFFECTS OF RHIZOSPHERE SOIL PHYSICOCHEMICAL CHARACTERISTICS ON PHYTOCHEMICALS AND ANTIOXIDANT ACTIVITY IN *TAXUS WALLICHIANA* OF GALIYAT-KHYBER PAKHTUNKHWA

ABSTRACT

Taxus wallichiana (TW) (Himalayan yew) is an exotic specie with low regeneration potential but high ethnobotanical and pharmacological value, making it an area of significant interest for researchers. Unfortunately, over the last few decades, this species has been under constant threat due to climate change, which may alter its antioxidant potential, soil conditions, nutrient distribution and availability, and lead to the extinction of beneficial plant growth-promoting rhizo-microbial communities and their richness. Information about soil conditions, nutrient availability, and microbial diversity in the rhizosphere of TW in the forests of Galiyat-Himalayan-Khyber-Pakhtunkhwa-Pakistan has not been previously reported. Furthermore, the beneficial effect of rhizosphere microbes as an inoculum on TW's antioxidant properties, phytochemical composition, soil conditions, and nutrient availability is scarce. This study aimed to prepare and apply a microbial formulation to rhizosphere soil to improve the physicochemical characteristics of the soil and the antioxidant activity of TW. To achieve this, the following objectives were studied

1. To evaluate the rhizosphere and non-rhizosphere soil physicochemical characteristics and phytochemical+antioxidant activity of TW at various altitudinal gradients.
2. To isolate, characterize, and identify different bacterial and fungal strains in the rhizosphere and non-rhizosphere soil at various altitudinal gradients.
3. To establish the response of isolated microbes alone, or as a consortium to enhance the soil physicochemical characteristics and antioxidant activity of TW.

The first objective is divided into three parts: A, B, and C. Part A, discussed in Chapter 2 of the thesis, focuses on the antioxidant and phytochemical activity of TW at various altitudinal gradients.

To achieve the parts B and C of the first objective, "To evaluate the rhizosphere and non-rhizosphere soil physicochemical characteristics of TW at variousaltitudinal gradients" around five replicates of rhizosphere and non rhizosphere (where found) was collected from the TW of the sampling sites. All soil samples were analyzed for organic carbon byloss on ignition method and available-N and P with Kjeldahl and Olsen methods.

Forest nutrient management was overlooked when sustainable forest management policies were documented. Therefore, for better forest management, a database of soil carbon, available-nitrogen,extractable-phosphorus and potassium, and some other phyto and anthropogenically trace elements such as Cu, Fe, Zn, and Cd, Pb need to be prepared. It was hypothesized in this study that organic carbon, nitrogen, phosphorus and their ratios with stoichiometric ratio C:N:P in the soils of TW, may be used as an indicator for proper nutrient management to mitigate climate change. The results showed that the rhizosphere of TW was enriched with organic carbon, available-Nitrogen (AN) and stoichiometric ratio of C:N:P than non-rhizosphere along altitudinal gradient. However, available-Phosphorus (AP) was increases with horizon thickness. Pearsons's correlation analysis showed that organic carbon was negatively correlated ($P<0.05$) with horizon thickness and positively correlated ($P<0.05$) with altitude, available-N and P. AP was negatively correlated with horizon thickness. There was no correlation between available-N and P. Nutrients

database was generated and Bagging and Random Forest 3 model was applied to predict the dependency of NPK model on other non-predictable variables like pH, EC, soil organic matterand moisture content, available Cd, Cu, Fe, Pb and Zn specifically. Knowledge about the changein the distribution of nutrient status and other physicochemical characteristics in the rhizosphereis in scarcity. The prediction made by NPK model was considered as best fitted prediction modelfor nutrient management of rhizosphere of TW in the moist temperate forests of Galiyat-Himalayan. The NPK model accurate and precise prediction will provide an automatic estimationof nutrients status of unsampled rhizosphere of TW with non-significant variations in their physicochemical characteristics. The bagging and random forest model shows that in this study the increase value of Mean Squared Error percentage (IncMSE%) was with pH, followed by Zn and organic matter, EC exhibiting the highest value (0.0002), this prominence was closely trailed by the notable contributions of potassium and Iron. Cadmium has lowest Increase in Node Purity, suggesting a relatively minor impact on the overall predictions. P showed slightly different metrics, in that pH showed the highest IcnMSE%. In this case, EC has the highest Node purity followed by Fe and Cd. For K, the highest IncMSE% and Node purity were observed for Zn and the lowest for P.

To achieve the second objective, "To isolate, characterize, and identify different bacterial and fungal strains in the rhizosphere and non-rhizosphere soil at various altitudinal gradients" soil samples were collected from the rhizosphere and non rhizosphere of TW of all sampling sites. The results show that *Bacillus sp*. was the dominant specie found in the rhizosphere of Ayubia, Baragali, and Murree (*Bacillus sp*. TS1223R, *Bacillus mycoides* strain TW14, *Perri bacillus simplex*). Whereas *Pseudomonas sp* was dominant in Kuldana rhizosphere. *Endophyte* sp was found at Dungagali rhizosphere and non rhizosphere. However non rhizosphere of Murree (low altitude) was *Pseudomonas* sp Tibet YD5003. *Phoma* and *Rhizopus* were the dominant fungi occurred in all rhizosphere and non rhizosphere soils of TW. *Phoma* and *Rhizopus* were dominant in Ayubia (high altitude) rhizosphere, whereas *Phoma* was in abundance in the rhizosphere soils of Kuldana and Murree. Whereas Rhizopus was found in the Baragali and Dungagali rhizosphere.

To achieve third objective," To establish the response of isolated microbes alone or as a consortium to enhance the soil physicochemical characteristics and antioxidant activity of TW" a pot experiment was conducted under nursery conditions.

Perri bacillus simplex (PBS) and *Pseudomonas Tibet* (PT) separately was inoculated to the autoclave soil of Burban in the pot over which TW seedlings was planted. The results show that PBS accelerate the antioxidant potential (DPPH%) to 93% than PT and control soils in the TW seedlings under nursery conditions after 75 days of incubation. *Perri bacillus simplex* accelerate the SOD, POD and ascorbate peroxidase (APX) % than PT and control, after 75 days of incubation under nursery conditions. Total organic matter and available nitrogen was slightly increased from
2.76 to 3.10% and 0.138% in control to 1.65% in the autoclave soil after inoculating with PBS and PT. Phosphorus remained same and had not been affected with PT and PBS whereas potassium content was increased from 76 to 97mg/kg in the rhizosphere soil with PT. The vegetative growth of TW seedlings after inoculating PT has increased the stem length to 2 inches and branch length to 1.5inches after 75 days of incubation. The microbial formulation PBS was considered as more efficient plant growth promoting rhizobacteria than PT whereas PT was effective in accelerating the vegetative growth than PBS and control of the TW seedlings after 75 days of incubation