Changes of enzyme activities and phytochemical constituents in small cardamom capsules caused by the infestation of thrips, *Sciothrips cardamomi* (Ramk.)

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Abstract
Thrips (Sciothrips cardamomi Ramk.) is a major pest of cardamom causing a considerable yield loss besides variation in colour and texture of the capsules. Present study was carried out to find out the phytochemical constituents changes occurring in thrips infested cardamom capsules. The results of this study revealed that there was a substantial variation in chemical constituents and enzyme activities in thrips infested capsules compared to healthy capsules.

The activity of enzymes like peptidase, trypsin like protease, peroxidase and essential oil constituent 1,8-cineole was higher in the thrips infested (itched) capsules than itch free healthy capsules. However, the concentrations of protein, total sugars, total phenols and peptidase activity on thrips infested capsules were found lesser compared to the itch free healthy capsules.

Keywords: Cardamom, healthy capsules, thrips infested capsules, phytochemical constituents, enzyme activity, volatile oil, GC-MS.

Introduction

Small cardamom, [*Elettaria cardamomum* (L.), Maton] is the queen of spices and has been most intensively cultivated in the tropical wet and moist forests in the Indian Cardamom Hills (ICH), Guatemalan Western Mountains, Sri Lankan Knuckle Hills and Tanzanian Usumbara Hills. The quality of cardamom capsules has been assessed based on colour, appearance, shape as well as the intensity of scented aroma of the cured cardamom capsules. All the through times, itch free capsules are preferred than the itched or scabbed capsules because of the perception that itched capsules would have fewer aromatic principles and reduced drying percentage besides some other biochemicals and enzymes[1,2].

At the same time, farmers have a feeling that pesticide application can increase the quality of cardamom by retaining the green colour and appearance there by, farmers were constantly motivated to apply more pesticides than the usual requirement[3]. Earlier reports have shown that the soils and water (surface) from the plantation areas were contaminated with toxic pesticides like phorate, chlorpyrifos, endosulfan and mercurial fungicides[2,4,5].

The present and future environmental sustainability of rain forest cardamom hill reserve (CHR) is in danger and needs immediate action to reduce the load of unwanted toxic pesticides. In light of this, we started working to analyze the itched capsules systematically for if there is any chemical changes occurred due to the infestation of thrips; there by, the perception of cardamom stakeholders on the quality preference of itched capsules can be changed and this can help to reduce the loads of pesticide consumption by cardamom .

Differences in the chemical compositions and bioactive metabolites of cardamom capsules from different parts of the world have earlier received an extensive analysis[3,6-10]. The essential oil (EO) of cardamom is an expensive ingredient in food preparation, beverages, perfumery and traditional medicines.[6] EO of cardamom capsules possess predominantly monoterpenic constituents such as 1,8-cineole, α-terpineol, α-pinene, linalool, linalyl acetate and nerolidol and ester constituent α-terpinyl acetate[2,7,10,11] all of which have several therapeutic benefits including antioxidant, anti-inflammatory, antifungal, antiviral anticancer, antidiabetic and gastroprotective activities[12,13].

Based on the earlier research accounts, it is apparent that EO of cardamom capsules has been attracting great interest by the researchers across the world. Therefore, the main objective of this study was to analyze itched (thrips infested) and healthy capsules for enzyme activities and phytochemical constituent changes and exploring possibilities for the reduction of pesticide usage in south India.

Material and Methods

Enzyme assays: Healthy and itch capsules under each category were assayed for acid phosphatase, peptidase and trypsin like activity profile using the substrate p-nitrophenyl phosphate and benzoyl-arginine p-nitroanilidine (BApNA) respectively.

Peroxidase activity: The assay mixture of 3 ml had 1.5 ml of 0.1 M phosphate buffer (pH 7), 1 ml freshly prepared 10 mM guaiacol, 0.1 ml enzyme extract and 0.1 ml of 12.3 mM H_2O_2. Initial absorbance was read at 636 nm and then increase in the absorbance was noted at the interval of 30 seconds on spectrophotometer. Enzyme activity was expressed as mol guaiacol oxidized min^{-1} g^{-1} protein[14,15].

Total sugars: The total sugar content of ethanol extract of well ground sample was determined according to the method described[16]. Aliquots (0.2 ml) of the extract were taken in a test tube and made up to the volume of 1 ml with distilled

water. Then 4 ml of Anthrone reagent (200 mg of Anthrone in 100 ml of ice-cold 95% sulphuric acid) was added sequentially in each tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 630 nm against the reagent blank.

Total phenols: The total phenolic content of ethanol extract of well ground sample was determined according to the method described[17]. Aliquots (0.2 ml) of the extract were taken in a test tube and made up to the volume of 3.5 ml with distilled water. Then 0.5 ml of Folin-Ciocalteu reagent (1:1 with water) and 2.0 ml of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the tubes were placed in the dark for 40 min and the absorbance was recorded at 650 nm against the reagent blank.

Proline: The proline content was assayed by the method described[18]. For the experiment, 0.5g of sample was homogenized in 10 ml of 3% aqueous sulphosalicylic acid. The homogenate was filtered through Whatmann number 2 filter paper. 2 ml of the filtrate was taken in a test tube and 2 ml of glacial acetic acid was added to it. To the mixture, freshly prepared 2 ml of acid ninhydrin was added. The final solution was subjected to heat for 1 hour in a boiling water bath. After 1 hour of boiling the reaction was terminated by placing the test tube in an ice bath. Now to the test tube 4 ml of toluene was added and stirred for 20-30 seconds.

Subsequently, the toluene layer was separated and the final mixture was again warmed to room temperature and the red colour (slightly red colour) was measured at 520 nm. The quantity of proline in the test sample with reference to standard curve was expressed in terms of µg/g fresh weight.

Estimation of volatile oil: Sufficient quantity (~100 g) of thrips infested and healthy cardamom capsules of variety Green gold were obtained from Cardamom Research Station, Pampadumpara. Both healthy and thrips infested green gold capsules were cleaned before they were used and are ground into fine powder without losing much of its volatile contents. 20 g of powdered sample was placed in the distillation flask. The distillation unit was cleaned before analysis. The hydro-distillation in Clevenger apparatus continued for 1.5 hours until the entire oil is liberated from the sample[11]. The essential oil was carefully collected and stored in a sealed glass vial for GC-MS analysis. Percentage of oil yield was expressed on volume/weight basis. The moisture content of the dry capsules was 10-11%.

Gas chromatography-mass spectroscopic analysis: Analytical GC was carried out on a Thermo GC- trace ultra-version 5.0, Thermo MS DSQ II gas chromatograph using a ZB 5-MS capillary standard non-polar column (30 m x 0.25 mm) with film thickness 0.25 µm. The carrier gas was used helium with a flow rate of 1 ml/min. The oven temperature was programmed as 70ºC for 15 min, and then gradually increased 6ºC/min to 260ºC and injection volume was 1µL. Identification of essential oil components was based on NIST and Wiley libraries as well as comparison of their retention time (RT)[10].

Results and Discussion

Variation of enzyme activities and chemical composition of healthy and thrips infested capsules are summarized in table 1. Maximum specific acid phosphatase activity was observed in fresh black seed stage for both healthy (184.11 nmoles pNP/min/mg) and thrips infested capsules (154.96 nmoles pNP/min/mg). The highest trypsin like protease activity in healthy cardamom capsules was observed in fresh white seeds and lowest in dried black seeds and similar trend was also observed in thrips infested capsule (Table 1).

Specific activity of peroxidase was greatest in the thrips infested fresh black seeds (20.0 g/minutes) compared to the healthy fresh black seeds (12.37 g/minutes) and it was confirmed with previous results of peroxidase activity of thrips infested onion plants[19]. The highest protein content was observed in the immature fresh white seeds of both healthy and thrips infested capsules and the lowest protein content was observed in thrips infested dried black capsules (Table 1).

The reduction of protein content in thrips infested mulberry leaves was reported by some other earlier researchers[20,21]. It was opined that decrease in protein content in infested leaves may be partly due to utilization by thrips at a faster rate for its multiplication. Hydrolysis of protein by proteolytic enzyme secreted by the pest itself may be the other cause of lowering the protein level[22].

Total phenols concentration was more in healthy capsules (0.318 mg/g in fresh and 0.626 mg/g in dried capsules) compared to the thrips infested capsules (0.228 mg/g in fresh and 0.328 mg/g in dried capsules). Salunkhe et al[23] reported that grains containing high amounts of polyphenolics are found to be resistant to insect attack and it was confirmed with our results. Similarly, the healthy capsules possessed more amount of total sugars compared to the thrips infested capsules (Table 1). Naik[24] also stated that total sugars and crude protein were significantly less in thrips infested leaves compared to that of healthy leaves. The proline content in the healthy capsules was less compared to the thrips infested capsules[25] which was confirmed with present study results.

Volatile oil profile of healthy and thrips infested capsules was presented in supplementary table 3 and volatile oil content did not differ significantly. The essential oil constituents of cardamom capsules are 1, 8-cineole (28.94 %) and α-terpenyl acetate (29.60%)[10] and it confirmed present study results. Furthermore, 1, 8 - cineole was found higher in thrips infested capsules (31.2%) than healthy capsules (26.10%). Nevertheless, healthy capsules recorded maximum α-terpenyl acetate content (44.8%) than thrips infested capsules (38.50%).

Table 1
Enzyme activity and chemical composition of healthy and thrips infested capsules

Particular	Specific activity of peroxidase (g/minutes)	Specific activity (nmole pNP/min/mg)			Chemical composition			
		Acid Phosphatase	Peptidase	Trypsin like protease	Protein (mg/g)	Total phenol (mg/g)	Total sugars (mg/g)	Proline (µ moles/g)
Healthy								
Fresh white seed	-	29.06	48.96	48.96	16.28	-	-	-
Fresh black seed	12.37	184.1	33.76	33.76	10.22	0.318	71.40	5.08
Dried black seed	4.73	133.8	11.60	11.63	11.90	0.626	308.63	16.04
Thrips infested								
Fresh white seed	-	22.75	42.02	42.02	12.95	-	-	-
Fresh black seed	20.0	154.96	36.03	36.03	10.55	0.228	41.383	6.69
Dried black seed	7.23	77.75	17.58	17.58	9.28	0.328	306.23	29.21
CD (P= 0.05)	1.17	5.06	2.79	2.79	0.97	0.01	8.06	1.74
CV (%)	7.61	3.34	5.83	5.83	5.45	1.17	3.212	8.84
SE	0.72	11.25	3.42	3.42	0.42	0	34.23	1.59

Abbreviations: CD, Critical difference; CV, Coefficient of variation; SE, Standard error

Table 2
Husk: Seed ratio, Volatile oil and major components of essential oil on healthy and thrips infested capsules

Particular	Husk: Seed	Volatile oil (% v/w)	1-8 cineole (%)	α-terpenyl acetate (%)
Healthy	41:60	6.63	26.10	44.80
Thrips infested	33:68	6.50	31.20	38.50

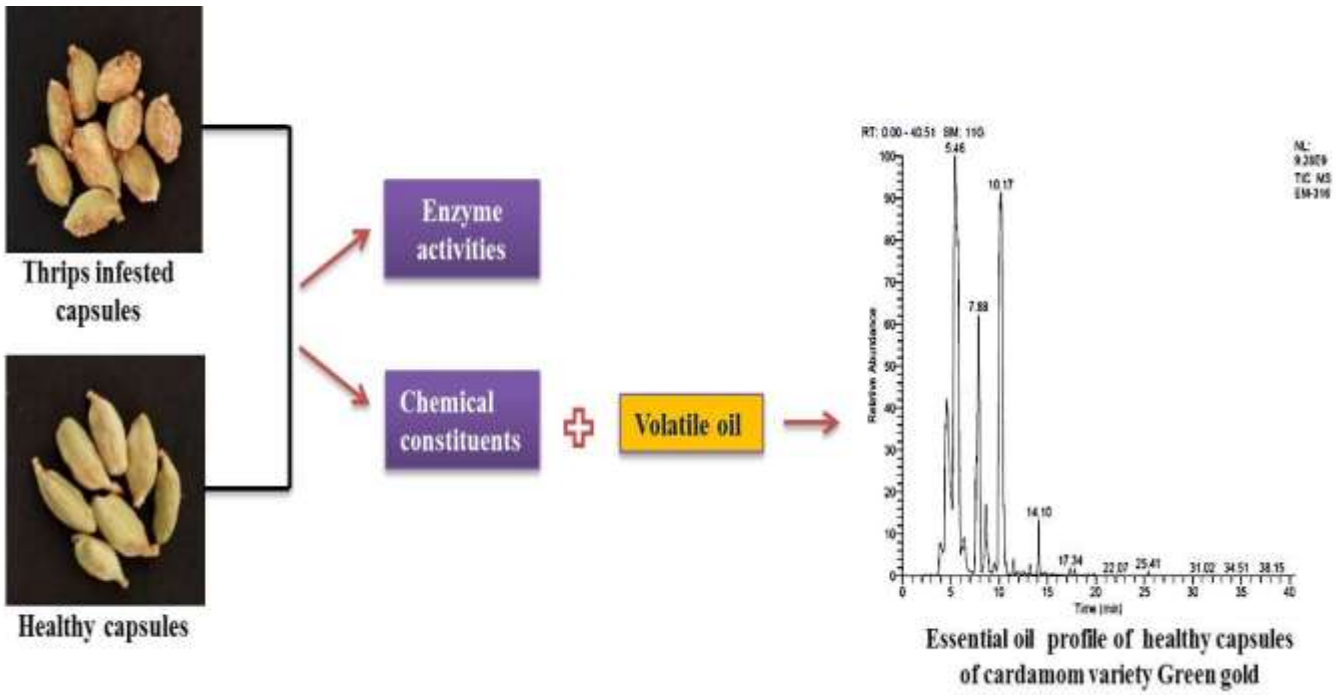

Fig. 1: Schematic representation of cardamom healthy and thrips infested capsules on enzyme activities and phytochemical analysis

Conclusion

From the study it is understood and concluded that the infestation of thrips affected qualitatively causing unacceptable appearance and colour to the capsules. The change in phytochemical parameters was significant only for 1-8 cineole content. The infestation had not therefore deteriorated the quality of the capsules because it adds fragrance and flavor. Although there were significant variations noticed for enzymes, primary and secondary metabolites between healthy and itched capsules. Hence, the stakeholders can use this information depending on their domains.

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References
1. Murugan M., Josephrajkumar A., Kurian S.P. and Backiyarani S., Essential oil of cardamom (*Elettaria cardamomum* M.), Effect of infestation by thrips (*Sciothrips cardamomi* Ramk.), *Indian Perfumer*, **46**, 321-323 **(2002)**

2. Murugan M., Josephrajkumar A., Sheeba B., Vasanthakumar K. and Ambikadevi D., Essential oil profile of elite small cardamom (*Elettaria cardamomum* M.) accessions and their interaction with thrips (*Sciothrips cardamomi* Ramk.) infestation, *Indian Perfumer*, **49**, 219-223 **(2005)**

3. Murugan M., Ravi R., Aanandhi A., Kurien S. and Dhanya M.K., Pesticide use in Indian cardamom needs change in cultivation practices, *Current Science*, **113**, 1058-1063 **(2017)**

4. Murugan M., Shetty P.K., Hiremath M.B., Ravi R. and Subbiah A., Occurrence and activity of cardamom pests and honeybees as affected by pest management and climate change, *International Multidisciplinary Research Journal*, **1(6)**, 03-12 **(2011)**

5. Dhakal B., Pinard M.A., Gunatilleke I.A.U.N., Gunatilleke C.V.S., Weerasinghe H.M.S.P.M., Dharmaparakrama A.L.S. and Bruslem D.F.R.P., Impacts of cardamom cultivation on Montane forest ecosystems in Sri Lanka, *Forest Ecology and Management*, **274**, 151-160 **(2012)**

6. Kumar A., Tandon S., Ahmad J., Yadav A. and Kahol A.P., Essential oil composition of seed and fruit coat of *Elettaria cardamomum* from south India, *Journal of Essential Oil Bearing Plants*, **8**, 204-207 **(2005)**

7. Kaskoos R.A., Ali M., Kapoor R., Akhtar M.M.S. and Mir S.R., Essential oil composition of the fruits of Eletteria cardamomum, *Journal of Essential Oil Bearing Plants*, **9(1)**, 81–84 **(2006)**

8. Saleem M., Mahmud S., Praveen Z., Waheed A. and Khanum R., Volatile constituents of *Elettaria cardamomum* Maton seed, *Pakistan Journal of Science*, **60**, 7-9 **(2008)**

9. Ashok kumar K., Pandian A., Murugan M., Dhanya M.K., Sathyan T., Sivakumar P., Surya R. and Warkentin T.D., Profiling bioactive flavonoids and carotenoids in select south Indian spices and nuts, *Natural Products Research*, DOI: 10.1080/14786419. 2018.1557179 **(2019a)**

10. Ashok kumar K., Murugan M., Dhanya M.K., Raj S. and Kamaraj D., Phytochemical variations among four distinct varieties of Indian cardamom *Elettaria cardamomum* (L.) Maton, *Natural Product Research*, DOI:10.1080/14786419.2018.1561687 **(2019b)**

11. Yashin A., Yashin Y., Xia X. and Nemzer B., Antioxidant activity of spices and their impact on human health: a review, *Antioxidants*, **6**, 70 **(2017)**

12. Hamzaa R. and Osman N., Using of coffee and cardamom mixture to ameliorate oxidative stress induced in γ-irradiated rats, *Biochemistry and Analytical Biochemistry*, **1**, 113-119 **(2012)**

13. Winarsi H., Sasongko N.D., Purwanto A. and Nuraeni I., Effect of cardamom leaves extract as antidiabetic, weight lost and hypocholesterolemic to alloxan-induced Sprague Dawley diabetic rats, *International Food Research Journal*, **21**, 2253-2261 **(2014)**

14. Putter J., Methods of Enzymatic Analysis, Academia Press, New York, **2**, 865 **(1974)**

15. Jebara C., Jebara M., Limam F. and Aouani M.E., Changes in ascorbate peroxidise, catalase, guaiacol peroxidise and superoxide dismutase activities in common bean (*Phaseolus vulgaris*) nodules under salt stress, *J Plant Physiol.*, **162**, 929-936 **(2005)**

16. Sadasivam S. and Manickam A., Biochemical Methods for Agricultural Sciences, Wiley Eastern Ltd., New Delhi, 242 **(1992)**

17. Makkar H.P.S., Becker K., Abel H. and Pawelzik E., Nutrient contents, rumen protein degradability and anti-nutritional factors in some colour and white flowering cultivars of *Vicia faba* beans, *Journal of the Science of Food and Agriculture*, **75**, 511-520 **(1997)**

18. Bates L.S., Waldren R.P. and Teare I.D., Rapid determination of free proline for water - stress studies, *Plant and Soil*, **39**, 205-207 **(1973)**

19. Sangamithra S., Jeyarani S. and Ramaraju K., Implication of oil based formulation of *Beauveria bassiana* vuillemin in inducing defense related enzymes in onion plants, *Journal of Entomology and Zoological Studies*, **5**, 766-772 **(2017)**

20. Etebari K., Jalali J. and Taksokhan M., First report on the presence of mulberry thrips, *Pseudodendrothrips moriniwa* (Thysanoptera: Thripidae) as a new species for insect fauna of mulberry field of north Iran, *Journal of Entomological Society of Iran*, **18**, 26 **(1999)**

21. Muthuswami M., Subramanian S., Krishnan R., Thangamalar A. and Indumathi P., Quantitative and qualitative damage caused in mulberry varieties due to infestation of thrips *Pseudodentrothrips mori* Niwa, *Karnataka Journal of Agricultural Science*, **23**, 146-148 **(2010)**

22. Sengupta D.J., Wickens M. and Field S., Identification of RNAs that bind to a specific protein using the yeast three-hybrid system, *RNA*, **5**, 596-601 **(1999)**

23. Salunkhe D.K., Chavan J.K. and Kadam S.S., Dietary tannins: Consequences and remedies, CRC Press, Boca Raton, Florida, USA **(1990)**

24. Naik S.L., Bio-ecology of thrips infesting mulberry, M.Sc. Thesis, University of Agricultural Sciences, Bangalore, India **(1997)**

25. Lawlor D.W. and Fock H.P., Water stress induced changes in the amounts of some photosynthetic products and respiratory metabolites of sunflower leaves, *Journal of Experimental Botany*, **18**, 329-337 **(1977)**.

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