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Impact of Seasonal Water Deficit on the Secondary Metabolite Profile and Antioxidant Defense System of *Andrographis paniculata* (Kalmegh) in Jharkhand's Forest Ecosystem

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ABSTRACT

Seasonal water deficiency is among the most significant non-living elements which disrupt the growth, activity and chemical composition of medicinal plants in the tropical forests. *Andrographis paniculata* (Burm. f.) Nees is among the most demanded plants among the large number of medicinal plants due to its biogenetic secondary metabolites as a diterpene andrographolide and other derivative compounds. The current study is expected to be focused on the study of effects of seasonal water scarcity on metabolite profile and photo-protection of A. paniculata that naturally grows in Jharkhand, India. The experiment was founded on field experiments, carried out using three different seasons, i.e. monsoon (enough water), post-monsoon (moderate lack of water) and summer (extreme lack of water). The total phenolic content, flavonoid, and andrographolide concentration and the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were assessed by scientists. the moderate level of stress caused the most significant increase in the quantity of secondary metabolites and antioxidant enzyme activities, and the so-called metabolic imbalance and the damage of the oxidative processes were observed during the periods of toxic droughts. The paper introduces the crude biochemical state to which A. paniculata has adapted due to the seasonal drought and in that, presents the agricultural and harvesting techniques which are dependent on the droughted-water situation as the primary benefit.

Keywords - *Andrographis paniculata*, seasonal drought, secondary metabolites, antioxidant enxymes.

1. Introduction

The traditional Indian curing of medicinal plants, which is inseparable with the biodiversity of forests, forms an important component of the traditional Indian culture. *Andrographis paniculata* (Kalmegh) is one of the most famous plants that have been reported to possess medicinal properties including; anti-inflammatory, antipyretic, hepatoprotective and immunomodulatory.

The general effectiveness of this herb is largely attributed to its secondary metabolites with the main being andrographolide, neo andrographolide, flavonoids and phenolic compounds. In the forest areas such as Jharkhand, the plants are exposed on daily basis to seasonal alterations of rain and moisture contents of the soil. One of the most

important non-biotic stresses that affects the metabolism of plants is water shortage caused by the dry summer months especially with a long duration.

As a result of water stress, physiological variations, reactive oxygen species (ROS) formation and subsequent regulation of secondary metabolite synthesis in plants occur as a defense system. Some of the research reports indicate that moderated drought stress may have the effect of triggering the production of medicinal compounds in plants, and low water supply may cause closure of all the metabolic routes and consequently low production of plants. Nevertheless, seasonal water shortage has scarcely been studied on A. paniculata in its natural forest. The given research work is, therefore, aimed at researching the changes in seasonal production of secondary metabolites, and antioxidant defense mechanism of A. paniculata in the forest ecosystem of Jharkhand.

2. Review Of Literature

The interest has been keen in the study of physiological and biochemical adaptations of medicinal plants to environmental stressors, particularly water shortage, which has been inconclusive in information. Most of the studies have established that the plants that experienced moderate level of drought stress might produce the secondary metabolites in their defense mechanism. As an example, under controlled water stress, the concentrations of phenolic and flavonoid compounds, and the activity of antioxidants such as superoxide dismutase (SOD) and catalase (CAT) have been increased with species such as Ocimum basilicum and Hypericum perforatum (Pandey et al., 2017; Chandra et al., 2019). The scope of these responses is usually linked with the stimulation of the prestigious biosynthetic pathways during oxidative stress that may enhance the therapeutic benefit of the plant.

However, when there is a prolonged or severe drought, there may be a break in the metabolism, sluggish growth, and reduced synthesis of phytochemicals; this is what was experienced with Withania somnifera and Coleus forskohlii that were left to experience extreme dry conditions (Rao & Patel, 2021). These experiments give rise to a more improved understanding of the factor-stress-factor-quality-of-metabolic-output interrelations- an idea that is quite necessarily at the core of the current studies on *Andrographis paniculata*.

The case of A. paniculata has had minimal research conducted which has involved the application of controlled greenhouse or laboratory conditions, the areas of study being irrigation regimes and simulated drought scenarios. Kumar and Singh (2019) argue that the content of Andrographolide in potted plants rose by 40% during water shortage, but Mehra et al. (2020) note that there was significant activity in antioxidant enzymes in moderate stress. However, there has been a huge gap in the ecological research on the anticipated effect of inter seasonal variation in water particularly in natural forests since sometimes the same would happen. The Jharkhand forests, characterized by recognizable monsoon, post-monsoon and summer seasons, therefore, offer a great natural laboratory to study the processes and explore their conditions in the field.

3. Materials and Methods

3.1 Study Area

The study which entailed a direct observation of the ecosystem was conducted in the various parts of the state of Jharkhand, India and to be specific, it was conducted within the districts of Ranchi, Gumla and Simdeba. In addition, the study sites belong to the tropical dry deciduous forest biome and are regarded as the ecosystem of the Chota Nagpur Plateau. Climate of these regions can sufficiently be characterized by a heavy change in season, which the Kolpen Geiger system classifies as AW; it passes through an undisputable sequence of wet and dry seasons. The annual rainfall ranges between 1100-1300 mm, and approximately 80-85mm of the total rainfall occurs during the southwest monsoon that

occurs between June and September. The rainfall and soil moisture decrease rapidly during the post-monsoon (October-November) season and this leads to the development of drought conditions. Summer (months of March-May) is characterized by hard hydrological pressure resulting in high heat (temperatures are usually more than 40C) and low relative humidity plus almost no precipitation. The selected areas in which the investigation was conducted included natural patches of forests, which were not disturbed and inhabited by the *Andrographis paniculata*. In such a manner the results would be credited to occurrences in nature in response to the ecological adaptations and not by the reaction of the cultivated plants. The appropriate state forest departments issued the required licenses of the collection and the study.

3.2 Plant Material Collection and Preparation

The natural and continuously varying water deficit was the cause of phenotypic and biochemical plasticity of A. paniculata as it was sampled at the culmination of each of the seasonal phases i.e. monsoon, post-monsoon, and summer which corresponded to the maximum and minimum water availability and water deficit, respectively. During the monsoon (high water supply), post-monsoon (late October; moderate water shortage), and summer seasons (mid-April; extreme water shortage), mature and disease-free plants at the same phenological stage were taken through a pre-flowering to early flowering process of harvesting. In each of the seasons at least fifteen individual plants were chosen randomly between three plots (five plants per plot), in order to take into account, the variability of the microhabitat. The third part was taken between 8:00 to 10:00 because fully expanded middle leaves of the plant were taken to reduce the effects of daily physiological differences.

The collected samples of the leaves were immediately analyzed in a field laboratory. Then they were extensively washed in distilled water intended to remove any epiphyte contaminants and dried by ballooning with care. In case of biochemical tests, which required a fresh tissue (antioxidant enzyme analysis), a single portion of the sample was flash-frozen in liquid nitrogen and stored in a freezer at -80 C until the analysis time. The remaining part of the leaves was placed in breathable paper bags, and dried at room temperature (25-30 C) in a warm, well-ventilated and dark room of approximately 10-15 days until a steady weight had been achieved. Then, the leaves were finely powdered using a mechanical grinder with a ceramic lining to prevent any contamination of metal and was subsequently sifted through a 60- mesh sieve to ensure consistency and then stored in airtight, light-proofed containers at 4 o C with desiccant until further phytochemical analysis.

3.3 Assessment of Water Stress Intensity

To be able to have a quantitative relationship between the biochemical reactions of the plant and the soil water status, the seasonal soil moisture content was closely monitored. The rhizospheric zone (0-20 cm depth) of the sampled plants was sampled by taking soil samples at the same time as the plants had been harvested. The reason why the gravimetric method was applied was that it is a reliable method with direct application to field conditions. To recap, fresh soil samples were weighed, dried at 105 degrees Celsius at 48 hours until constant mass was attained and the weight of the sample was recorded again. Percentage of moisture in the soil was calculated as: [(Fresh weight - Dry weight)/ Dry weight) x 100]. The results of this experiment gave an indirect estimate of the three experimental conditions, monsoon (unstressed, soil moisture >25%), post-monsoon (moderate stress, soil moisture 12-18%), and summer (acute stress, soil moisture <8%).

3.4 Estimation of Secondary Metabolites

The quantification of major secondary metabolites was carried out according to the protocols:

Total Phenolic Content (TPC): The TPC values of the leaf extracts were obtained by the Folin-Ciocalteu reagent method using gallic acid as the standard. In short, a mixture of 0.5 mL of the methanolic extract (obtained from 100 mg of dried powder) and 2.5 mL of 10% Folin-Ciocalteu reagent was prepared and allowed to react for 5 minutes. Following this, 2 mL of 7.5% sodium carbonate solution was introduced to the mixture. The solution was cultivated in the dark under room temperature for 60 minutes, and absorbance was taken at 765 nm on a UV-Vis spectrophotometer. The results were given as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total Flavonoid Content (TFC): Quercetin was used as the reference standard in the aluminum chloride colorimetric assay used to determine the TFC. 0.3 mL of the 5% $NaNO_2$ solution was added after 1 mL of the plant extract and 4 mL of distilled water were combined. 0.3 mL of the 10% $AlCl_3$ solution was added after 5 minutes, and 2 mL of the 1 M NaOH solution was added after 6 minutes. The absorbance of the pink complex was measured right away at 510 nm after the mixture was diluted to 10 mL with distilled water. Milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW) were used to calculate and report the TFC.

Content of Andrographolide: As mentioned earlier, the primary bioactive compound, andrographolide was detected in its quantifiable amount by means of reverse-phase High-Performance Liquid Chromatography (HPLC). The C18 column (250 mm x 4.6 mm, 5 µm particle size) was used and the detection was through a photodiode of a specific wavelength of 223 nm. The mobile phase was a mixture of acetonitrile and water flowing at a rate of 1.0 mL/min. The leaf powder was extracted with methanol in a Soxhlet apparatus till it was completely done. After that, the extract was filtered, concentrated under vacuum, and dissolved in the mobile phase. The calorimeter for andrographolide was created using pure andrographolide (≥98%, Sigma-Aldrich). The content of andrographolide in the samples was determined through retention time, UV spectra and standard comparison and it was stated in terms of percentage of dry weight (% w/w).

3.5 Antioxidant Enzyme Analysis

Fresh plant tissue (0.5 g) frozen at -80°C was ground up in a mixture of 5 mL of liquid nitrogen phosphate buffer (50 mM, pH 7.0) which included 1% (w/v) polyvinylpyrrolidone (PVP) and 1 mM EDTA. Then, the homogenate was subjected to centrifugation at 15,000 × g for 20 minutes at 4°C, and the supernatant obtained was utilized directly as the crude enzyme extract for upcoming assays:

Superoxide Dismutase (SOD; EC 1.15.1.1): The method for SOD activity measurement was illuminated by the assay that relied on the capability of SOD to restrain the transformation of nitro blue tetrazolium (NBT) to formazan in the presence of riboflavin and methionine under light conditions. One SOD activity unit was determined as the volume of enzyme that caused a halt of 50% to NBT reduction, which was monitored at 560 nm.

Catalase (CAT; EC 1.11.1.6): CAT activity was measured by the monitoring of H_2O_2 depletion at 240 nm (extinction coefficient 39.4 mM^{-1} cm^{-1}). The reaction mixture consisted of phosphate buffer (50 mM, pH 7.0) and a concentration of 10 mM H_2O_2. The absorbance drop per minute was noted.

Peroxidase (POD; EC 1.11.1.7): The enzymatic activity of POD was evaluated with the guaiacol substrate. The rise of absorbance at 470 nm resulting from the tetraguaiacol formation (extinction coefficient 26.6 mM^{-1} cm^{-1}) was documented for two minutes. The components of the reaction were phosphate buffer (50 mM, pH 6.5), 10 mM guaiacol, 5 mM H_2O_2, together with the enzyme extract.

The content of protein in the enzyme extract was measured through the Bradford method, which was based on bovine serum albumin as the standard. The activities of all enzymes were expressed in terms of units per milligram of protein (U mg^{-1} protein).

3.6 Statistical Analysis

Each plant sample (n=15 per season) was analyzed biochemically in triplicate. The results were statistically processed through SPSS software (Version 25.0). A one-way analysis of variance (ANOVA) was used to assess the impact of seasonal water deficit (independent variable with three levels: monsoon, post-monsoon, summer) on all the dependent variables (TPC, TFC, andrographolide, SOD, CAT, and POD). The variance homogeneity was checked with Levene's test. Where ANOVA showed significant differences ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) test at a 95% confidence level was used for post-hoc comparisons of seasonal means. The data is given as mean ± standard deviation (SD) for all cases. OriginPro software was employed to prepare graphical representations.

4. Results

4.1 Seasonal Variation in Secondary Metabolites

The secondary metabolite profile of *Andrographis paniculata* in its ecosystem was significantly ($p < 0.05$) impacted by the seasonal water deficit in a variety of ways. The one-way ANOVA and the post-hoc Tukey's HSD test showed that the plants' moderate water stress had a significant impact on the production of secondary metabolites during the post-monsoon season, making it a potent elicitor of the biosynthesis process.

There was a clear hormetic response in the Total Phenolic Content (TPC). With a mean of 42.7 ± 3.1 mg GAE/g DW, it was at its lowest during the monsoon season (when there was plenty of water). Under moderate post-monsoon stress, TPC drastically increased by about 52% to reach 65.1 ± 4.8 mg GAE/g DW, which may be a sign of the activation of the phenylpropanoid pathway, a well-known reaction to oxidative stress. The extreme water shortage during the summer caused the TPC to drop to 48.3 ± 3.9 mg GAE/g DW, which is comparable to the monsoon season but significantly lower than the post-monsoon peak. This suggests that the harsh conditions caused a shutdown in the biosynthetic process.

Total Flavonoid Content (TFC) showed the same pattern. A floor of 28.5 ± 2.4 mg QE/g DW was established during the monsoon season. TFC increased to 45.9 ± 3.7 mg QE/g DW, a remarkable 61% increase brought on by post-monsoon conditions. In contrast, the flavonoid content drastically changed during the summer, falling to 22.1 ± 2.1 mg QE/g DW, a level much lower than that of the monsoon and post-monsoon periods. This suggests that prolonged and severe drought inhibits this pathway, most likely due to carbon shortage or enzyme inactivation, whereas mild drought increases flavonoid synthesis as one of the antioxidant and UV-protective tools.

The main bioactive substance, andrographolide, exhibited a clear pattern of stress-mediated enhancement followed by inhibition. During the monsoon season, the average amount of andrographolide was 2.1 ± 0.3% of dry weight. It increased by almost 70% to 3.5 ± 0.4% DW in the post-monsoon season, supporting and extending earlier findings from studies conducted in controlled environments to a natural ecosystem. The significant increase suggests that the plant has specifically chosen to invest in its primary defensive diterpenoid, which is the least harmful, during the moderate abiotic challenge. However, andrographolide production was nearly completely destroyed by the severe summer drought, and it was only 1.4 ± 0.2% DW—much less than the baseline set by the monsoon. This decline in output indicates a threshold above which the plant's metabolic resources are redirected to basic survival processes or perhaps even the compromised biosynthetic machinery, rather than being used to produce specialized compounds.

4.2 Antioxidant Defense Responses

The secondary metabolites, the oxidative stress conditions had a major impact on the activity of the important antioxidant enzymes that were crucial to the ROS-detoxification process; this fact emphasizes the relationship between oxidative signaling and metabolic regulation once more.

Monsoon samples had the lowest levels of Superoxide Dismutase (SOD) activity (15.2 ± 1.8 U mg^{-1} protein), which is the primary mechanism for removing superoxide radicals. But under post-monsoon stress, it increased dramatically by an astounding 58% (24.0 ± 2.5 U mg^{-1} protein), indicating that the detoxification system was successfully activated through enzymatic means in response to the rising oxidative pressure. In contrast, in the summer, when the oxidative load was extremely

On the other hand, SOD activity decreased to 18.5 ± 2.1 U mg^{-1} protein during the summer, when the oxidative load was extremely high. This was thought to indicate that the enzyme may have been inactivated or downregulated during long-term, toxic stress conditions.

The enzyme catalase (CAT) that disintegrates hydrogen peroxide followed a similar trend in activity. The maximum activity occurred during the post-monsoon season with 14.7 ± 1.6 U mg^{-1} protein, which is 73% higher than the monsoon season's 8.5 ± 0.9 U mg^{-1} protein level. A very strong reaction is needed to deal with $H2O_2$, which can act as a signaling molecule in moderate amounts but becomes toxic at high concentrations. CAT activity. Summer saw catabolic activity fall exceedingly low at a mere 6.8 ± 0.8 U mg^{-1} protein, a value way below that of the monsoon. This could mean either a drastic drop in the cellular energy required for the enzyme system's functioning or heavy oxidative damage to the system.

The activity of peroxidase (POD), which is a key player in various stress responses such as lignin production and H_2O_2 neutralization, was the parameter that reacted the most. Its value changed from 12.1 ± 1.4 U mg^{-1} protein during the monsoon to 38.5 ± 3.9 U mg^{-1} protein in the post-monsoon period, thus underscoring its crucial function in the adaptation of the plant to moderate-stress conditions. In contrast, the summer period saw a sharp drop to 9.3 ± 1.1 U mg^{-1} protein, which once again signalled the antioxidant defense system's inability to cope under the most severe stress conditions.

the findings show a distinct hormetic response in A. paniculata. A mild post-monsoon water stress acts as a eustressor, resulting in the simultaneous upregulation of the production of valuable secondary metabolites and the antioxidant defense system. Conversely, the extreme summer drought acts as a distress signal, resulting in metabolic suppression, decrease in bioactive compound stringency, and collapse of the enzymatic defenses which is, in turn, reflected as oxidative damage and impaired physiological function.

5. Discussion

The findings resulting from this field experiment clearly depict an ecophysiological story *of Andrographis paniculata*'s biochemical modulation due to a natural variation in water stress. The remarkable increase in total phenolics, flavonoids, and andrographolide at the cotton-picking stress of post-monsoon (12-18% soil moisture) among others, is indicative of strategic adaptive response. "Stress-induced defense" is the concept that best fits this situation, whereby mild abiotic stress is said to act as an elicitor, signaling the plant to activate protective secondary metabolic pathways. On an antioxidant system, phenolics and flavonoids are considered the most potent non-enzymatic antioxidants, and their increased production together with the rise in the activities of SOD, CAT and POD, has been termed as a coordinated defense against oxidative stress caused by drought. The increase in the production of andrographolide—the primary bioactive diterpenoid—by 70% is of special pharmacological importance. It suggests that moderate, natural drought stress can be used as a method to reinforce the medicinal potency of wild A. paniculata material without the involvement of either genetic or chemical methods.

the drastic drops in metabolite concentrations and antioxidant enzyme activities resulting from severe summer drought (<8% soil moisture) indicate a definite tolerance threshold. In the case of water deficit becoming very high and continuation of the same, the plant's physiological resources are expected to be shifted from the energy-intensive

production of specialized metabolites to essential survival processes, or the biosynthetic pathways themselves may become impaired due to cellular damage. The reduction in the activities of key enzymes points to the defeat of the defense system by oxidative damage, which results in the metabolic state of exhaustion. This two-phase response—increase followed by decrease—provides very strong support for a hormetic model of plant stress response.

The results of this study not only support but also broaden the scope of previous studies carried out under controlled conditions on A. paniculata and other medicinal plants, confirming that the extent of stress is an important factor in the production of phytochemicals. In terms of conservation and sustainable harvesting, this means that the best time for harvesting trees in Jharkhand's forests to obtain the highest quantity of bioactive compounds is the period after monsoon rains. Furthermore, crop management methods may take up regulated deficit irrigation to imitate this mild stress and possibly enhance the medicinal quality of Kalmegh grown in the field.

5. Conclusion

The research provides irrefutable evidence that the seasonal water deficit is the most important abiotic factor which regulates the biochemical activity of *Andrographis paniculata* in the Jharkhand forest ecosystem. The results show a distinct hormetic response where the level of stress decides the amount of phytochemicals produced. The moderate water stress in the post-monsoon season serves as a powerful biochemical activator that causes a huge increase in the non-specific compounds of medicinal value such as total phenolics, flavonoids, and above all, andrographolide. In addition, the strong activation of the antioxidant enzymes SOD, CAT, and POD occurs simultaneously with the production of the bioactive compounds. This coordinated production, therefore, is a perfect adaptive mechanism that not only protects the plant against oxidative stress but also increases its medicinal value.

In contrast, extreme summer droughts with the intensity such that they exceed the tolerability of the plants cause a drastic switch of their physiological state. The noted drop in secondary metabolism compounds and the complete halt of antioxidants' enzymes' activities indicate the disturbance in metabolism, the shifting of resources to cope with the situation, and the possible occurrence of oxidative damage. This marks the boundary of the plant's endurance water shortage in the wilderness.

The ecological insights gained have direct and highly significant practical applications. The post-monsoon period is highlighted to be the best time for sustainable wild harvesting to increase yield and bioactive compound content simultaneously, thus conserving and making the best use of the natural resources. These conclusions suggest that the irrigation protocols for cultivation should be established that would make the plants undergo the same moderate stress of the post-monsoon season as if they were grown under RDI conditions, thus improving the crop quality without affecting its survival. Eventually, this study stresses that the Eco physiological comprehension is to be considered in the management of medicinal plants for the purpose of making their use, conservation, and therapeutic potential more sustainable during climate changes.

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