Malaysian Journal of Microbiology, Vol 9(4) 2013, pp. 335-340
http://dx.doi.org/10.21161/mjm.52813

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

SHORT COMMUNICATION

Effect of fungal infection on phenolic compounds during the storage of coffee beans

Amal Abdulaziz Al-Juraifani*, Amira Hassan Al-Abdalall

Department of Biology, College of Science, University of Dammam, P.O.Box 383.Dammam31113, Kingdom of Saudi Arabia.
Email: land8080@gmail.com

Received 16 May 2013; Received in revised form 17 July 2013; Accepted 17July 2013

Aims: This work was undertaken to study the effect of *Aspergillus* infection on phenolic compounds in beans from four cultivars of the coffee plant (*Coffea arabica* L.). The effects of storage conditions of the coffee beans were also examined.
Methodology and results: Beans from four varieties of coffee were artificially infected with three species of *Aspergillus*: *A. niger*, *A. melleus* and *A. alliacus*, and stored at 0, 8 and 25 ± 2 °C). After 3, 6 and 9 months, the contents of phenolic compounds in the beans were determined using high performance liquid chromatography (HPLC).
Conclusion, significance and impact study: The results of this study showed that phenolic compounds were qualitatively and quantitatively higher in the inoculated beans as compared with the uninfected control beans, reflecting a possible induced defense mechanism in the infected beans. Increased storage periods resulted in higher levels of phenols, but the average total, bound and free phenols did not differ between the cultivars tested. Effective control of *Apergillus* infection in coffee beans can prevent such changes in phenolics that may affect their commercial value.

Keywords: coffee beans, *Aspergillus niger*, *Aspergillus melleus, Aspergillus alliacus*, phenols, storage condition

INTRODUCTION

Coffee, a drink prepared from the roasted and ground beans (seeds) of the coffee plant, *Coffea arabica*, is among the most commonly consumed beverages in the world. For example, about 70% of Brazilians drink coffee daily (Pozza, 2000). Coffee is a rich source of bioactive phytochemicals, including methylxanthines, amino acids, phenolic acids and polyphenols, while caffeine, the primary methylxantine in the beverage, is well known for its stimulatory and metabolic effects (Acheson *et al.*, 1980).

Several factors directly affect the quality of coffee. The chemical composition of the bean, for instance, is influenced by a combination of genetic, cultural and environmental factors, the preparation process and its storage. Temperature and humidity play critical roles in storage of the coffee bean since inappropriate storage conditions provide opportunity for microbial infection that is detrimental to the appearance of the coffee bean, its taste and aroma. In such situations, the question of product safety also arises. The fruit (peel, pulp and seed), being a rich source of carbon and nitrogen present in the form of cellulose, hemicellulose, pectin, reducing sugars, sucrose, starch, oils acids, protein and caffeine, serves as substrate for the growth of bacteria, yeasts and filamentous fungi. Among the microflora of coffee, filamentous fungi are the most relevant group, being more frequently encountered. The biochemical transformations in the beans resulting from their infection can result in significant crop losses (Pimenta and Vilela 2003; Pasin *et al.*, 2011). Variation in the concentrations of plant phenolics and increased activities of oxidative enzymes in response to microorganism infection is a common phenomenon in plants (Batista, 2003; Owen–Going *et al.*, 2012).

The objective of this research was to examine the effect of infection by different *Aspergillus* fungi on the phenolic contents of raw coffee beans.

MATERIALS AND METHODS

Sample collection and experimental design

The four most important varieties of coffee beans, viz. harari, lukkmaty, habbashy and barry, were collected from local markets of Dammam, Al-Khobar, Al-Qatif, Rastanura and Al-Hassa in the eastern region of Saudi Arabia.

The coffee beans were divided into four groups, with 36 boxes containing 250 coffee beans per group. Coffee

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

beans in each group were infected with the fungi *Aspergillus niger***,** *A. melleus* or *A. alliacus*. These fungi were chosen in this study because they are the most frequently isolated fungi from raw coffee beans (Table 1). The fourth group that served as control comprised uninfected beans. Following infection, the boxes of beans together with the controls were stored at 0, 8 or 25 ± 2 °C, with 3 replicates per coffee bean variety per temperature treatment. Observations were made after 3, 6 and 9 months from fungal infection.

Preparation of plant extracts

Coffee beans were pulverized into fine powder using a grinder, and 25 g of the powder were extracted with 250 mL 80% aqueous acetone for 48 h on a mechanical shaker. After filtration, acetone was removed under reduced pressure in a rotary evaporator and the remaining aqueous solution was lyophilized by freeze-drying.

Determination of total phenolic content

The Folin-Ciocalteu reagent method of Singleton *et al.* (1999) was used to determine total phenolic content. The extract was mixed with 0.5 mL of Folin-Ciocalteu agent and 10 mL of 1 M sodium carbonate. Photometric detection at 750 nm was performed after 1 h. Three replicate extractions were performed for each determination and gallic acid (GAE) was used for calibration. Phenolic compounds that were detected were further analyzed by high performance liquid chromatography (HPLC), equipped with a diode array detector.

Statistical analysis

The SPSS16 software was used for data analyses and significant differences between the treatment means were determined at p=0.05 (Norusis, 1999).

RESULTS AND DISCUSSIONS

Fungal infection on seed and grain may occur during all stages of crop production, including post-harvest and storage. Post harvest losses tend to be high in tropical countries; they can range between 25-30% (Sudha *et al*., 2007*).*

In the present study, changes in phenol content of four varieties of coffee beans that had been artificially infected with three species of fungi and stored under different temperatures are shown in Tables 2, 3 and 4. Following infection by *Aspergillus melleus*, *A. alliaceus* and *A. niger*, total phenolics were increased in all the fungal-infected samples. Free phenolic compounds of coffee beans stored at 0 °C increased significantly to 58.18, 60.22 and 60.94 mg/100 g respectively for the three *Aspergillus* species, as compared with 57.38 mg/100 g in the control group. Bound phenols similarly increased significantly with *A. alliceus* and *A. niger* infection (18.06, 17.24 mg/100 g respectively) although a significant decrease was observed after infection with *A.melleus* (14.87 mg/100 g). It could be surmised that the change in phenol content was due to the activity of the infecting fungi. Similar results resulting from fungal infection of apple have been reported by Schovankova and Opatova (2011) and De Lima *et al.* (2012).

Table 1: The frequency of occurrence of the fungi isolated from the four coffee varieties.

Isolated fungi	Varieties				Total of isolated fungi	Frequency%
	Harari	Lukkmaty	Hbashy	Barry		
A.niger	540	458	304	187	1489	74.89
A.alliaceus	82	34	26	4	146	7.34
A.melleus	9	41	1	34	85	4.27
A.tubingensis	22	19	21	17	79	3.97
F.solani	32	9	14	14	69	3.47
A.flavus	10	4	18	8	40	2.01
P.oxalicum	6	16	1	11	34	1.71
A.alternate	-	-	26	-	26	1.30
E.nidulans	10	-	-	7	17	0.85
P.variotii	-	2	-	1	3	0.15
Total of isolates	711	583	411	283	1988	

The phenolic content in infected coffee beans increased during storage at different temperatures, with both total and free phenols rising when bean samples were infected with *A. alliaceus* and *A. niger.* However, a significant decrease in free phenols was observed following infection of beans with *A. melleus* at 0 °C, although the free and total phenols rose for all bean varieties under storage at 8 and 25 °C (Tables 3 and 4).

Changes in the concentration of plant phenolics and increased activities of oxidative enzymes in response to infection of coffee plants are common phenomena

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 2: Effect of artificial infection with tested fungi on phenolic compounds of coffee beans stored at (0 °C) for different periods.

Varieties / Fungi		Phenols after 3 month			Phenols after 9 months			Storage average of phenols			Average of variety
		free	bound	total	free	bound	total	free	bound	total	
Harari	*A. niger*	**64.39**	**28.12**	**92.51**	**75.64**	**06.20**	**81.84**	**20.52**	**17.16**	**36.68**	free **74.57**
	A. alliaceus	**71.36**	**84.10**	**55.47**	**56.83**	**68.24**	**24.108**	**14.60**	**76.17**	**89.77**	bound **84.16**
	A. melleus	**89.35**	**76.10**	**65.46**	**45,81**	**43,24**	**88,105**	**67.58**	**60.17**	**26.76**	total **58.74**
	control	**29.39**	**39.10**	**68.49**	**67.80**	**33.21**	**102**	**98.59**	**86.15**	**84.75**	
Lukkmaty	*A. niger*	**96.36**	**12.8**	**08.45**	**67.82**	**15.18**	**82.100**	**81.59**	**13.13**	**95.72**	free **35.59**
	A. alliaceus	**50.32**	**04.14**	**54.46**	**89.82**	**8.35**	**69.118**	**69.57**	**92.24**	**61.82**	bound **35.18**
	A. melleus	**29.28**	**07.11**	**36.49**	**56.83**	**17.24**	**73.107**	**92.60**	**62.17**	**54.78**	total **71.77**
	control	**52.34**	**39.10**	**91.44**	**47.83**	**11.25**	**58.108**	**99.58**	**75.17**	**74.76**	
Habashy	*A. niger*	**34.38**	**94.9**	**28.48**	**38.79**	**59.20**	**97.99**	**86.58**	**26.15**	**12.74**	free **78.58**
	A. alliaceus	**68.40**	**91.10**	**59.51**	**89.87**	**56.23**	**45.111**	**28.64**	**23.17**	**52.81**	bound **27.16**
	A. melleus	**49.37**	**42.7**	**91.44**	**91.81**	**22.16**	**13.98**	**7.59**	**82.11**	**52.71**	total **05.75**
	control	**59.34**	**74.13**	**33.48**	**99.69**	**8.27**	**79.97**	**29.52**	**77.20**	**06.73**	
Barry	*A. niger*	**62.35**	**06.14**	**68.49**	**12.88**	**79.34**	**91.122**	**87.61**	**42.24**	**30.86**	free **66.60**
	A. alliaceus	**24.41**	**67.8**	**91.49**	**34.76**	**05.16**	**39.92**	**79.58**	**36.12**	**15.71**	bound **83.15**
	A. melleus	**54.48**	**35.9**	**89.57**	**44.80**	**49.15**	**93.95**	**49.64**	**42.12**	**91.76**	total **67.76**
	control	**6.35**	**63.8**	**23.44**	**88.80**	**61.19**	**49.100**	**24.58**	**12.14**	**36.72**	
	Storage period average	**87.37**	**66.10**	**53.48**	**50.80**	**99.22**	**48.103**	**17.59**	**82.16**	**76**	
Average/ fungi	*A.niger*	**64.37**	**1.11**	**74.48**	**73.78**	**40.23**	**12.102**	**18.58**	**24.17**	**44.75**	
	A.alliaceus	**78.37**	**11.11**	**90.48**	**67.82**	**02.25**	**69.107**	**22.60**	**06.18**	**30.78**	
	A.melleus	**05.40**	**65.9**	**70.49**	**84.81**	**07.20**	**91.101**	**94.60**	**87.14**	**80.75**	
	control	**00.36**	**78.10**	**79.46**	**75.78**	**46.23**	**21.102**	**38.57**	**13.17**	**51.74**	
L.S.D.		**88.40**	**85.20**	**22.56**	**13.54**	**33.15**	**38.43**				

(Mazzafera, 1999; Mazzafera and Robinson, 2000; Ramiro *et al*., 2006; Ferruzi, 2010). In the present study, total phenols, free phenols and bound - phenols in infected coffee beans increased after storage periods of 3 to 9 months, in agreement with similar results previously reported by Schovankova and Opatova (2011) and De lima *et al*. (2012). The most abundant phenolic compounds in the coffee bean are those of the

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 3: Effect of artificial infection with tested fungi on phenolic compounds of coffee beans stored at (8 °C) for different periods.

Varieties	Fungi	Phenols after 3 month			Phenols after 9 months			Storage average of phenols			Average of variety
		free	bound	total	free	bound	total	free	bound	total	
Harari	*A. niger*	**04.41**	**93.8**	**97.49**	**81.77**	**94.16**	**75.94**	**42.59**	**94.12**	**36.72**	free 57.80
	A. alliaceus	**44.39**	**01.9**	**45.48**	**22.66**	**13.15**	**35.81**	**83.52**	**07.12**	**9.64**	bound 13.05
	A. melleus	**62.39**	**82.8**	**44.48**	**99.79**	**8.17**	**79.97**	**80.59**	**31.13**	**12.73**	total 70.86
	control	**31.38**	**01.9**	**32.47**	**99.79**	**81.18**	**80.98**	**15.59**	**91.13**	**06.73**	
Lukkmaty	*A. niger*	**68.39**	**44.9**	**12.49**	**99.78**	**8.18**	**79.97**	**34.59**	**12.14**	**46.73**	free 60.90
	A. alliaceus	**22.41**	**7.9**	**92.50**	**12.80**	**85.18**	**97.98**	**67.60**	**27.14**	**94.74**	bound 13.04
	A. melleus	**87.40**	**63.7**	**50.48**	**56.81**	**22.15**	**78.96**	**21.61**	**42.11**	**64.72**	total 73.94
	control	**44.41**	**19.8**	**63.49**	**34.83**	**47.16**	**81.99**	**39.62**	**33.12**	**72.74**	
Habashy	*A. niger*	**73.36**	**29.13**	**02.50**	**67.72**	**03.26**	**97.98**	**7.54**	**80.19**	**50.74**	free 55.66
	A. alliaceus	**07.40**	**12.11**	**19.51**	**67.76**	**29.21**	**96.97**	**37.58**	**20.16**	**57.74**	bound 16.92
	A. melleus	**09.36**	**67.10**	**76.46**	**87.69**	**66.20**	**53.90**	**98.52**	**66.15**	**64.68**	total 72.59
	control	**43.37**	**62.10**	**05.48**	**78.75**	**5.21**	**28.97**	**60.56**	**06.16**	**66.72**	
Barry	*A. niger*	**04.36**	**51.11**	**55.47**	**89.68**	**99.21**	**88.90**	**47.52**	**75.16**	**21.69**	free 54.87
	A. alliaceus	**97.35**	**47.12**	**44.48**	**99.68**	**91.23**	**90.92**	**48.52**	**19.18**	**67.70**	bound 14.76
	A. melleus	**85.38**	**6.9**	**45.48**	**69.73**	**2.18**	**89.91**	**27.56**	**9.13**	**17.70**	total 69.63
	control	**78.37**	**61.6**	**39.44**	**77.78**	**79.13**	**56.92**	**28.58**	**2.10**	**47.68**	
	Storage period average	**78.38**	**78.9**	**58.48**	**84.75**	**1.19**	**94.94**	**30.57**	**44.14**	**75.71**	
Average / fungi	*A.niger*	**37.38**	**79.10**	**17.49**	**59.74**	**00.21**	**60.95**	**48.56**	**90.15**	**38.72**	
	A.alliaceus	**17.39**	**57.10**	**75.49**	**00.73**	**80.19**	**80.92**	**08.56**	**18.15**	**28.71**	
	A.melleus	**85.38**	**18.9**	**03.48**	**28.76**	**97.17**	**25.94**	**56.57**	**58.13**	**14.71**	
	control	**74.38**	**60.8**	**35.47**	**47.79**	**64.17**	**11.97**	**10.59**	**12.13**	**22.72**	
L.S.D.		**29.80**	**35.22**	**72.16**	**53.58**	**72.22**	**17.79**				

chlorogenic acid family of biomolecules, which may account for up to 12% of the dry matter of green coffee beans Ky *et al*., 2001. These compounds are mainly esters of hydroxycinnamic acids that include caffeic acid (3,4-dihydroxycinnamic acid), *p*-coumaric (4-hydroxycinnamic acid) and sinapic acid (3,5-dimethoxy-4-hydroxycinnamic acid) (Ky *et al*., 2001; Manach *et al*., 2004; Zhu *et al*., 2006). Chlorogenic acid has also important physiological functions in the coffee plant, contributing to the control of seed germination and cell

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 4: Effect of artificial infection with tested fungi on phenolic compounds of coffee beans stored at (25 ± 2 °C) for different periods.

Varieties	Fungi	Phenols after 3 month			Phenols after 9 months			Storage average of phenols			Average of variety
		free	bound	total	free	bound	total	free	bound	total	
Harari	*A. niger*	**09.40**	**42.9**	**51.49**	**86.79**	**77.18**	**63.98**	**97.59**	**09.14**	**07.74**	free 57.86
	A. alliaceus	**31.40**	**41.8**	**72.48**	**58.76**	**98.15**	**56.92**	**44.58**	**20.12**	**64.70**	bound 13.03
	A. melleus	**88.37**	**78.9**	**66.47**	**67.67**	**47.17**	**14.85**	**77.52**	**62.13**	**4.66**	total 70.88
	control	**78.41**	**46.8**	**24.50**	**67.78**	**92.15**	**59.94**	**22.60**	**19.12**	**42.72**	
Lukkmaty	*A. niger*	**13.39**	**59.8**	**72.47**	**77.79**	**51.17**	**28.97**	**45.59**	**05.13**	**5.72**	free 59.07
	A. alliaceus	**42.36**	**6.8**	**02.45**	**99.80**	**12.19**	**11.100**	**71.58**	**86.13**	**57.72**	bound 13.26
	A. melleus	**6.36**	**31.8**	**91.44**	**84.79**	**12.18**	**96.97**	**22.58**	**22.13**	**44.71**	total 72.33
	control	**93.38**	**39.8**	**32.47**	**87.80**	**42.17**	**29.98**	**9.59**	**91.12**	**81.72**	
Habashy	*A. niger*	**99.40**	**13.8**	**12.49**	**77.79**	**83.15**	**60.95**	**38.60**	**98.11**	**36.72**	free 60.40
	A. alliaceus	**94.39**	**11.8**	**05.48**	**33.79**	**1.16**	**43.95**	**63.59**	**10.12**	**74.71**	bound 12.26
	A. melleus	**97**	**86.7**	**50.43**	**89.82**	**27.18**	**16.101**	**27.59**	**06.13**	**33.72**	total 72.66
	control	**95.41**	**01.8**	**96.49**	**67.82**	**79.15**	**46.98**	**31.62**	**9.11**	**21.74**	
Barry	*A. niger*	**93.38**	**45.8**	**38.47**	**88.81**	**76.17**	**64.99**	**41.60**	**11.13**	**51.73**	free 60.60
	A. alliaceus	**24.38**	**90.7**	**14.46**	**45.75**	**59.15**	**04.91**	**85.56**	**75.11**	**59.68**	bound 12.44
	A. melleus	**03.42**	**20.7**	**23.49**	**66.87**	**02.15**	**68.102**	**85.64**	**11.11**	**96.75**	total 73.03
	control	**26.41**	**43.9**	**69.50**	**32.79**	**13.18**	**45.97**	**29.60**	**78.13**	**07.74**	
	Storage period average	**38.39**	**44.8**	**82.47**	**58.79**	**05.17**	**63.96**	**47.59**	**75.12**	**23.72**	
Average / fungi	*A.niger*	**79.39**	**65.8**	**43.48**	**32.80**	**47.17**	**79.97**	**05.60**	**06.13**	**11.73**	
	A.alliaceus	**72.38**	**26.8**	**98.46**	**09.78**	**70.16**	**79.94**	**41.58**	**48.12**	**89.70**	
	A.melleus	**03.38**	**29.8**	**32.46**	**52.79**	**23.17**	**73.96**	**78.58**	**76.12**	**52.71**	
	control	**98.40**	**57.8**	**55,49**	**38.80**	**82.16**	**20.97**	**68.60**	**70.12**	**37.73**	
L.S.D.		**68.77**	**85.51**	**36.92**	**90.75**	**12.53**	**82.89**				

growth through the regulation of indolacetic acid levels (Clifford, 1985). It is also involved in numerous plant functions such as those related to pest and disease resistance (Farah and Donangelo, 2006; Belay, 2011).

Geraldo *et al.* (2006) have earlier reported on the existence of a large variation in the coffee bean phenolic contents in different coffee cultivars. However, the contents of phenols in the present study did not differ between the coffee varieties harari, lukkmaty, habbashy and barry. Phenol contents increased significantly in the *Aspergillus*-infected beans as compared with the control beans, and they rose in tandem with the duration of storage at temperatures between 8 and 25 °C.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

The change in phenol content in coffee beans, that is a possible defense mechanism, may be influenced by the cultivar, maturation and storage conditions (relative humidity and temperature) which encourage fungal infection and proliferation. Such changes can influence the taste and flavor of coffee beans, and thus affect their quality and market value.

CONCLUSION

From the results observed in this work, we conclude that *Aspergillus* infection increases the phenolic content in coffee beans at temperatures between 8 and 25 °C. Phenolics rose in tandem with the increase in storage temperature, this being indicative of a possible induced defense mechanism reacting to the fungal infection.

ACKNOWLEDGEMENT

The authors would like to thank Dammam University for supporting this work undertaken in the Science College.

REFERENCES

Acheson, K. J., Zahorska-Markiewics, B., Pittet, P., Anantharaman, K. and Jequier, E. (1980). Caffeine and coffee: Their influence on metabolic rate and substrate utilization in normal weight and obese individuals. *American Journal of Clinical Nutrition* **33, 989-997.**

Batista, L. R., Chalfoun, S. M., Prado, C. R., Schwan, R. F. and Wheats, A. E. (2003). Toxigenic fungi associated with processed (green) coffee beans (*Coffea arabica* L.). *International Journal of Food Microbiology* **85 (3), 293-300.**

Belay, A. (2011). Some biochemical compounds in coffee beans and methods developed for their analysis. *International Journal of the Physical Sciences* **6 (28), 6373-6378.**

Clifford, M. N. (1985). Chlorogenic acids. Coffee. Springer Netherlands, London. **pp. 153-202.**

De Lima, L. M ., Pozza, E. A. and Santaons, F. D. S. (2012). Relationship between incidence of brown eye spot of coffee cherries and the chemical composition of coffee beans. *Journal of Phytopathology* **160(4), 209-211.**

Farah, A. and Donangelo, C. M. (2006). Phenolic compounds in coffee. *Brazillian Journal of Plant Physiology* **18 (1), 23-36.**

Ferruzi, M. G. (2010). The influence of beverage composition on delivery of phenolic compounds from coffee and tea. *Physiology and Behavior* **100(1), 33-41.**

Geraldo, A. M., Milton, M. S. and Paulo, M. (2006). Polyphenoloxidase activity in coffee leaves and its role in resistance against the coffee leaf miner and coffee leaf rust. *Phytochemistry* **67, 277-258.**

Ky, C. L., Louarn, J., Dussert , S., Guyot, B., Hamon, S. and Noirot, M. (2001). Caffeine, trigonelline, chlorogenic acids and sucrose diversity in wild *Coffea arabica* L. and *C. canephora* P. accessions. *Food Chemistry* **75, 223-230.**

Manach, C., Scalbert , A., Morand, C., Remesy, C. and Jimenez, L . (2004). Polyphenol food: Source and bioavilability. *American Journal of Clinical Nutrition* **79, 727-747.**

Mazzafera, P. (1999). Chemical composition of defective coffee beans. *Food Chemistry* **64, 547-554.**

Mazzafera, P. and Robinson, S. P. (2000). Characterization of polyphenol oxidase in coffee. *Phytochemistry* **55, 285-296.**

Norusis, M. J. (1999). SPSS/PC+ Statistics 6.0 for the IBM PC/XT/AT and PS/2. Prentice Hall, Library of Congress, USA.

Owen-Going, T. N., Beninger, C. W., Hall, J. C. and Sutton, J. C. (2012). Infection by *Pythium aphanidermatum* increases production of phenolics in hydroponically grown peppers and predisposes healthy plants to root rot. *European Journal of Plant Pathology* **132, 341-352.**

Pasin, L. A. H. P., De Abreu, M. S. and Souza, I. P. C. (2011). Influence of the fungi population on the physicochemical and chemical composition of coffee (*Coffea arabica* L.). *Food Science and Technology* **31(3), 681-687.**

Pimenta, J. C. and Vilela, E. R. (2003). Composicao microbiana e ocratoxina. A no café (*Coffea arabica* L.) submetido a diferentes tempos de espera antes da secagem. *Ciência e Agrotecnologia'* **27(6), 1315-1320.**

Pozza, A. A. A. (2000). A Qualidade do café opcoes para o consumo.EPAMIG. *Belo Horizonte*.

Ramiro, D. A., Gerreiro-Filho, O. and Mazzafera, P. (2006). Phenol contents, oxidase activities, and the resistance of coffee to the leaf Miner. *Leucoptera coffeella*. *Journal of Chemistry Ecology* **32, 1977-1988.**

Schovankova, J. and Opatova, H. (2011). Changes in phenols composition and activity of phenylalanine-ammonia lyase in apples after fungal infection. *Horticultural Science (prague)* **38(1), 1-10.**

Singleton, V. L., Orthofer, R. and Lamuela_Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu-Reagent. *Methods in Enzymology* **299, 152-178**.

Sudha, R., Amutha, R., Muthulaksmi, S., Baby Rani, W., Indira, K. and Mareeswari, P. (2007). Influence of pre and post harvest chemical treatments on physical characteristics of Sapota (*Achras sapota* L.) var. PKM 1. *Research Journal of Agriculture and Biological Sciences* **3(5), 450-452.**

Zhu, H., Shako H., Zhang, Z., Wang, W. and Yao, S. (2006). Laser flash photolysis study on antioxidant properties of hydroxycinnamic acid derivative. *Radiation and Environmental Biophysics* **45, 73-77.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538