JEB
Journal of Environmental Biology

Journal Home page : www.jeb.co.in
E-mail : editor@jeb.co.in

ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Impacts of biotic and abiotic stress on major quality attributing metabolites of coffee beans

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Publication Info

Paper received:
27 November 2013

Revised received:
10 April 2014

Re-revised received:
06 June 2014

Accepted:
27 August 2014

Abstract

Biotic stress factors such as *Rhizopus oligosporus* and *Aspergillus niger* mycelial extracts and abiotic elements methyl jasmonate (MJ) and salicylic acid (SA), when administered through floral spray to *Coffea canephora*, showed significant influence on major bioactive metabolites of beans. Up to 42% caffeine, 39% theobromine and 46% trigonelline, along with 32% cafestol and kahweol content elevation was evident under respective elicitor treatments. Over all, the surge in respective metabolites depends on elicitor stress type and concentration. Abiotic factors MJ and SA were found to be efficient at 1 to 5 µM concentration in augmenting all the metabolites, compared to *R. oligosporus* and *A. niger* spray at 0.5 -2.0 % wherein the response was moderate as compared to abiotic stress, however significant compared to control. Though this elevation in caffeine, theobromine, cafestol and kahweol is not warranted from quality point of view, increase in trigonelline improves coffee quality. Besides increase in metabolites, stress mediated augmentation of bioactive compounds in coffee has a wide scope for studying gene expression pattern.

Key words

Cafestol, Caffeine, Coffee, Kahweol, Nicotinic acid, Trigonelline

Introduction

Secondary metabolites production in plants is influenced by various extrinsic and intrinsic factors (Ramakrishna and Ravishankar, 2011). Extrinsic factors like environmental and biotic factors such as fungi, bacteria and algae are reported to be good in eliciting production of various secondary metabolites (Prasad *et al*., 2006). Recent studies have demonstrated the influence of this elicitor stress mediated approach to augment food value metabolites such as annatto pigment in *Bixa orellana* (Giridhar and Parimalan, 2010; Giridhar *et al*., 2012), phenyl propanoid intermediates and capsaicin in Chilli (Gururaj *et al*., 2012).

Coffee is rich in bioactive compounds such as caffeine, trigonelline, nicotinic acid and diterpenes cafestol and kahweol. Coffee consumption has been correlated with reduced risk of colon rectal cancer (Lee *et al*., 2007), Type 2 diabetes (Campos and Baylin, 2007) and Alzheimer's disease (Barranco *et al*., 2007). Caffeine (1,3,7 trimethyl xanthine) is one of the major secondary metabolite in coffee and some other plants and is reported to combat physical and biotic stress factors such as pathogens and predators (Kim *et al*., 2011). Similarly, coffee also contains trigonelline which is considered as the second abundant alkaloid compound and it thermally gets converted to nicotinic acid and some flavour compounds during roasting (Shimizu and Mazzafera, 2000). In addition, it is considered important for taste and nutrition (Ashihara, 2006). Other important components that are associated with lipid fraction of coffee are diterpenes- cafestol (Caf), kahweol (Kah) which are unique to coffee, and they are mainly pentacyclic diterpene alcohols based on kaurene skeleton (Urgert *et al*., 1995) and only a small portion (~ 1–3%) is present in free-form. High level of caffeine produced by coffee seedlings exhibit allelopathy there by inhibiting germination of other seeds in the vicinity of growing plants (Peneva, 2007). The production of purine alkaloid caffeine was shown to be stimulated by stressors such as high light intensity and high NaCl concentration (Frischknecht and Baumann, 1985). Though sporadic reports are available on biotic elicitors influence on *in vitro* cultures of *Coffea* for caffeine production, no such reports are available on *Coffea* grown *ex vitro*, and also during ontogeny of fruits. Such studies pertaining to trigonelline, cafestol and kahweol production in

green beans of *C. canephora* are lacking. Moreover, various microbes incidence on coffee berries is reported (Velmourougane *et al*., 2011; Velazquez Aradillas *et al*., 2011) which may influence the quality of bean. In the present investigation, the influence of abiotic elicitors (salicylic acid and methyl jasmonate) and biotic elicitors (*Aspergillus niger* and *Rhizopus oligosporus*) on caffeine alkaloids, trigonelline and antinutritioal diterpenes cafestol and kahweol production in green beans of *C. canephora* under elicitor stress was studied.

Materials and Methods

Preparation of biotic and abiotic spray : Biotic elicitors were prepared using two fungal cultures viz., *Aspergillus niger* and *Rhizopus oligosporus* which were procured from microbial culture facility of Food Microbiology Department of CFTRI. Fresh cultures of *A. niger* and *R. oligosporus* were made on PDA slants and incubated for 7 days. Then the spores of the respective fungi were used to prepare spore suspension in 0.1% sodium lauryl sulphate (w/v) and diluted with sterile distilled water under sterile conditions to obtain a spore density of ~ 2.5 X 10^6 spore mL^{-1}. Later, the same was inoculated into 40 ml of PDA broth contained in 150 ml Erlenmeyer conical flasks and the cultures were incubated in dark for 10days. After culture growth, the cultures were autoclaved and the mycelium was separated from the culture broth by filtration and their fresh weight was recorded. An aqueous extract was made by homogenizing in mortar and pestle using neutralized sand. The extract was filtered through Whatman no. 1 filter paper. Then they were made up to known concentration and kept as stock solution from which the individual fungal mycelial extracts at a working concentration of 0.5, 1.0 and 2% % w/v (wet weight of fungal mycelium in 100 ml of distilled water) were prepared in sterile water and used for elicitation experiment. Abiotic elicitors viz. salicylic acid (SA) and methyl jasmonate (MJ) (Sigma, USA) were dissolved in distilled H_2O and diluted to three concentrations (1.0, 2.5 and 5.0 µM) for study.

Biotic and abiotic stress floral administration : 5 years old plants of *Coffea canephora* CxR were used to study the influence of different elicitors at different concentrations. To avoid interactive effect, plants sprayed with a type of elicitor were not used for another type of elicitor in the present study. All these plants were under cultivated in an area of 50 X 40 ft at Plant Cell Biotechnology Department, of this institute, with a spacing of 4 X 4 ft. The prepared biotic (*A. niger, R. oligosporus*) and abiotic elicitors methyl jasmonates (MJ) and salicylic acid (SA) were sprayed on the flowers during anthesis (between 9-11 hrs). The ripened red fruits of the respective treatments were harvested at maturity (after 9 months) and used for extraction of respective molecules and their analysis.

Extraction and analysis of caffeine : Fruit pulp was removed and beans were dried to attain 12-15% moisture content. To determine caffeine, beans (5g fresh) were used for alkaloid extraction and the tissue was ground with 80% ethanol using mortar and pestle and resultant slurry was homogenized using neutralized sand and the extract was centrifuged for 10min at 8000 rpm and the supernatant was collected after centrifugation. The extract was flash evaporated to dryness and dissolved in 1ml of 80% ethanol, prior to estimation of caffeine and metabolites by HPLC (Ashoor *et al*., 1983). HPLC analysis was performed on Shimadzu LC 20 A (Shimadzu Corp., Kyoto, Japan) equipped with CLASS-VP integrator software for data processing. The C-18 column used was 250 mm in length, with an internal diameter 4.60 mm, particle size 5.0 µm, pore size 110A°, (Gemini column of Phenomenex, USA). HPLC separation was performed at ambient temperature by applying isocratic mobile phase consisting of methanol/ water (25:75 v/v), with a flow rate of 0.8 ml min^{-1}. The mobile phase was degassed by vacuum filtration through a 0.22 µm filter and the detector wavelength was set at 270 nm. The compounds were identified by their retention times, chromatographic comparisons with authentic caffeine standard (Sigma-Aldrich, USA), and their UV spectra. Quantification was based on external standard method.

Extraction and analysis of trigonelline and nicotinic acid : To 5 g powder of coffee beans 25 ml water was added, and the mixture was autoclaved for 20 min at 120º C (Taguchi *et al.,* 1985). After cooling, the sample was centrifuged for 10 min at 1,300 xg. To the precipitate, 5 ml of water was added and the well-mixed solution was centrifuged again before the supernatant was collected. The extraction procedure and washing of precipitate were conducted twice and the supernatants were pooled, filtered (0.2µm. pore size filter) and directly analysed using HPLC with C-18 column, 5.0 µm, 4.6 mm x 250 mm column (Sunfire column of Waters, USA) with a solvent system methanol: water (3:1 v/v) and chromatograms were recorded at 268 nm. The compounds were identified by their retention times, chromatographic comparisons with authentic standards and their UV spectra.

Extraction and analysis of diterpenes cafestol and kahweol : Known quantity of ground coffee sample (grounded green beans) was weighed (10gms) and kept for soxhlet extraction, with tert-butyl methyl ether as solvent (volume of 160 ml for each extraction) for 6 hr for extraction of cafestol and kahweol separately.

Upon performing saponification, the residue was collected (fatty acid free part) dried and evaporated using rotavapor (Laborota-4000, Heidolph, Germany) under dark conditions. Respective extracts stored at -20°C were reconstituted in 1ml of methanol (v/v) for estimation of diterpene levels just before analysis.

HPLC analysis was performed (Kolling-Speer *et al*., 1992) on Shimadzu LC 20 A (Shimadzu Corp., Kyoto, Japan) using Nucleosil 120-3, C18, 250/4 column (Macherey – Nagel, Gmbh, Germany) with UV absorbance at 230 for cafestol and 290 for kahweol. The mobile phase used was acetonitrile: water:

glacial acetic acid (70/29.5/0.5 v/v) with a ~ pH 3.1 and flow rate of 0.6 ml min^{-1} for 30 min. Identification of compound was based on peak elution of compound *i.e.* retention time (RT) comparison and co-elution with authentic standards of cafestol and kahweol (Sigma- Aldrich, USA).

Statistical analysis : Data obtained were subjected to statistical analyses for the significance of the study, using one-way analysis of variance and the means were separated using Duncan's multiple range test. Data from all five replicate determinations were analysed statistically by SPSS 17.0 software through one-way ANOVA and homogenous subsets were determined to separate the mean values of different samples and developmental stages. Statistically significant (different subsets) means was marked with different alphabets.

Results and Discussion

Increase in respective secondary metabolites of coffee beans was evident under different types of stress which vary with the elicitor and its concentration. Both biotic and abiotic factors were influenced in a similar way. However, the number of folds of rise in metabolites was better in abiotic elicitors. Analysis of respective molecules in extracts was done by HPLC method. Retention times for different standards were; theobromine (RT 5.54 min) and caffeine (RT 13.56 min), nicotinic acid (RT 2.18 min), trigonelline (RT 2.75 RT), Kahweol (RT 7.95 min) and cafestol (RT 12.68 RT). The purine alkaloid caffeine content was maximum (2900 mg 100g f. wt.) at 2.5 µM MJ, which was 42% more than that of control (Fig.1a). At 2.5 µM SA treatment also, an increase in 39% caffeine content was found. At par with caffeine content, theobromine content too increased at respective elicitor treatments and maximum production under 2.5 µM SA spray was noticed which was 38% more than control. When biotic factors, *R. oligosporus* and *A. niger* were sprayed at 1% concentration as elicitors to flowers, there was maximum enhancement of 20% and 18% in trigonelline, 25% and 21% in caffeine, respectively (Fig.1b), which was less than that of abiotic elicitors SA and MJ .

The content of pyrimidine alkaloid trigonelline and its precursor nicotinic acid too were influenced by respective elicitors (Fig.1c, d). In case of trigonelline and its precursor nicotinic acid, a similar trend was noticed, wherein trigonelline production was maximum at 1.0 µM MJ. Though there was an increase in nicotinic acid content it was in the range of 4-5 mg 100 g f. wt. as compared to control (3.78 mg $100g^{-1}$). At higher concentrations of SA (2 µM) and MJ (2 µM) there was significant decrease in caffeine, theobromine, trigonelline content.

Free diterpenes cafestol and kahweol profiles were influenced less by elicitor treatments as compared to other metabolites of the study viz., caffeine, theobromine and trigonelline. There was 12, 26 and 18% increase in cafestol content under lower to high concentration of SA treatments (Fig.1e) as compared to control (156.4 mg $100gm^{-1}$). But under 1.0, 2.5, 5.0 µM MJ spray, increase in cafestol content was more than SA treatments, and it was 17, 32 and 22% for MJ low to high concentration treatments. Similarly, both *R. oligosporus* and *A. niger* triggered moderate enhancement of cafestol compared to abiotic stress treatments and it was 4%, 14% and 10% for *R. oligosporus* and 6, 15 and 18% for *A. niger* respectively at 0.5, 1 and 2% concentration as compared to control. A similar trend was evident for kahweol content enhancement with maximum kahweol production at 2.5 µM SA treatment (Fig. 1f).

The significant entity in the present study was the augmentation of major bioactives under *ex-vitro* conditions through floral administration of respective biotic or abiotic stress factors which is a novel method with reference to natural compounds of any category. The bioactive metabolites of coffee beans investigated in the present study were compounds that were not only associated with important traits of the plant itself, with reference to coffee quality, resistance to pests and diseases, but also proved to be functionally important at physiological level in consumers (Van Dam *et al*., 2006). Elicitors of both microbial and abiotic elicitors (Parimalan *et al*., 2011) are known for triggering varied responses in plants including augmented levels of secondary metabolites for value addition (Ramakrishna and Ravishankar, 2011), which upon contact with higher plant cells, trigger the increased production of phytoalexins (Giridhar and Komor, 2002). Also they trigger pigments (Mahendranath *et al*., 2011) and isoflavones (Saini *et al*., 2013) etc. Similarly, the role of biotic elicitors on increased production of alkaloid in *Brugmansia candida* was reported (Pitta-Alvarez *et al*., 2000).

Treatment with jasmonates (MJ) can provoke accumulation of several classes of alkaloids (Zabetakis *et al*., 1999), phenolics (Lee *et al*., 1997) and capsacin in *Capsicum* (Prasad *et al*., 2006). Moreover, a mechanism by which MJ induced-gene expression, involved in plant secondary metabolites biosynthesis at molecular level, was demonstrated (Suzuki *et al*., 2005). But such reports are scanty with reference to augmentation of coffee metabolites. Hence, the present study is first of its kind, wherein, floral application of MJ could increase major secondary metabolites content in robusta coffee beans as discussed. Similarly, recent studies have demonstrated the importance of SA in alkaloid production in *Stemona curtisii* hairy root cultures (Chotikdachanarong *et al*., 2011) and trigonelline production in *Trigonella foenum-graceum* cell cultures (Mathur and Yadav, 2011).

Production of caffeine is stimulated by stress factors such as high light intensity and high NaCl concentration (Frischknecht and Baumann, 1985). Exogenous calcium influences alkaloids in *C. arabica* (Ramakrishna *et al*., 2011). In the present study, biotic elicitors (*A. niger, R. oligosporus* (0.1% w/v) enhanced ~ 15% of cafestol and kahweol than control. The level of caffeine in developing seeds are important though information is available on the actual role of this in developing fruits unlike polyamines

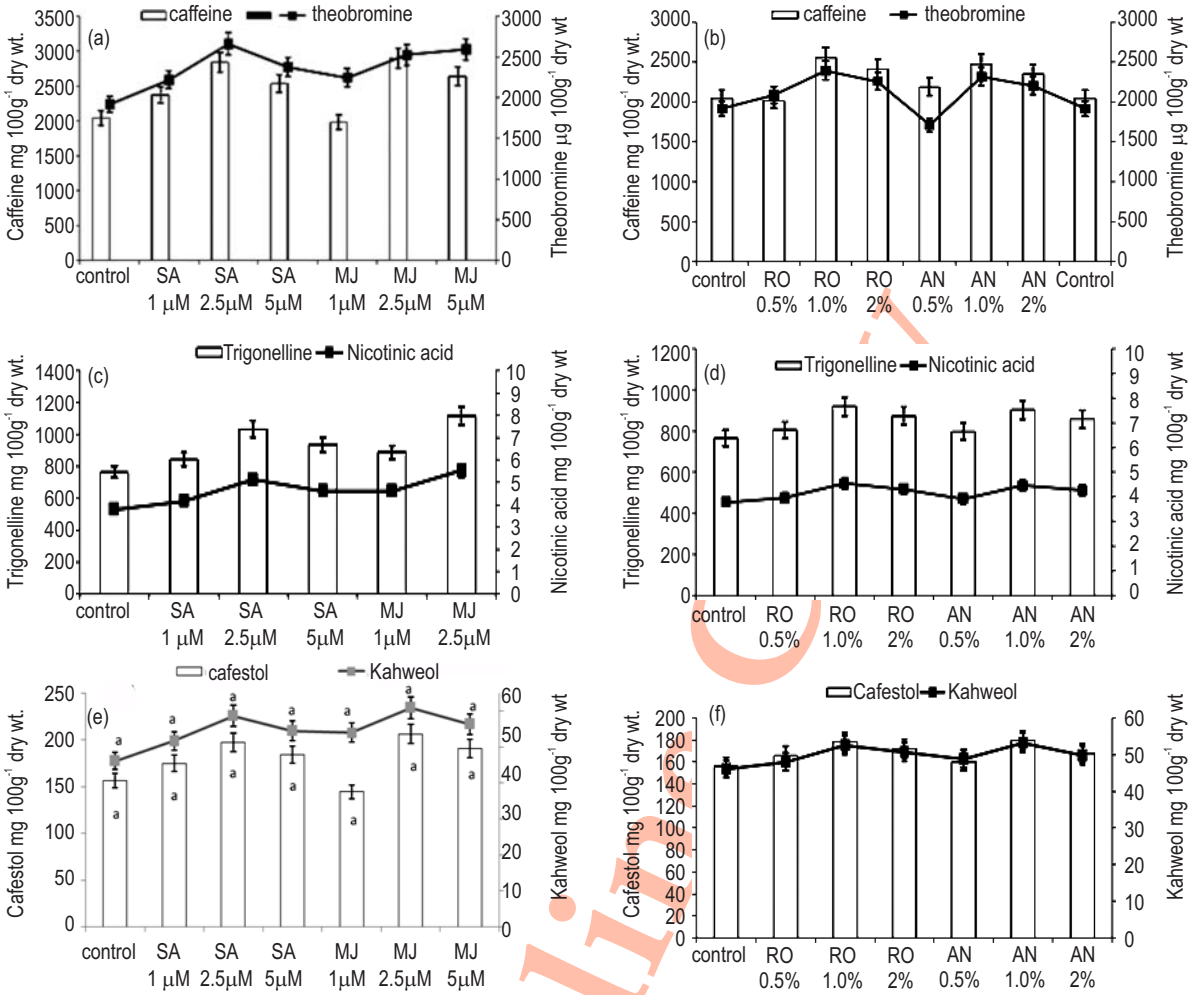

Fig. 1 : Metabolites profile of harvested beans of *C.canephora* plants (values are mean ± SD of five analyses; significant at p< 0.05); (a) Caffeine and theobromine under abiotic stress; (b) Caffeine and theobromine profiles under biotic stress; (c) Trigonelline and nicotinic acid under abiotic stress; (d) Trigonelline and nicotinic acid under biotic stress; (e) Cafestol and kahweol under abiotic stress and (f) Cafestol and kahweol under biotic stress

and free diterpenes (Sridevi *et al*., 2009; 2010). The caffeine synthesized in pericarp gets translocated to endosperm of seed where, it accumulates apart from its own caffeine content, and once further caffeine synthesis stops in pericarp in ripened fruits, caffeine content rather stabilizes in matured seeds of harvested fruits (Koshiro *et al*., 2006). So the actual elicitor response triggers during the initial developmental stage and it possibly lasts till fruit maturity stage.

Nicotinic acid acts as a precursor for trigonelline production. The trigonelline data in the present study, at all elicitor treatments, was in direct relation with nicotinic acid levels. Trigonelline is normally synthesized in almost all parts of coffee plant and its accumulation is higher in young tissues as reported by Zheng and Ashihara (2004). In general, post-harvest roasting conditions and brewing methods significant by influence the metabolic profile of beans. Other metabolites such as caffeine and diterpenes cafestol and kahweol profiles diminish upon roasting (Sridevi *et al*., 2011).

Generally, diterpenes Caf and Kah are associated with creamy fat part of the coffee brew. Though there are no reports available on direct or indirect influence of elicitor stress on lipid content and diterpenes of beans, increase in cafestol and kahweol content may be due to higher activity of gibberellic acid pathway enzymes, where ent-kaurene is the precursor for both cafestol and kahweol (Sridevi *et al*., 2010). Generally, higher concentrations of elicitor signals have impact on secondary metabolites production (Ramakrishna and Ravishankar, 2011). A part from elicitor stress, other associated factors such as climate,

temperature, and availability of light and water play a crucial role during the ripening stage of fruit with special reference to metabolite profiles of beans (Bertrand *et al*., 2006). Although low content of caffeine, theobromine, cafestol and kahweol contribute to coffee quality and taste, an increase in their content adversely effect consumers health. Especially, their elevation under various types of stress would have impact on coffee quality.

The data obtained in the present study clearly shows the influence of elicitor mediated augmentation of major bioactive metabolites of coffee, which is important from quality aspect and also has a wider scope for studying the expression pattern of major biosynthetic pathway genes of caffeine, trigonelline and diterpenes in coffee.

Acknowledgment

The authors are thankful to the Department of Science and Technology, New Delhi for financial assistance.

References

Ashoor, S.H., G.J. Seperich, W.C. Monte and J. Welty: High performance liquid chromatographic determination of caffeine in decaffeinated coffee, tea and beverage products. *J. Assoc. Off. Anal Chem*., **66,** 606-609 (1983).

Ashihara, H.: Metabolism of alkaloids in coffee plants. *Braz. J. Plant Physiol*., **18**, 1-8 (2006).

Barranco, Q.J.L., M.E. Allam, A.R. Serrano Del Castillo and C.N.R Fernández: Alzheimer's disease and coffee a quantitative review. *Neur. Res*., **29**, 91-95 (2007).

Bertrand, B., P. Vaast, E. Alpizar, H. Etienne, F. Dvarieux and P. Charmetant: Comparison of bean biochemical composition and beverage quality of *Arabica* hybrids involving Sudanese-Ethiopian origins with traditional varieties at various elevations in Central America. *Tree Physiol*., **26**, 1239-1248 (2006).

Campos, H. and A. Baylin: Coffee consumption and risk of type 2 diabetes and heart disease. *Nutr. Rev.,* **65**, 173-179 (2007).

Chotikadachanarong, K.S., A. Dheeranupattana, S. Jatisatienr, P. Wangkarn, S.G. Mungkornasawakul, A.T. Pyne Ung and T Sastraruji: Influence of salicylic acid on alkaloid production by root cultures of *Stemona curtisii* Hook. *Curr. Res. J. Biol. Sci*., **3**, 322-325 (2011).

Frischknecht, P.M. and T.W. Baumann: Stress induced formation of purine alkaloids in plant tissue culture of *Coffea arabica*. *Phytochem.*, **24,** 2255–2257 (1985).

Giridhar, P. and R. Parimalan: A biotechnological perspective towards improvement of annatto colour production for value addition – the influence of biotic elicitors *Asia Pac. J. Mol. Biol. Biotechnol.,* **18**, 77-79 (2010).

Giridhar, P. and E. Komor: Phytoalexins: Characteristics, defense mechanism and their elicitation by fungi. In: Frontiers in Plant Pathology and Biotechnology (Ed.: D.K. Dubey, C. Manohara Chary, S. Ram Reddy, M.A. Singara Charya and S. Girisham) 2002. Scientific Publishers, Jodhpur, India. pp., 105-115 (2002).

Giridhar, P., G. Mahendranath, V. Akshatha and G.A. Ravishankar: Enhanced yield of food colourant Annatto from seeds of *Bixa orellana* L: The efficacy of polyamines floral spray. *Proc. Nat. Acad. Sci. India, Sec B, Biol. Sci.,* **82**, 553-556 (2012).

Gururaj, H.B., P. Giridhar and G.A. Ravishankar: Laminarin as a potential non-conventional elicitor for enhancement of *capsaicinoid* metabolites. *Asian. J .Plant. Sci. Res*., **2**, 490-495(2012).

Kim, Y.S., S. Lim, H. Yoda, C.S. Choi, Y.U. Choi and H. Sano: Simultaneous activation of salicylate production and fungal resistance in transgenic chrysanthemum producing caffeine. *Plant. Signal. Behav*., **6**, 409–412(2011).

Kolling-Speer, I., S. Strohscneider and K. Speer: Determination of free diterpenes in green and roasted coffee *J. High. Res. Chr*., **22**, 43-46 (1992).

Koshiro, Y., X.Q. Zheng, M. Wang, C. Nagai and H. Ashihara H: Changes in content and biosynthetic activity of caffeine and trigonelline during growth and ripening of *Coffea arabica* and *Coffea canephora*. *Plant Sci*., **171,** 242-250 (2006).

Lee, J., T. Vogt, J. Schmidt, B. Parthier and M. Lobler: Methyljasmonate-induced accumulation of coumaroyl conjugates in barley leaf segments. *Phytochem.*, **44**,589–592 (1997).

Lee, K.J., M. Inouem T. Otani, M. Iwasaki S. Sasazuki and S. Tsugane: Coffee consumption and risk of colorectal cancer in a population – based prospective cohort of Japanese men and women. *Inter. J. Cancer*, **121**, 1312-1318 (2007).

Mahendranath, G., V. Akshatha, P. Giridhar and G.A. Ravishanakar: Improvement of Annatto pigment yield in achiote through laminarin spray: An ecofriendly approach. *Int. J. Agri. Env. Biotec*., **4**,163-166 (2011).

Mathur, L. and R.K. Yadav: Effect of salicylic acid on trigonelline production in *Trigonella foenum-graecum* L. cell suspension culture. *Inter. Ref .Res. J*., **17**,137-138 (2011).

Parimalan, R., G. Mahendranath, P. Giridhar and G.A Ravishankar: Abiotic elicitor mediated augmentation of annatto pigment production in standing crop of *Bixa orellana* L. *Ind. J. Fund. Appl. Life. Sci*., **1**, 229-236 (2011).

Peneva, A.: Allelopathic effect of seed extracts and powder of coffee *Coffea arabica* L. on common cocklebur (*Xanthium strumarium* L.). *Bulgarian. J. Agri. Sci*., **13**, 205-211(2007).

Pitta-Alvarez, S.I., T.C. Spollansky and A.M. Giulietti: The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of *Brugmansia candida*. *Enzyme Microb. Technol.,* **26**, 252-258 (2000).

Prasad, B.C.N., H.B. Gururaj, Vinod Kumar. P. Giridhar and G.A. Ravishankar: Valine pathway is more crucial than phenylpropanoid pathway in regulating biosynthesis in *Capsicum frutescens* Mill. *J. Agric. Food. Chem*., **54**, 6660-6666 (2006).

Ramakrishna, A and G.A. Ravishankar: Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling. Behav.,* **6**, 1720 – 1731(2011).

Ramakrishna, A., P. Giridhar and G.A. Ravishankar: Calcium and calcium ionophore A23187 induce high-frequency somatic embryogenesis in cultured tissues of *Coffea canephora P ex Fr. In-Vitro Cell. Dev. Biol. - Plant*, **47**, 667–673 (2011).

Saini, R.K., M.K. Akithadevi, P. Giridhar and G.A. Ravishankar: Augmentation of major isoflavones in *Glycine max* L. through the elicitor-mediated approach. *Acta Bot. Croat.,* **72**, 311-322 (2013).

Shimizu, M.M. and P. Mazzafera: A role for trigonelline during imbibition and germination of coffee seeds. *Plant Biol.,* **2**, 605-611(2000).

Sridevi, V., P. Giridhar and G.A. Ravishankar: Free diterpenes cafestol and kahweol in beans and *in vitro* cultures of *Coffea species*. *Curr. Sci*., **99**: 1101-1104 (2010)

Sridevi V., P. Giridhar and G.A. Ravishankar: Endogenous polyamine profiles in different tissues of *Coffea sp* and their levels during the ontogeny of fruits. *Acta. Physiol. Planta*., **31**, 757–764 (2009).

Sridevi, V., P. Giridhar and G.A. Ravishankar: Evaluation of roasting and brewing effect on antinutritional diterpenes-Cafestol and Kahweol in coffee. *Global. J. Medical. Res*., **11**, 17-22 (2011).

Suzuki, H., M.S. Reddy, M. Naoumkina, N. Aziz, G.D. May, D.V. Huhman, L.W. Sumner, J.W. Blount, P. Mendes and R.A. Dixon: Methyl jasmonate and yeast elicitor induce differential transcriptional and metabolic re-programming in cell suspension cultures of the model legume *Medicago truncatula. Planta*, **220**, 696-707(2005).

Taguchi, H., M. Sakaguchi and Y. Shimabayashi: Trigonelline content in coffee beans and the thermal conversion of trigonelline into nicotinic acid during the roasting of coffee beans. *Agric. Biol. Chem.,* **49**, 3467–3471(1985).

Urgert, R., G. vander Weg, T.G. Kosmeijer-Schuil, P. vander Bovenkamp, R. Hovenier and M.B. Katan: Levels of the cholesterol-elevating diterpenes cafestol and kahweol in various coffee brews. *J. Agri. Food Chem*., **43**, 2167-2172 (1995).

Van Dam, R.M.: Coffee and Type 2 Diabetis: From beans to beta cells. *Nutr. Metabolism .Cardiovasc. Dis* ., **16**, 69-77(2006).

Velázquez Aradillas, J.C., J. Toribio-Jiménez, M. González Chávez, C.A. de, F. Bautista, M.E. Cebrián, F.J. Esparza-García and R. Rodríguez–Vázquez: Characterisation of a bio surfactant produced by a *Bacillus cereus* strain tolerant to cadmium and isolated from green coffee grain. World. *J. Microbiol. Biotechnol.,* **27**, 907-913(2011).

Velmourougane, K., R. Bhat, T.N. Gopinandhan and P. Panneerselvam: Impact of delay in processing on mold development, ochratoxin-A and cup quality in arabica and robusta coffee. *World. J. Microbiol. Biotechnol*., **27**, 1809-1816 (2011).

Zabetakis, I., R.O' Edwards and D. Hagan: Elicitation of tropane alkaloid biosynthesis in transformed root cultures of *Datura stramonium Phytochem*., **50**, 53–56 (1999).

Zheng, X.Q. and H. Ashihara: Distribution biosynthesis and function of purine and pyridine alkaloids in *Coffea arabica* seedlings. *Plant Sci*., **166**, 807-813(2004).