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INFLUECE OF LIGHT AND DEVELPOMENTAL STAGES ON ACTIVE PRINCIPLES OF *Andrographis paniculata* (Burm.f.) Wall. ex Nees

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ABSTRACT

***Andrographis paniculata* is one of the most important medicinally important plants grown worldwide. The plant is exploited for its medicinally important compound i.e. Andrographolide. But the impact of actual growth stages and proper light condition for extraction of the metabolite have not been worked out till date. The present investigation aims to study the effect of two different light conditions such as Sunlight and Shade and different growth stage i.e. Vegetative, Flowering and Fruiting on the physiology of the plant including the changes in enzymes i.e. Peroxidase (POX), Polyphenoloxidase (PPO), Succinate Dehydrogenase (SDH) and Aspartate Aminotransferase (AAT). The changes in the concentration of flavonoids and Andrographolide were also worked out. The study revealed a close relationship of metabolite concentrations with light and growth.**

KEY WORDS: POX, PPO, SDH, AAT, andrographolide, flavonoids

Andrographis paniculata is commonly known as "Kalmegh". It is also known as 'King of Bitters' due to its bitter taste and weak odor. *A. paniculata* is available abundantly in India, Pakistan, Sri Lanka, East and West Indies, Mauritius, China, Java, Thailand and Indonasia. It is widely distributed and exploited as medicinal plant in almost all regions of India such as Himachal Pradesh, Assam, Bihar, West Bengal, Chhattisgarh, Madhya Pradesh, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala. Long known in traditional Asian medicine as an immune system booster, *Andrographis* has demonstrated significant activity in fighting common cold, flu and upper respiratory infections (Vijaykumar et al.,2007). The constituents found in *A. paniculata* are diterpene lactones and their glycosides i.e. andrographolide, deoxyandrographolide, 11, 12-dedehdro-14-deoxyandrographolide, neoandrographolide. Diterpenoids and flavonoids are the main chemical constituents of the plant and are also responsible for the biological activities of the plant (Patarapanich et al., (2007)). The active principle levels may vary as a function of the plant's developing stage and/or the edapho-climatic conditions where it grows. Higher plants have the ability to respond towards the amount of light available during their growth. Optimum photoperiod is a key factor for obtaining higher yield. As an important defence mechanism against the deleterious effects of solar radiation, long term adaptation of higher plants involves synthesis of relatively compounds capable of serving as light screens and internal traps. Of the vast number of medicinal plants used a small number have received considerable interest and use over the past few years.

MATERIALS AND METHODS

For the present investigation the plants of *Andrographis panniculata* were planted in two sets with each set receiving different light intensities. The plot receiving light intensity of $1.44x10^3$-$2.24x10^3$ µmole. photons.m^{-2}.sec^{-1} was termed as "Sunlight" condition and those receiving a light intensity of $0.24x10^3$-$0.96x10^3$ µmole. photons.m^{-2}.sec^{-1} as "Shade" condition. During the present investigation the effect of light on different developmental stages of *A.paniculata* was studied by standard methodologies. The enzymes such as peroxidases (POX) were estimated by methodology of Reuveni et al.,(2002), polyphenoloxidases (PPO) by Mayer et al., (1965) method, succinate dehydrogenases (SDH) by Cooper and Beevers ,(1969) and aspartate dehydrogenase (AAT) by Bermeyer and Bernt ,(1974) method. The active

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principle i.e. andorgapholide was estimated through HPLC by the method of Vijay Kumar et al., (2007). Since leaf is the primary source of all the metabolites, therefore, all the estimations were carried out from the leaves of the plants. For proper validation of the results the obtained values were subjected to statistical analysis by SPSS 12.0 software.

RESULTS AND DISCUSSION

In the present investigation it was observed that the active principles of *Andrographis paniculata* are greatly affected by factors such as change in light intensities and growth stages. Flavonoids are the most common secondary metabolites in vascular plants. In the present work maximum flavonoid content was recorded in the leaves of sunlight grown plants i.e. 2.66 µmol/l as compared to their shade counterparts which recorded a concentration of 0.35 µmol/l (Fig. 1). The present result might due to the fact that light is considered one of the most important factors in the control of flavonoid synthesis acting upon a complex system involving several photoreceptors: UV, blue, phytochrome and the photosynthetic system (Awad et al., 2001).

In the present investigation it was observed that light condition and growth stages have a profound effect on the POX activity of the plant. The leaves of the plants grown under sunlight showed higher activity of POX compared to other plant parts i.e. stem and root. The POX activity was recorded the highest as 97.22 µmol $min^{-1}.g^{-1}$FW in fruiting stage of sunlight condition whereas 55.77 µmol $min^{-1}g^{-1}$FW in shade condition (Fig. 2). The result may be due to the fact that under adverse conditions photoinhibition, photoxidation and photorespiration occur. Plants are particularly susceptible to photoinhibition when exposed to bright light. Under environmental conditions where the photon energy is in excess of CO_2 assimilation, photosystem II is the primary target for photo inhibition, while PSI is more than PSII, receiving a damage usually less significant and strictly related to the rate of electron flow from PSII and presence of oxygen (Sofo et al., 2004).

During the study period the leaves showed the maximum PPO activity in vegetative stage followed by gradual decline in flowering and fruiting stages. Although the plants grown in shade conditions had recorded higher enzyme activity compared to plants grown in sunlight conditions. The highest activity was recorded as 2.44 changes in activity $min^{-1}.g^{-1}$ FW in the vegetative stage of the sunlight condition and in the shade condition it was 0.81 changes in activity $min^{-1}g^{-1}$ FW in the vegetative stage (Fig. 3). In the present study there existed an inverse relation of the enzymes with aging. Gooding et al., (2001) depicted parallel observations where PPO activity was shown to be the highest in young tissues and in meristamatic regions and genes expression generally decreased during development and maturation of plant tissue. Also the results obtained in this study confirm that light intensity plays a key role in the functionality of plants subjected to water stress conditions. The repairing of damage due to oxidative stress generated by drought stress and high irradiance levels was associated with a different antioxidant response in plants grown in semi shade conditions or under environmental irradiance (Sofo et al., 2004).

The SDH activity showed similar pattern as that of polyphenoloxidase enzyme activity where leaves showed higher activity compared to other parts of the plants. The enzyme activity showed a higher value in the plants grown under shade condition compared to their sunlight counterparts (Fig. 4). In the present work the SDH activity was also recorded higher in the plants grown under lower light intensity while those grown at higher light intensity showed a decreased level of the enzyme. This is very well substantiated with the findings of Popov et al., (2007) where SDH activity in *Arabidopsis* was recorded lower in light condition compared to those cultivated in shade. They also stated that the exposure of plants to light suppressed the enzyme activity almost by a factor of 6 as compared to that in plants grown in darkness. The light conditions were found to have a great effect on the SDH activity in the plants.

The AAT, a key enzyme involved in nitrogen and carbon metabolism, catalyzes the reversible transamination reaction between aspartate and ketoglutarate to form oxaloacetate and glutamate. During the study the highest activity in the leaves was recorded in the sunlight grown plants compared to the shade condition plants (Fig. 5). In a study carried out by Schnarrenberger et al., (1971), the specific activity of the enzyme in the microbodies increased

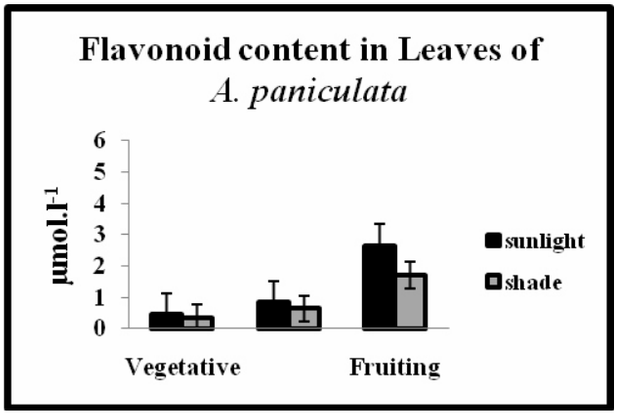

Fig. 1

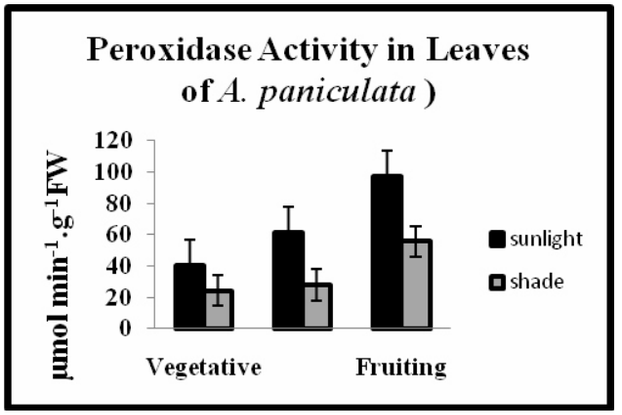

Fig. 2

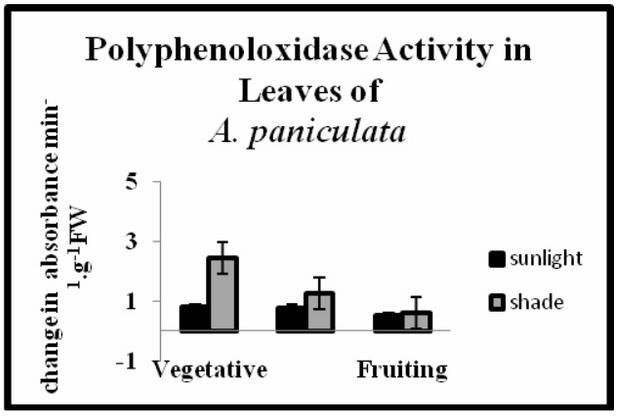

Fig. 3

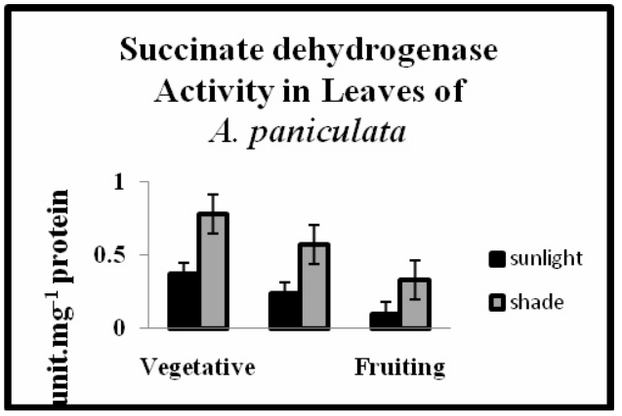

Fig. 4

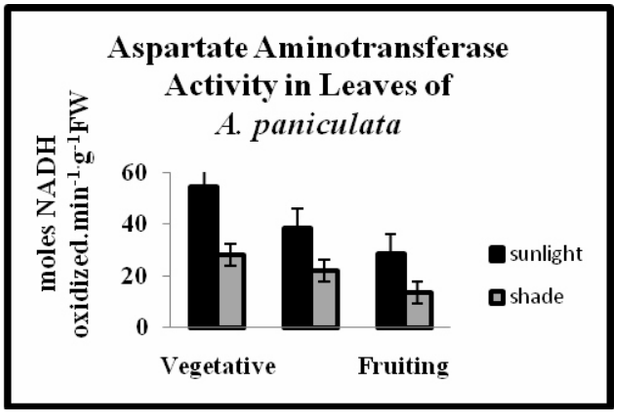

Fig. 5

HPLC CHROMATOGRAM OF ANDROGRAPHLOIDE EXTRACTED FROM LEAVES AT DIFFERENT GROWTH STAGES WITH DIFFERENT LIGHT CONDITIONS

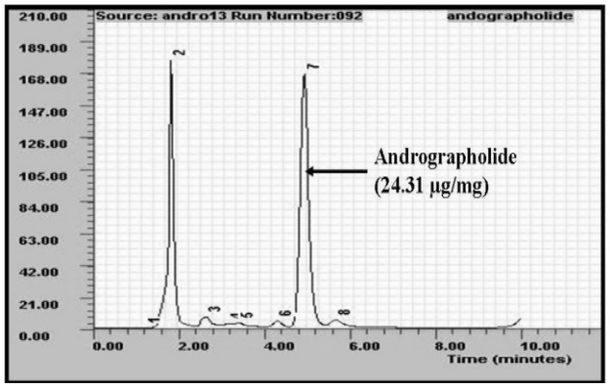

Fig.: 6.1.1 Leaves of Sunlight grown plant in vegetative stage

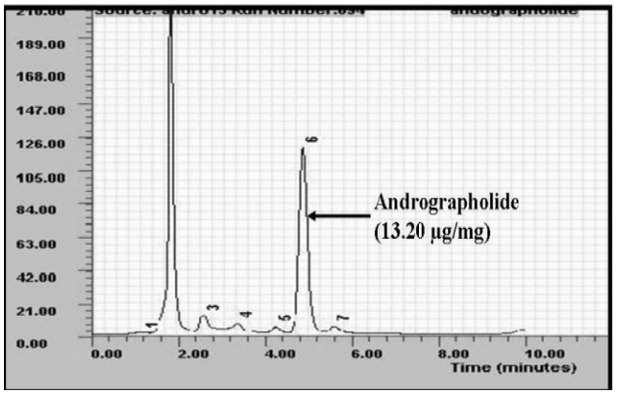

Fig.: 6.1.2: Leaves of Shade grown plant in vegetative stage

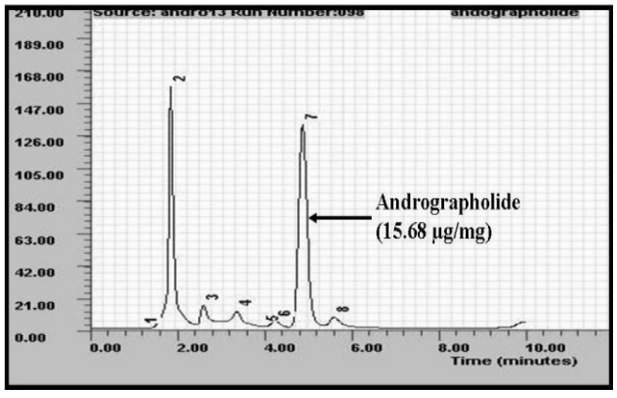

Fig.: 6.2.1 Leaves of Sunlight grown plant in Flowering stage

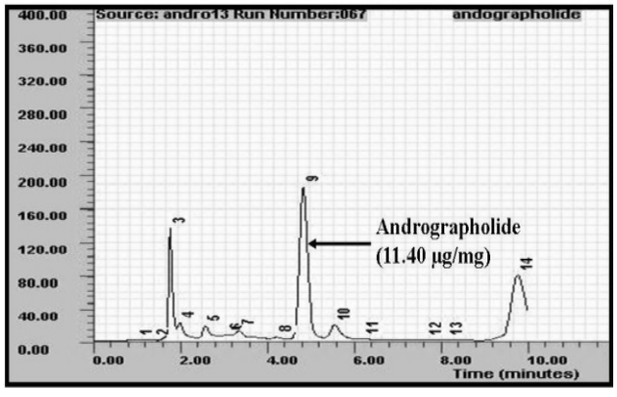

Fig.: 6.2.2 Leaves of Shade grown plant in Flowering stage

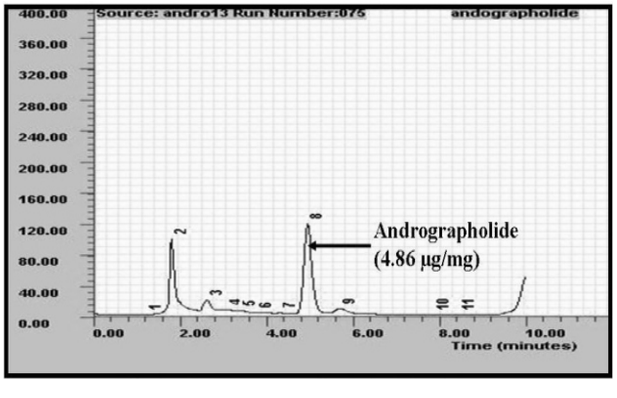

Fig.: 6.3.1 Leaves of Sunlight grown plant in Fruiting stage

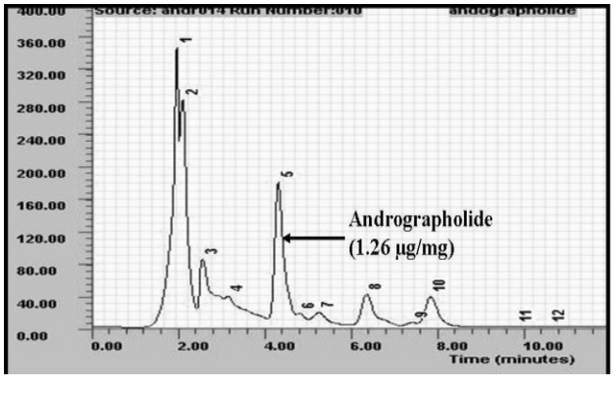

Fig.: 6.3.2 Leaves of Shade grown plant in Fruiting stage

tenfold in the light over dark condition, whereas the specific activity of the enzymes in the mitochondria was about 50% lower in the light grown seedlings as compared to dark. Similar results were obtained in the present findings where in an increased concentration of AAT was observed in the plants grown under higher light intensity. The present result might be due to the stress induced reductions in NADP-malate dehydrogenase and / or pyruvate Pi dikinase which limits photosynthetic carbon metabolism, an increase in the concentration of their substrates or build up of amino acids such as aspartate and alanine.

During the study it was observed that the plants grown under sunlight condition showed the highest concentration of the alkaloid compared to that of shade condition. The maximum percentage of the andrographolide was recorded as 2.43%±0.1279 in the leaves of sunlight grown plants during vegetative stage. The alkaloid concentration was found minimum 1.56%± 0.0450 during fruiting stage. In the shade condition the value was lower with 1.32%±0.1279 during vegetative stage (Fig 6.1.1 6.3.2). The present result may be due to the fact that environmental conditions such as light and temperature affect alkaloid formation and in particular the presence of light is in some cases necessary for the onset of alkaloid accumulation (Waller et al., 1978). Patarapanich et al.,(2007) reported that with different location of planting areas, different amount of each diterpenoid lactone was obtained. They also reported that highest total diterpenoid lactone content was found in March-April, while the lowest total contents was in October-November. Similar findings were obtained in the present findings where the concentration of andrographolide and total lactones were observed during the vegetative stage i.e. during May-June and lowest in the fruiting stage i.e. during November.

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