Journal of Stress Physiology & Biochemistry, Vol. 10 No. 1 2014, pp. 222-229 ISSN 1997-0838

ORIGINAL ARTICLE

Influence of Growth Regulators on Secondary Metabolites of Medicinally Important Oil Yielding Plant *Simarouba glauca* DC. under Water Stress Conditions.

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Received October 27, 2013

One year old seedlings of *Simarouba glauca* were subjected to water stress for 4, 8, 12 and 16 days. The foliar sprays of 50 ppm salicylic acid (SA) and 10 ppm Putriscine, Gamma amino butyric acid (GABA) and Abscisic acid (ABA) were applied before and after water stress. It was observed that polyphenols, tannins, alkaloid and flavonoid contents were increased with increasing water stress treatments. Foliar applications of growth regulators ameliorate water stress and exhibits induction of secondary metabolites like coumarins, sterols, xanthoproteins, cardiac glycosides and saponins. It was also noticed that foliar application of SA, GABA, ABA considerably increases all these secondary metabolites which will help to improve the medicinal potential of Simarouba glauca under water stressed condition.

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Key words: Flavonoids, polyphenols, secondary metabolites.

Simarouba glauca is an edible oil seed bearing tree. It is commonly known as `Laxmitaru' or `Paradise tree' belonging to family Simaroubiaceae. This plant is well known for its different types medicinal and pharmacological properties. *Simarouba glauca* tree has an ablity to grow well even in marginal wasteland or dry land with degraded soil (Govindaraju *et al.,* 2009). According to Jaleel *et al*. (2007), biotic and abiotic stresses exert a considerable influence on the production of several metabolites in plants. Dash and Mohanty (2001) suggested drought is one of the most important abiotic stress factors. Drought alters biochemical properties of plants (Zobayed *et al*., 2007). Drought stress is also known to increase secondary metabolite production in variety of medicinal plants, like artemisinin in leaves of *Artemisia annua* (Charles *et al*., 1993), hyperforin in *Hypericum perforatum* leaf tissue (Zobayed *et al*., 2007) and ajmalicin in *Cathranthus roseus* roots (Jaleel *et al*., 2008a). In the light of these observations it was thought worthwhile to study effect of water stress on secondary metabolites of *Simarouba glauca*.

MATERIALS AND METHODS

One year old seedlings of *Simarouba glauca* DC. were transplanted in earthen pots. Seedlings were

settled by watering regularly in polyhouse of Botany Department of Shivaji University. After one month plants were subjected to water stress for 4, 8, 12 and 16 days interval. The control plants were watered every after two days. Foliar sprays of 50 ppm SA and 10 ppm Putriscine, GABA and Abscisic acid at the mid interval of and before 4, 8, 12 and 16 days of stress. Polyphenols were analysed according to method of Folin and Denis (1915) and total flavonoids content was estimated by AlCl3 method described by Luximan – Ramma *et al*. (2002). The method described by Singh *et al*. (2004) was used to determine alkaloid content. Tannins were determined by method of Schanderi (1970). The methanolic extract were screened for the presence of alkaloids, cardiac glycosides,coumarins, phenols,saponins, sterols, tannins, Xanthoproteins following the methods of Trease and Evans(1985); Bhindra *et al.*, (1981) and Lala(1993).

RESULTS AND DISCUSSION

Effects of SA, GABA, Putriscine and Abscisic acid on polyphenol content in leaves of *Simaruba glauca* grown under water stressed conditions is shown in Fig. 1. It is observed that total polyphenols increases with increasing water stress treatments up to 16 days. Further foliar application of these growth hormones exert positive influence on accumulation of polyphenols under water stressed condition. Among these SA and GABA exhibit marked accumulation of polyphenols under water stressed conditions. Kirakosyan *et al.,* (2004) observed enhancement in the levels of polyphenolics in the leaves of two hawthorn sp. (*Crataegu s slaevigatta* and *C. monogyna*) due to water deficit. Nivedithadevi and Somasundaram (2012) showed increase in total phenolic compounds in ABA treated plants followed by SA treated plants. According to Shahram and Zare (2001) tannin like phenolics are defence metabolites increased under stress condition. Pritchard *et al*. 1997 reported increase in total phenolic and tannins due to elevated co_2 under both water stressed and well-watered conditions. In leaves of *Simarouba glauca* total polyphenols were increased by two folds due to SA, GABA, ABA and Putresceine treatments. This will helps to increase the recovery of phenolics and improve the bioactive potential of *Simaruba glauca.*

Effect of growth hormones on flavonoid content of *Simaruba glauca* grown under water stressed conditions is shown in Fig. 2. It is observed that total flavanoids increase with increasing water stress treatments upto 16 days and foliar sprays of growth regulators. It is also evident that the accumulation of flavonoids is 3 to 4 fold higher than the control plants. Yaginuma *et al*. (2003) noticed that under the stressed conditions content of flavonoid glucosides in foliage of safflower seedlings markedly increased on 2^{nd} day then decreased to the initial level before stress loading on 5^{th} day. Increased total flavonoids due to ABA treatment than SA were observed by Nivedithadevi and Somasundaram (2012).

Effect of foliar sprays of growth regulators on alkaloid content of *Simarouba glauca* under water stressed condition is shown in figure 3. As compared to control alkaloid content increases by 2 to 3 folds in all stressed as well as sprayed plants. Liu (2000) and Jaleel *et al.* (2008 b) reported increased levels of secondary metabolites due to water stress. Hoft *et al*. (1996) observed increase in several alkaloid content in response to drought in *Tabemaemontana pachysiphon*. Pitta *et al.* (2000) and Spolansky *et al.* (2000) reported that SA can be used as effective strategy to increase the production of important alkaloids in cell and organ

culture.

The phytochemical research based on ethnopharmacological information is generally considered as effective approaches in the discovery of new anti-infective agent from higher plants. (Duraipandiyan *et al.,* 2006). The qualitative screening of phytochemical constituents of leaf extract of *Simarouba glauca* reveals the presence of phenols, coumerines, sterols, alkaloid, tannins, xanthoproteins, cardiac glycosides, saponins and terpenoids. Phenols, sterols, alkaloids, tannins and saponins were accumulated in stressed plant as compared to control plants. Except putresceine similar results were observed in SA, GABA and ABA sprayed plants .In control plants coumarines and cardiac glycosides were less than stressed and sprayed plants. The plant contains many bioactive constituents are analgesic and antispomodic alkaloid (Stray 1998), anti-inflametory tannins(Westendary 2006), anticoagulants saponins (Sadipo *et al*., 2000) and many antioxidants like flavonoids (Salah *et al.,* 1995). In the light of these observations *S. glauca* is potent medicinal plant used against various diseases. The application of water stress and foliar spray of SA, putrescine , GABA, and ABA induces the synthesis of secondary metabolites. The foliar application of PGRs under stressed conditions ameliorate a water stress and results in the induction of synthesis of several bioactive compounds which will improve medicinal potential of *Simaruba glauca*. Further *S. glauca* can be grown successfully in dry areas with the application of these growth regulators to reduce the water stress effects.

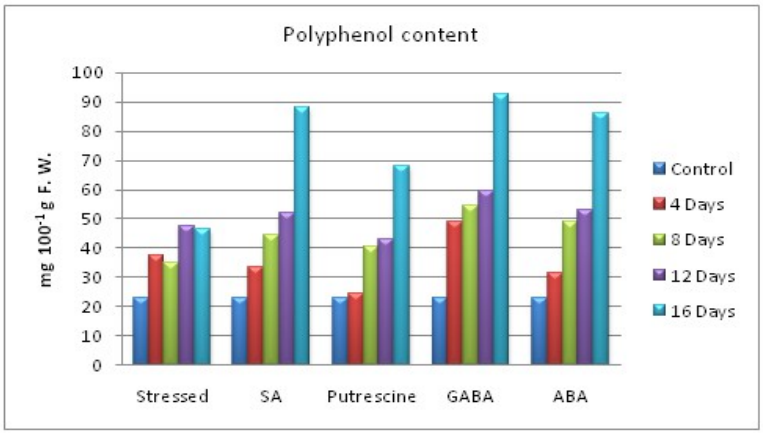

Figure 1 : Effect of foliar sprays of growth regulators on polyphenol content in the leaves of *Simarouba glauca* grown under water stress condition.

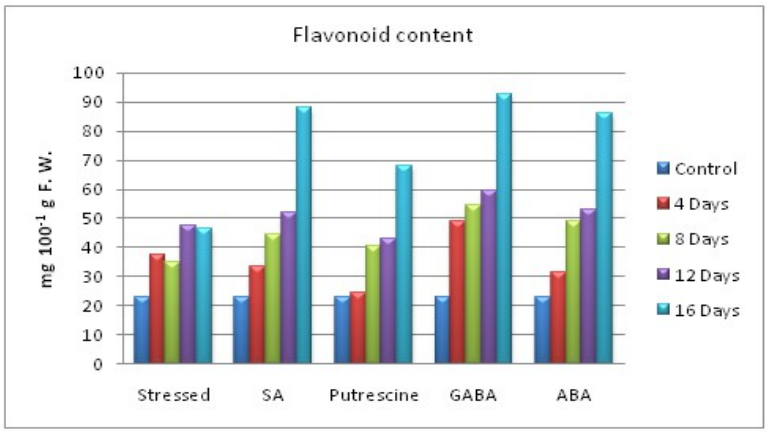

Figure 2 : Effect of foliar sprays of growth regulators on flavonoid content in the leaves of *Simarouba glauca* grown under water stress condition.

Table 1 : Qualitative screening of phytochemicals of methanolic extract in leaves of *Simarouba glauca* grown under water stressed condition.

Secondary metabolites	Treatments	Control	Stressed	Salicylic acid (50 ppm)	Putriscine (10 ppm)	GABA (10 ppm)	Abscisic acid (10ppm)
Phenols	4 Days	++	+++	+++	++	+++	++
	8 Days	++	+++	++	++	+++	+++
	12 Days	++	+++	+++	++	+++	+++
	16 Days	++	+++	+++	++	+++	+++
Coumarines	4 Days	+	++	+++	+	++	++
	8 Days	+	++	+++	++	+++	+++
	12 Days	+	++	+++	++	+++	+++
	16 Days	+	++	+++	++	+++	+++
Sterols	4 Days	++	+++	++	+	++	++
	8 Days	++	+++	+++	++	+++	++
	12 Days	++	+++	++	+++	+++	++
	16 Days	++	+++	++	+	+++	++
Alkaloids	4 Days	++	+++	+++	++	++	+++
	8 Days	++	+++	++	++	+++	+++
	12 Days	++	+++	++	++	++	+++
	16 Days	++	+++	+++	+	+++	+++
Tannins	4 Days	++	+++	++	++	+++	+++
	8 Days	++	+++	+++	++	+++	+++
	12 Days	++	+++	+++	++	+++	+++
	16 Days	++	+++	+++	+	+++	+++
Xanthoproteins	4 Days	++	++	+++	++	++	+
	8 Days	++	++	++	++	++	++
	12 Days	++	++	++	++	++	++
	16 Days	++	++	++	+	++	++
Cardiac Glycosides	4 Days	++	++	+++	++	++	+
	8 Days	+	+++	++	++	++	+
	12 Days	+	++	+++	++	+++	++
	16 Days	+	+++	+++	+	+++	++
Saponins	4 Days	++	+++	+++	++	+++	++
	8 Days	++	+++	++	++	++	+++
	12 Days	++	+++	+++	+	+++	+++
	16 Days	++	+++	+++	++	+++	+++
Terpenoids	4 Days	++	++	+++	+	+++	+
	8 Days	++	++	+++	++	+++	+
	12 Days	++	++	+++	++	+++	+++
	16 Days	++	++	+++	++	+++	+++

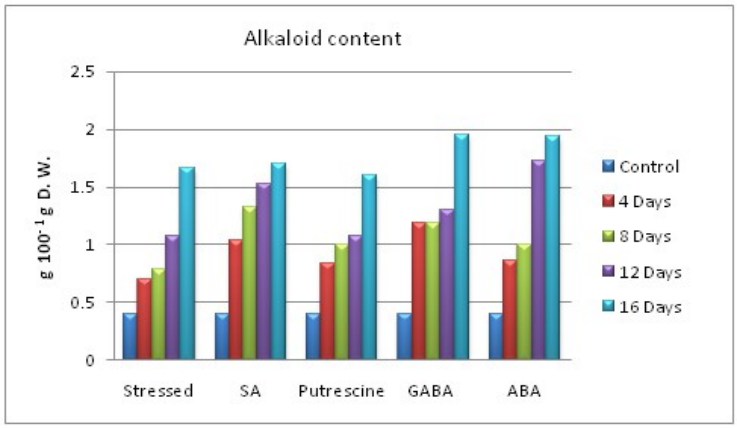

Figure 3 : Effect of foliar sprays of growth regulators on alkaloid content in the leaves of *Simarouba glauca* grown under water stress.

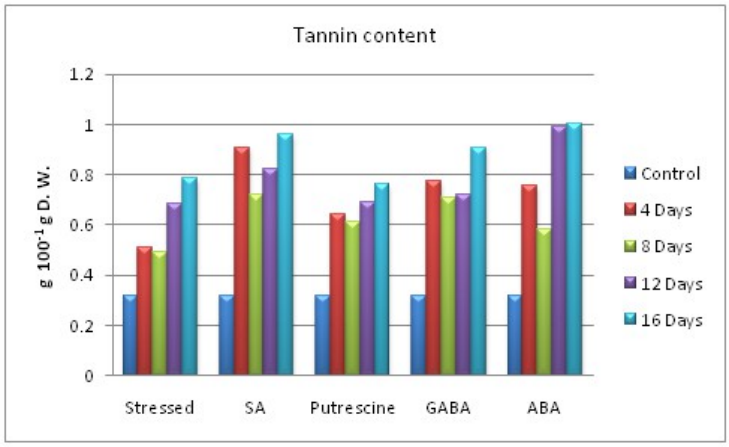

Figure 4 : Effect of foliar sprays of growth regulators on content tannin content in the leaves of *Simarouba glauca* grown under water stress.

ACKNOWLEDGEMENT

One of the authors (Patil M. S.) is thankful to Head, Department of Botany, Shivaji University Kolhapur for providing the internet facility and Departmental Library facilities. She is also thankful to the Librarian, Br. Balasaheb Khardekar Library, Shivaji University Kolhapur for providing the necessary valuable books, thesis, research journal and articles etc.

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