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SHORT COMMUNICATION

CHANGES IN POLYPHENOL AND ARECOLINE CONTENTS IN *ARECA CATECHU* GENOTYPES DURING MATURITY

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The consequence on different maturity stages and genotypes on polyphenol, condensed tannins and arecoline content of arecanut were investigated. The content of polyphenol, condensed tannins and arecoline showed significant differences among months and decrease in content of polyphenol was found during fruit attaining maturity or ripe stage in arecanut genotypes. The arecanut genotypes showed a marked increase in condensed tannins (CT) content beginning from the 3 month (very tender stage) while a marked decrease 20% to 40 % in CT was observed in semi ripe and fully ripen stages. The ratio of CT to total polyphenol (TP) was found to be 40-50 % for entire growth period which gives an indication of the relative proportions of CT. Ripe arecanut showed high arecoline content than unripe arecanut. Moderate arecoline content was recorded in at semiripe stage (6 month) in arecanut. With respect to arecanut maturity stages, tender nut stage (6-7 month) may be ideal stage for arecanut chewing purpose which also showed lower arecoline content and this stage may also be ideal for optimum extraction of polyphenol and CT for pharmacological uses.

Key Words: **Arecanut genotypes, arecoline, maturity, pharmacology, polyphenols**

Areca catechu is an important commercial crop cultivated in India and southeastern countries. The arecanut or betel nut is mainly used for masticatory properties. The arecanut seed or nut contains polyphenol, alkaloids, polysaccharide, fat, protein. Main polyphenol subgroups in arecanut are proanthocyanidins, catechin, and epicatechin. Proanthocyanidins are high molecular weight flavonoid polymers that are also referred to as 'condensed tannins' (Nonaka *et al*. 1981). Accumulation of phenolic compounds varies strongly in relation to the physiological state of the fruit, being a result of equilibrium between biosynthesis and further metabolism including turnover and catabolism. The outer layer coats of the plant seeds contain different polyphenolic compounds, e.g. flavonoids phenolic acids, coumarins and anthocyanins. Their content and composition is strongly influenced by different factors (Weidner and Paprocka 1996).

Arecanut is reported to have pharmacological properties which may be mainly attributed to its biochemical components. Arecanut extract has been found to have potent anti-oxidative activity (Kim *et al.* 1997) and inhibition of free radicals and reactive oxygen species (Ohsugi 1999). Arecanut extract was shown *in vitro* inhibitory effect of on H_2O_2 induced RBC hemolysis. *Areca catechu* extract reported to have *in vitro* inhibitory activity on intestinal alpha–glucosidase enzymes such as maltase and sucrase and followed by *in vivo* suppressive effect on post-prandial elevation in

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blood glucose in maltose tolerance test in rats. Preventive activity of *A. catechu* extract against ethanol induced gastric mucosal ulcer was reported which was ascribed to the presence of, polyphenolic compounds such as catechin, epicatechin could exert direct antioxidant action on ethanol induced gastric ulcer (Senthil Amudhan and Hazeena Begum 2005, 2008). Both arecoline and polyphenol fractions of arecanut were found to exhibit wound healing effect on incision and excision wounds in rats (Shamina Azeez *et al*. 2007). *A. catechu* extract have been found to have exhibited strong inhibitory activities against pancreatic cholesterol esterase (pCEase) and found to decrease in absorption of cholesterol oleate (Jeon *et al*. 2000) and intestinal free cholesterol (Park *et al*. 2002). Biochemical changes in polyphenol and arecoline contents in *Areca catechu* during maturity were reported by Mathew *et al*. (1965). For several years there has been growing interest in antioxidants present in plant product. Studies were therefore conducted with an objective to find out arecanut biochemical changes due to both maturity stage and genotypes. This will make possible to extract optimum concentration of polypheonl at particular maturity stage for its pharmacological significance.

Four genotypes of *Areca catechu* nut were harvested subsequently for three years (2000-03) from Central Plantation Crops Research Institute, (CPCRI), Vittal, and Karnataka. Maturity stages were identified for collecting nuts were 3-4 months (very tender), 6-7 months (semi ripe) and 9-10 months (mature). Each arecanut palm of genotypes was tagged. Tender nut stage 10-25 nuts and semi ripe 5-20 nuts were collected which was found to contain minute quantity, whereas these nuts were dried and powered then mixed as one replication for estimation of respective maturity stage.

The total phenolic content of ethanol arecanut extract was determined by method of Swein and Hills (1959). Proanthocyanidins or condensed tannin was determined by a butanol-HCl assay (Porter *et al*. 1986). Arecoline was estimated by steam distillation method given by Nambuidri (1968). Results were expressed as miligrarm equivalents per gram on dry weight basis.

The design of the experiment was arranged as a factorial randomized block design with six replications and two way ANOVA was carried out. Comparison of means were done by LSD to differentiate means at $P < 5\%$.

The changes in the content of total polyphenol during maturity are indicated in Table 1. There were considerable differences in the contents of polyphenolic compounds among genotypes of arecanut varieties. Phenolic compounds are important components of arecanut and they contribute to sensory characteristics such as colour, flavour, astringency and hardness of it. The polyphenol content of arecanut varied depending on maturity stages. From this point of view, significant differences have been noted among months on the content of total phenols in arecanut varieties. There was no statistical difference in total phenol changes among the arecanut varieties. The lowest polyphenol content were found at the ripen maturity stage. Regarding the arecanut varieties, the decrease in content of total polyphenol compounds was noted during fruit attaining maturity or ripens stage. It is speculated that an increase in the degree of polymerization of tannin or polyphenol occurs in arecanut fruit with months and results in a decrease of extractable tannin.

Result of this study and other numerous investigations having confirmed that concentrations of phenolic compounds are generally higher in young fruits and tissues (Britton 1983, Macheix *et al.* 1990). In fruits, the total phenol content (mg/g f.w.) falls during growth, but two distinct phenomena can be observed. Either the level continues to fall steadily, as in the case of white-coloured species and varieties, e.g. white grape cultivars, mango and banana, or it rises at the end of maturation as in the case of red fruits in which anthocyanins or flavonoids accumulate (Macheix *et al.* 1990). Recent studies have shown that phenols and polyphenols are stronger antioxidants than the vitamin antioxidants (Rice-Evens *et al.* 1997). Areca nut polyphenol, which could be a rich source of antioxidant potential, may be used as food preservatives or additives.

Contents of extractable condensed tannins (CT) in arecanut and the changes during months are depicted in Table 1. Condensed tannins (CTs) have antiparasitic effects (Waghorn and McNabb 2003). It is speculated that plants containing condensed tannins evolved over time to implore them as a defense mechanism, which protected them against pathogenic microorganisms and against being consumed by insects or grazing animals (Swain 1979). Condensed tannins (CT) can be beneficial to ruminants as anthelminthics and by binding to plant proteins to decrease ruminal bacterial metabolism of protein and arecanut has already been used anthelminthics which may be due to presence of condensed tannins. The arecanut varieties showed a marked increase in CT content beginning from the 3 month (very tender stage) while the later semi ripe and fully ripen stages showed a marked decrease about 20% to 40% in condensed tannins (CT). The difference between varieties and months were statistically significant. CT content among arecanut varieties, which studied maximum was found in 3 month stage in Sumangala (118.85 mg /g d.w) and Mohitnagar (116 mg/ g d.w) respectively. In fully ripe arecanut maximum CT content present in Sumanagala (87.19 mg/ g d.w.) followed by Sreemanagala (83.95 mg/g d.w.). The ratio of condensed tannins (CT) to total polyphenol (TP) was found to be 40-50 % for entire growth period which gives an indication of the relative proportions of condensed tannins. CT concentration is primarily controlled by genetic factors and secondarily by environmental variables (Miller and Ehlke, 1996; McMahon *et al.* 2000).

Arecoline levels in arecanut has undergone distinct changes during month of maturity are presented in Table 1. Arecoline derivatives, as muscarinic agonist which is an agent that enhances the activity of the muscarinic acetylcholine receptor, had useful profiles in treatment of Alzheimer's disease (AD) on basis of cholinergic hypothesis. Arecoline is known to be a vasodilator (Goto *et al*. 1997). Alkaloid content in arecanut constantly changed throughout the fruit maturity period and alkaloids have been found to be the intermediate forms of nitrogen metabolism, in which these arecoline was accumulated in increasing tendency. Our results are in consistent with previous report on arecanut (Mathew *et al.* 1965). There were considerable differences in the contents of arecoline content among genotypes of arecanut varieties, but there were not statistically different among arecanut varieties. During fruit development in various month of

Table 1. Changes in polyphenol (mg /g d.w[#]), condensed tannins (mg/g d.w) and arecoline (mg/ g d.w) contents during maturity of arecanut genotypes*.

(Pooled data for three years)

Parameter	Polyphenol			Condenced tannins			Arecoline		
				Months					
Genotypes	**3-4**	**6-7**	**9-10**	**3-4**	**6-7**	**9-10**	**3-4**	**6-7**	**9-10**
Mangala	241.7	211.71	168.32	109.88	87.53	67.61	0.128	0.212	0.324
Sreemangala	268.3	225.02	156.71	113.82	103.54	83.95	0.130	0.219	0.327
Sumangala	272.30	203.3	160.02	118.85	105.60	87.19	0.132	0.217	0.329
Mohitnagar	281.71	221.3	141.71	116.5	85.66	65.73	0.126	0.215	0.328
For Comparing	LSD at 5 %			LSD at 5 %			LSD at 5 %		
Genotype	NS			4.88			NS		
Months	8.09			4.23			0.057		
Genotype x months	NS			8.46			NS		

*Each value represents means of six replicates NS-non-significant
#d.w-dry nut weight

maturity arecoline content showed statistically significant different in arecanut varieties. Ripe arecanut showed high arecoline content than unripe arecanut. Semi ripen stage arecanut are considered to be lower arecoline content.

In conclusion, biochemical content of arecanut was dependent on genotype and maturity stage of arecanut. Even though biochemical content during maturity among arecanut varieties were not found statistically significant, Sreemangala showed higher polyphenol and condensed tannin. Sumangala showed higher condensed tannin. With respect to arecanut maturity stages, tender nut stage (6-7 month) may be ideal stage for arecanut chewing purposes, which also showed lower arecoline content and this stage may also be ideal for optimum extraction of polyphenol and condensed tannin for pharmacological uses.

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