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HPLC analyses of phenolics in the stigma of fruit bearing and fruitless trees of *Kigelia pinnata* (Jacq.) DC. syn *K. africana* (Lamk.) Benth.

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ABSTRACT

HPLC analyses of phenolics in the stigma of fruit bearing and fruitless plants of *Kigelia pinnata* syn. *K. africana* (Bignoniaceae) was made. The HPLC chromatograms showed higher peaks and higher number (9) phenolic compounds in the stigmas of fruitless plants at Agra. On the other hand, the peaks were low and there were only 8 and 7 phenolics in the stigmas of fruit bearing trees at Jammu and Dehra Dun respectively. The quantity of phenolic compounds in the fruitless plants was significantly higher as compared to that in the fruit bearing plants. Presence of higher number of phenolics and their higher quantity in the stigmas of fruitless plants inhibit pollen germination and render the plants fruitless at Agra.

Keywords: Bignoniaceae, phenolics, HPLC, pollen germination.

INTRODUCTION

Kigelia pinnata (Jacq.) DC. syn *K. africana* (Lamk.) Benth. (Tribe: Crescentieae; Family: Bignoniaceae) is native of Central Africa extensively planted in the tropics. The plants are large (10-15 m tall) commonly called as sausage tree. It is planted in the gardens, avenues and as a road side tree for its beautiful pinnately compounds leaves and large showy scarlet flowers pollinated by bats. The trees flower profusely at Agra but fail to produce fruits, while at Jammu and Dehra Dun, it produces large number of big fruits.

Earlier investigations into the causes of fruitlessness in this important tree pointed out that high temperature (42-47°C) during flowering period at Agra induces pollen sterility to a considerable extent (Chauhan 1995). There are morphological differences in the structure of stigma and stigmatic papillae, cuticle-pellicle layer in fruit and fruitless plants (Chauhan *et al.* 1987, Rana & Chauhan 1994, Rana *et al*. 1996, Rana 2009). Biochemical studies pointed out that large quantity of phenolics accumulate in the stigmas of fruitless plants as compared to that of fruit bearing plants (Rana & Chauhan 1996).

Present investigation has been undertaken to make qualitative and quantitative analysis of phenolics by HPLC in the stigmas of fruitless and fruit bearing *Kigelia pinnata* plants growing at Agra and Jammu and Dehra Dun respectively.

MATERIALS & METHODS

Stigmas from fresh open flowers in the morning hours (0600-0700 h) were collected from Agra (26º 44´ and 77º 55´ N and 78º 32´E), Jammu (32.73ºN, 74.87ºE) and Dehra Dun (29 °58' and 31°2 'N and 77° 34' E) and dried in hot air Owen at 35° and powdered. 1 g of each sample was extracted with 50% methanol (1 x 10 ml) and

hydrolyzed with HCL (1.2 N) by refluxing on a water bath for 1 h (Singh *et al.* 2009a). The hydrolysates were processed and subjected to qualitative and quantitative analysis by using a Shimadzu LC-10A (JASCO, Kyoto, Japan) HPLC system coupled to an ultraviolet (UV) detector (Singh *et al.* 2009b). The mobile phase was water containing 1% glacial acetic acid (solvent A) and acetonitrile (solvent B). Solvent B changed during the gradient program as follows: 18% to 32% for 15 min, and 32% to 50% in 40 min. The flow rate was 1 ml/min. 20 µl samples were injected and time of each run was 30 minutes. Results (µg/g dry wt) were obtained by comparing the peak areas (254 and 290 nm) of the samples with those of standards (Class VP series software, Shimadzu, Japan). Quantities of total phenolics in the stigmas by HPLC were compared statistically by analysis of variance (ANOVA) using the MSTAT-C software.

RESULTS & DISCUSSION

The HPLC chromatograms of the stigmatic tissue of both fruitless and fruit bearing plants (Figs. 1a, 1b, 1 c); the phenolics identified, their quantity and the retention time of the peaks are shown in Table 1.

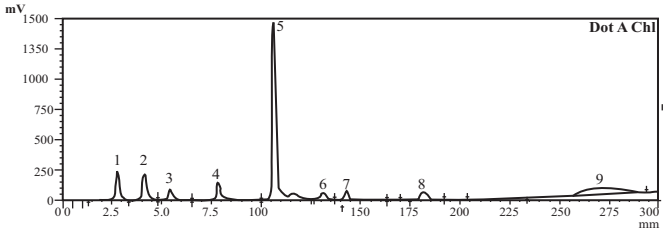

Fig. 1a. HPL Chromatogram from the stigmas of fruitless *Kigelia pinnata* plants growing at Agra.

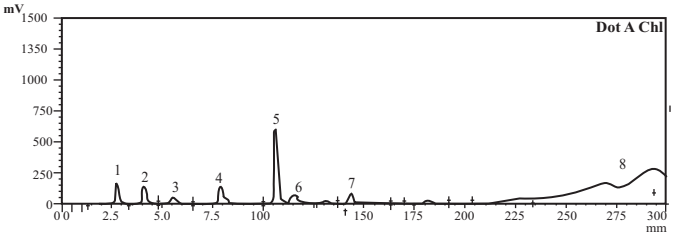

Fig. 1b. HPL Chromatogram from the stigmas of fruit bearing *Kigelia pinnata* plants growing at Jammu.

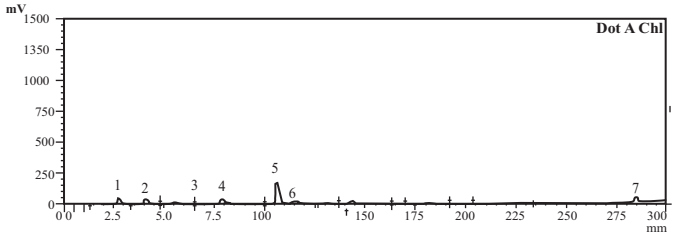

Fig. 1c. HPL Chromatogram from the stigmas of fruitbearing *Kigelia pinnata* plants growing at Dehra Dun.

Table 1- Quantity of phenolics, retention time of the peaks in the stigma of *Kigelia pinnata* plants growing at Agra, Jammu and Dehra Dun

Phenolics	Agra			Jammu			Dehra Dun		
	PN	RT	Quantity*	PN	RT	Quantity*	PN	RT	Quantity*
Shikimic acid	1	2.77	4.40**	1	2.35	3.011	1	2.47	3.008
Gallic acid	2	4.11	0.26	2	3.70	0.210	2	4.09	0.220
t-Chlorogenic acid	3	5.44	0.18**	-	-	Absent	3	6.16	0.032
Synringic acid	-	-	Absent	3	7.6	0.005	-	-	Absent
Ferulic acid	4	14.33	0.06	4	13.5	0. 059	4	14.11	0.060
p-coumaric acid	-	-	Absent	5	11.3	0.012	-	-	Absent
Quercetin	-	-	Absent	6	21.9	0.015	-	-	Absent
Kaempferol	5	27.19	0.27**	7	27.15	0.162	-	-	Absent
Synaptic acid	6	13.17	0.04	8	12.90	0.036	5	12.88	0.033
Salicylic acid	7	19.74	0.02	-	-	Absent	6	19.25	0.020
Rutin	8	7.89	0.16	-	-	Absent	7	7.64	0.121
Cinnamic acid	9	12.10	0.007	-	-	Absent	-	-	Absent

PN: Peak number; RT: Retention time; *Quantity (µg/mg dry weight)
*significantly higher at P=0.01

The data in Table 1 shows that there are a total of twelve phenolics namely, shikimic acid, gallic acid, *t*-chlorogenic acid, syringic acid, ferulic acid, *p*-caumaric acid, quercetin, kamepferol, synaptic acid, salicylic acid, rutin and cinnamic acid in various combinations and different quantities in the stigmas of both the fruitless and fruit bearing plants growing at different places. In the stigmas of the fruitless plants at Agra there were nine phenolic acids (shikimic acid, gallic acid, *t*-chlorogenic acid, ferulic acid, kamepferol, synaptic acid, salicylic acid, rutin and cinnamic acid), while in the stigmas of fruit bearing plants at Jammu there were eight phenolic acids (shikimic acid, gallic acid, *t*-chlorogenic acid, ferulic acid, kamepferol, synaptic acid, salicylic acid and rutin) and seven phenolic acids (shikimic acid, gallic acid, *t*-chlorogenic acid, ferulic acid, synaptic acid, salicylic acid and rutin) in the stigmas of Dehra Dun plants. Only shikimic acid, gallic acid, ferulic acid and synaptic acid were present in the stigmas of fruitless (Agra) and fruit bearing (Jammu & Dehra Dun) plants. However, the peaks and the quantity of these phenolics were significantly higher in the stigmas of fruitless plants of Agra. Kaempferol was present in the stigmas of Agra and Jammu plants. Rutin, *t*-chlorogenic, and salicylic acid were present in the stigmas of Agra and Dehra Dun plants. Synringic acid, *p*-coumaric acid and quercetin were present only in the stigmas of fruit bearing plants of Jammu. Cinnamic acid was found only in the stigmas of fruitless plants (Agra). Mass spectrometric analyses made by Rana & Chauhan (1996) also revealed the presence of derivatives of cinnamic acids (*p*-hydroxy cinnamic acid, di-hydroxy cinnamic acid and hydroxyl-methoxy cinnamic acid at 164, 180 and 194 MW respectively) in the stigmas of fruitless plants. Presence of higher peaks in the chroamograms, higher number of phenolics and their higher quantity in the stigmas of fruitless plants at Agra can be attributed to significantly high temperature (42-47°C) during the flowering period as the phenolic compounds are known to accumulate under stress (Harborne 1997). Accumulation of higher quantity of phenolics in the stigmas of some seedless plants of the family Bignoniaceae are known to lead to boron deficiency inhibiting pollen germination on stigmatic surface (Dhakre *et al*. 1994, Chauhan *et al.* 2004, 2005).

Thus, in the light of earlier and present observations, it is concluded that accumulation of phenolics in large quantity in the stigmatic tissues of fruitless plants inhibit pollen germination and render the *Kigelia pinnata* plants fruitless at Agra.

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LITERATURE CITED

Chauhan SVS 1995 Seedlessness in some Bignoniaceae. In: Chauhan YS & Pandey AK (eds.) *Advances in Plant Reproductive Biology.* Narendra Pub., House, Delhi. Pp. 173-196.

Chauhan SVS, Veena & Yadav DK 1987. Studies into the causes on seedlessness in some Bignoniaceae. *J. Exp. Bot.* **38** 173-177.

Chauhan SVS, Singh J & Tahara Satoshi 2004. Role of phenolics and boron in the reproductive success of seasonally transient sterile *Tecoma stans* L. *Indian J. Exp. Biol.* **42** 197-201.

Chauhan SVS, Rana Anita & Chauhan Seema 2005. Role of simple phenolics and boron in pollen-pistil interaction in some seedless and seed bearing Bignoniaceae. In: Gautam SP, Bansal YK & Pandey AK (eds.) *Biological Diversity: Current trends.* Shree Publishers & Distributors, New Delhi. Pp. 356-366.

Dhakre G, Singh J & Chauhan SVS 1994. Analysis of phenolics and boron in the stigma of seedless *Campsis grandiflora* K. Schum. *Indian J. Exp. Biol.* **32** 816-818.

Harborne JB 1997 *Biochemical plant ecology*. In: Dey PM & Harborne JB (eds.). *Plant Biochemistry,* Harcourt Asia Pvt Ltd., Singapore Pp. 503-516.

Rana Anita 2009 Morphological differences in the stigma of fruit bearing and fruitless plants of *Kigelia pinnata* DC. *J. Plant Repro. Biol.* **1**(1) 43-48.

Rana Anita & Chauhan SVS 1994. Histochemical localization of non-specific esterases and acid phosphatase in the stigma of seedless and ssed bearing *Kigelia pinnata* DC. *Plant Cell & Incompatibility Newsletter* **26** 48-52.

Rana Anita & Chauhan SVS 1996. Quantitative and qualitative analysis of phenolics in the stigma of seedless and seed bearing *Kigelia pinnata* DC. *J. Indian Bot. Soc*. **75**(1&2) 65-67.

Rana Anita, Singh J. & Chauhan SVS 1996. Morphological differences in the stigma of seedless and seed bearing *Kigelia pinnata* DC. *J. Indian bot. Soc.* **75**(3&4) 281-292.

Singh BN, Singh BR, Singh RL, Prakash D, Dhakarey R, Upadhyay G & Singh HB 2009a. Antioxidant and anti-quorum sensing activities of green pods of *Acacia nilotica* L. *Food Chem. Toxicol.* **47** 778-786.

Singh BN, Singh B.R, Singh RL, Prakash D, Dhakarey R, Upadhyay G & Singh HB 2009b. Oxidative DNA damage protective activity, antioxidant and antiquorum sensing potentials of *Moringa oleifera*. *Food Chem. Toxicol*. **47** 1109-1116.