Journal of Stored Products and Postharvest Research

Volume 7 Number 7 September 2016

ISSN 2141-6567

Academic Journals

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Journal of Stored Products and Postharvest Research

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academicJournals

Vol. 7(7), pp. 61-68, September 2016
DOI: 10.5897/JSPPR2016.0221
Article Number: 985D59660444
ISSN 2141-6567

http://www.academicjournals.org/JSPPR

Journal of Stored Products and Postharvest Research

Full Length Research Paper

Effect of hot water treatment on reduction of chilling injury and keeping quality in tomato (*Solanum lycopersicum* L.) fruits

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Received 3 May, 2015; Accepted 3 June, 2015

Chilling injury is a physiological disorder caused by the exposure of fruits and vegetables to low temperature above the freezing point. Chilling can delay fruit ripening in tomato fruits. The objective of this study was to determine the effect of hot water treatment on reduction of chilling injury and keeping quality of tomato fruits. The experiment was done in post-harvest physiology laboratory of Jimma University using Complete Randomised Design (CRD) arrangement of treatments replicated three times. The experiment had three treatments: green mature tomato treated in water at 40°C and 50°C both for 20 min and control (non-treated) fruits. Results have indicated that 40°C treatment for 20 min resulted in reduced weight loss and chilling injury index but increased fruit firmness during storage. Moreover, shelf life was better than control by three and half days when fruits were treated by hot water at 40°C for 20 min. With regard to chemical quality attributes, 50°C treatment for 20 min was better for higher lycopene content compared to other treatments. Significant differences were not detected among the treatments for total soluble solids, pH and ß-carotine. Hence, hot water treatment before storage can alleviate chilling injury and improving some quality characteristics of tomato fruits.

Key words: Chilling injury, chlorophyll, hot water treatment, lycopene, quality, tomato.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an important agricultural commodity worldwide. Tomatoes and tomato-based products are considered as healthy foods for several reasons. They are low in fat and calories, cholesterol-free, and good source of fiber. In addition, tomatoes are rich in vitamin A and C, ß-carotene, lycopene and other antioxidants (Yahia et al., 2007). However, they are chilling sensitive at temperatures below 10°C if kept for longer than two weeks or at 5°C for longer than six to eight days (Suslow and Cantwell, 2002 cited in El Assi, 2004).

Ethylene, in association with other hormones, plays a key role in the ripening process of climactric fruits like tomato (Chaves and Mello-Farias, 2006). The ripening process involves decreasing firmness as a result of structural changes in the principal cell wall components

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that is, cellulose, hemicellulose and pectin. It also involves the accumulation of sugars such as glucose, fructose and organic acids in vacuoles and the production of complex volatile compounds that are responsible for the aroma and flavor of the fruit.

Chilling injury is a physiological disorder caused by the exposure of fruits and vegetables to low temperature above the freezing point (Soto-Zamora et al., 2005). Consequences of chilling injury are failure to ripen and develop full color and flavor, irregular color development, premature softening, surface pitting, browning of the seeds and increasing decay (Verlinden et al., 2004). Moreover, according to Lelièvre et al. (1997), chilling injury interferes with gene expression for ethylene biosynthesis depending on species, cultivar, developmental stage as well as duration of the chilling treatment. In addition, cell ultrastructure is altered following prolonged chilling (Kratsch and Wise, 2000). These symptoms appear when the fruits are transferred to non-chilling temperature (above 10 to 13°C) (Wang, 1994). Another study reported that tomato showed less sensitivity to chilling injury as ripening proceeds and ethylene production increases (Ben-Amor et al., 1999).

The extent of chilling injury is related to the duration of exposure to a particular temperature, post-chilling conditions, pre-temperature treatment, harvesting stage, degree of ripeness of fruits and cultivar (Saltveit, 2005). Changes in surface colour of mature green fruits are not correlated with the sensitivity of cultivars to chilling. However, within the standard and cherry (very small size) types, chilling-tolerant fruits change surface colour when subjected to chilling unlike their chilling-sensitive counterparts when picked early in the season. This shows that early harvested standard and cherry types are less sensitive to chilling effect when harvested early (Dodds et al., 1991).

Hot water treatment is considered to be better than air treatment in reducing chilling injury (Lurie et al., 1997). According to Zhang et al. (2005), heat treatments that increase chilling tolerance are thought to be related with induced synthesis and accumulation of specific heat shock proteins (HSPs). Krishnan et al. (1989) reported that these proteins can cause thermo-tolerance on the tissue in which they are formed and hence subsequent exposure to chilling temperature does not cause damage. The study of Soto-Zamora et al. (2005) indicated that cherry tomato fruits exposed to hot air at 34°C for 24 h prior to storage at 10°C for up to 30 days showed the least loss in antioxidant content and fruit colour developed adequately.

In addition, studies have shown that subjecting tomato fruits to heat-shock treatments prior to chilling reduces the incidence and severity of chilling injury, reduces skin damage during storage, extends shelf life and inhibits ripening processes (Lu et al., 2010). Tomato fruits that are heat-treated with warm air at 38°C for 2 to 3 days can be kept for one month at 2°C without being affected by chilling injury (Sabehat et al., 1996). However, some tomato quality attributes can be negatively affected by hot water treatment. For instance, pre-heating (at 38°C or higher) inhibits lycopene synthesis in tomatoes (Whitaker, 1994). Lycopene content was more than eight-fold higher in pericarp from non-preheated tomatoes compared with heat-treated tomatoes (Whitaker, 1994). This was attributed to the inhibition of transcription of mRNA for lycopene synthase, a key enzyme in the pathway (Sabehat et al., 1996) but the tomato recovers after heat removal (Lurie et al., 1996). Heat-treated tomatoes showed a wide variation in ripening stage ranging from breaker to orange whereas non pre-heated tomatoes were uniformly red ripe (Whitaker, 1994).

The previous works mentioned above in the field revealed the effect of higher temperature treatment on the quality where the treatments were limited to about 40°C with treatment durations from several hours to a few days. However, from practical point of view, treating tomato fruits for several hours or a few days would demand more resource if it is considered in large scale. Therefore, there was a gap in information about treatment of fruits with relatively higher temperature than previously proposed (for example about 50°C) and shorten the time of treatment. In addition, previous studies about the effect of the temperature treatment on chilling injury lack information on how the quality attributes of fruits were affected. Therefore, the objective of this study was to determine the effect of hot water treatment on chilling injury and quality attributes of tomato fruits when they are stored for longer period of time after treatment.

MATERIALS AND METHODS

Study area

The study was conducted in post-harvest physiology laboratory of Jimma University College of Agriculture and Veterinary Medicine.

Experimental material and treatments

Tomato fruits of local cultivar 'Cochoro' grown in greenhouse at Jimma University College of Agriculture and Veterinary Medicine were harvested at the mature green stage. Fruits were dipped in water at 40 and 50°C for 20 min, excessive water was drained off and fruits were dried in cold air. Both hot water treated fruits and non-treated (control) fruits were then packed in polyethylene film bags and stored at 4°C for 16 days and then were transferred to 20°C and stay there for 16 days to allow ripening. Samples were removed every 4 days during the ripening period for analysis. Each treatment had 20 fruits and was replicated three times.

Data collection

Chlorophyll, carotenoids and lycopene content

The content of chlorophyll, lycopene and carotenoids pigments were extracted by mixing 1 g of extracted tomato juice with 15 ml

acetone and hexane mix solution (4:6 ratio) at once, then the absorbance of the supernatant containing those pigments were measured at 663, 645, 505 and 453 nm using spectrophotometer at the same time. From these values the content of chlorophyll, lycopene and ß-carotene in tissues were estimated by the following Equations 1, 2 and 3:

$$\text{Chlorophyll(mg/100 ml)} = 0.999A663 - 0.0989A645 \quad (1)$$

$$\text{Lycopene(mg/100 ml)} = -0.0458A663 + 0.204A645 + 0.372A505 - 0.0806A453 \quad (2)$$

$$\text{ß} - \text{carotene(mg/100 ml)} = 0.216A663 - 1.22A645 - 0.304A505 + 0.452A453 \quad (3)$$

Where, A663, *A*645, *A*505 and *A*453 are the absorbance at 663, 645, 505 and 453 nm, respectively.

Color of the fruits

During storage, tomato fruits were taken at specified time intervals for color measurements (*L*, *a* and *b* values), which were measured with colorimeter (model: ACCUprobe, HH06, USA). It was calibrated using white reference plate (a = -409, b = 867, L = 8269). Tomato fruits were scanned for color at two different locations to determine the average L, a and b values during colorimetric measurements. Then color index, chroma and hue angle were calculated from *L*, *a* and *b* values scale during storage by using the following Equation 4, 5 and 6:

$$\text{CI} = \frac{21.6a - 7.5b}{\text{La}} X100 \quad (4)$$

Where CI= color index

$$\text{Chroma} = (a^2 - b^2)^2 \quad (5)$$

$$\text{Hueangle} = \tan^{-1}(b/a)^2 \quad (6)$$

Firmness

The firmness of the fruits was measured by using texture analyzer (model: TA.XT. plus). The firmness was determined by the maximum force exerted to compress the tomato fruit down to 5 mm at 10 mm/s speed from lowering the probe until it touched the tomato skin.

Weight loss

Weight loss during post-harvest storage was determined by subtracting sample weights from their previous recorded weights and presented as percentage of weight loss compared to initial weight using the following Equation 7.

$$\text{Weightloss(percent)} = \frac{\text{Initialweightoftomato}(g) - \text{weightafterinterval}(g)}{\text{Initialweightoftomato}(g)} X100 \quad (7)$$

Chilling injury

Chilling injury index (CII) was visually assessed by the scale of skin lesion that was estimated as percentage of affected surface area where 0 = no injury (no signs), 1 = slight (<20% of surface area), 2 = moderate (20-50% of surface area), and 3 = severe (>50% of surface area). CII was calculated using Equation 8.

$$\text{CII} = \frac{(\sum(\text{scale} \times N))}{\text{totalfruitnumber}} \quad (8)$$

Where N is the number of fruits on the corresponding scale.

Shelf life

Shelf life of the fruits was recorded in days at 30% spoilage level on percent basis.

Total soluble solids (TSS)

Total soluble solid was measured from the already extracted tomato juice using hand refractometer (model: 45-02).

Titratable acidity (TA)

Tomato juice was extracted from the sample with a juice extractor and clear juice was used for the analysis of TA by the methods described by Maul et al. (2000). Finally, the percentage acidity was determined by using the following Equation 9:

$$\text{Percentageofacid} = \frac{\text{TiterX0.0064(citricacidfactor)}}{\text{1mljuice}} X100 \quad (9)$$

pH

The pH value of tomato juice was measured by pH meter. To determine the pH value of tomato juice, the probe and meter was calibrated following the manufacturer's instruction. The pH measurement of each sample was read from the probe according to the manufacturer's specifications.

Data analysis

All data were analyzed using GenStat statistical package 14th Edition (VSN International, 2012). Analysis of variance (ANOVA) was used to determine variations among the treatment effects for the variables recorded.

RESULTS AND DISCUSSION

Effect of hot water treatment weight loss and firmness of the fruit

There was a significant ($p < 0.05$) difference in weight loss between control and hot water treated (40 and 50°C for 20 min) fruits. Weight loss of fruits in the treatments gradually increased from 0 to 11.2%, 23.3 and 38.4% in 40, 50°C and control treated fruits, respectively during the 16 days storage time (Figure 1A). 40°C treatment for 20 min generated the least weight loss compared to control (12.1% higher) and 50°C treatment for 20 min (27.2% higher). According to Lurie et al. (1997) the outer pericarp tissue of hot water treated fruits tomatoes had higher phospholipids, lower sterol contents, less saturated fatty

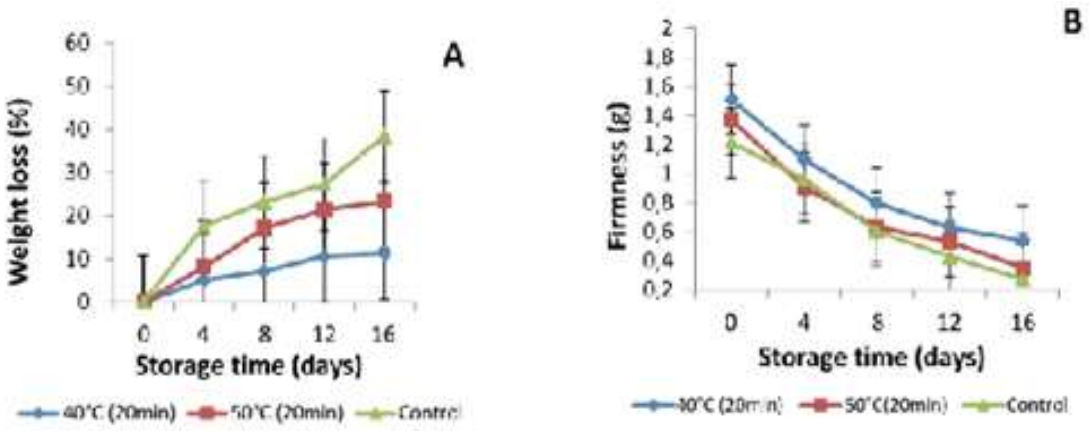

Figure 1. Effect of hot water treatments on weight loss (A) and firmness (B) of the fruit during storage at 20°C. Data are means ± SE. Control (—▲—), 40°C (20min) (—◆—) and 50°C (20min)(—■—).

acid than unheated fruits, and this might be to the reason for the treated fruit to have less weight loss compared to control. The basic mechanism of weight loss from fresh fruit and vegetables is due to vapor-phase diffusion caused by difference in water vapor pressures at different location where the product is stored (Yaman and Bayo-indirli, 2002) and besides that respiration also results in loss of weight of fruits as it involved degradation and loss of carbon atom from the fruit (Bhowmik and Pan, 1992).

The firmness of the fruits was significantly ($p < 0.05$) reduced with storage time for both hot water treated and control fruits (Figure 1B). However, there was no significant difference observed between tomato fruits treated at 40 and 50°C for 20 min (Figure 1B). At the end of the storage period, control fruits had lower firmness values than that of the treated ones. On the other hand, the maximum firmness was maintained by treated fruits. Similar result is reported by (Tigist et al., 2012) which showed that the firmness of control fruit decreased more than that of treated fruits. Fruit softening results from cell structure deterioration and changes in composition of cellular material and cell wall (Seymour et al., 2002). This is a biochemical process involving pectin and starch hydrolysis by enzymes like wall hydrolases. Depolymerization (shortening of chain length of pectin substances) occurs with an increase in pectinesterase and polygalacturonase activities during fruit ripening (Yaman and Bayoindirli, 2002).

Effect of hot water treatment on reduction of chilling injury and extension of shelf life of tomato fruit

Chilling injury index was significantly ($p < 0.05$) higher in control fruits than treated ones (Table 1). Fruits treated by hot water showed the lowest outbreak of skin lesion than control. Chilling injury in tomato fruits was observed as susceptibility to skin lesion, and failure to ripe. These symptoms mainly occurred upon removal from chilling to a warm, non-chilling temperature. Therefore, the extent of CII could be measured as the subsequent increases in skin lesion after chilling and ripening. According to Manurakchinakorn et al. (2014) heat treatment that increases chilling tolerance is believed to work through the induced synthesis and accumulation of specific heat shocked proteins (HSPs). Beside, Li et al. (2003) suggested that these proteins confer thermo-tolerance on the tissue in which they are formed and hence subsequent exposure to chilling temperature does not cause damage.

Shelf life of hot water treated fruits significantly ($p < 0.05$) higher than control fruit (Table 1). This might be due to the valuable additional features of hot water treatment to enhance the quality of fruit result in shelf life extension and food safety.

Effect of hot water treatment on total soluble solid (TSS), titratable acidity (TA) and pH of the fruit

A statistically significant differences ($p < 0.05$) was observed among the treatments in TSS. Higher TSS was recorded in hot water treated fruits than in control fruits (Figure 2A). This might be due to the fact that the extent of chilling injury was higher to cause skin lesion in fruits of control treatment. This in turn reduces the synthesis and utilization of metabolites resulting in lower TSS. It has been reported that fresh tomatoes showed more TSS than stored ones, with or without treatment (Kagan- Zur and Mizrahi, 1993). As shown in Figure 2A there was a

Table 1. Assessment of chilling injury and shelf life in tomato fruits stored at 4°C for two weeks (16 days) and subsequent ripening at 20°C for 16 days.

Treatment	Parameter	
	Chilling injury index	Shelf-life (day)
40°C (20 min)	0.193[a]	17.33[bc]
50°C (20 min)	0.299[b]	16.67[b]
Control	0.305[bc]	14.00[a]
CV (%)	15.0	5.1

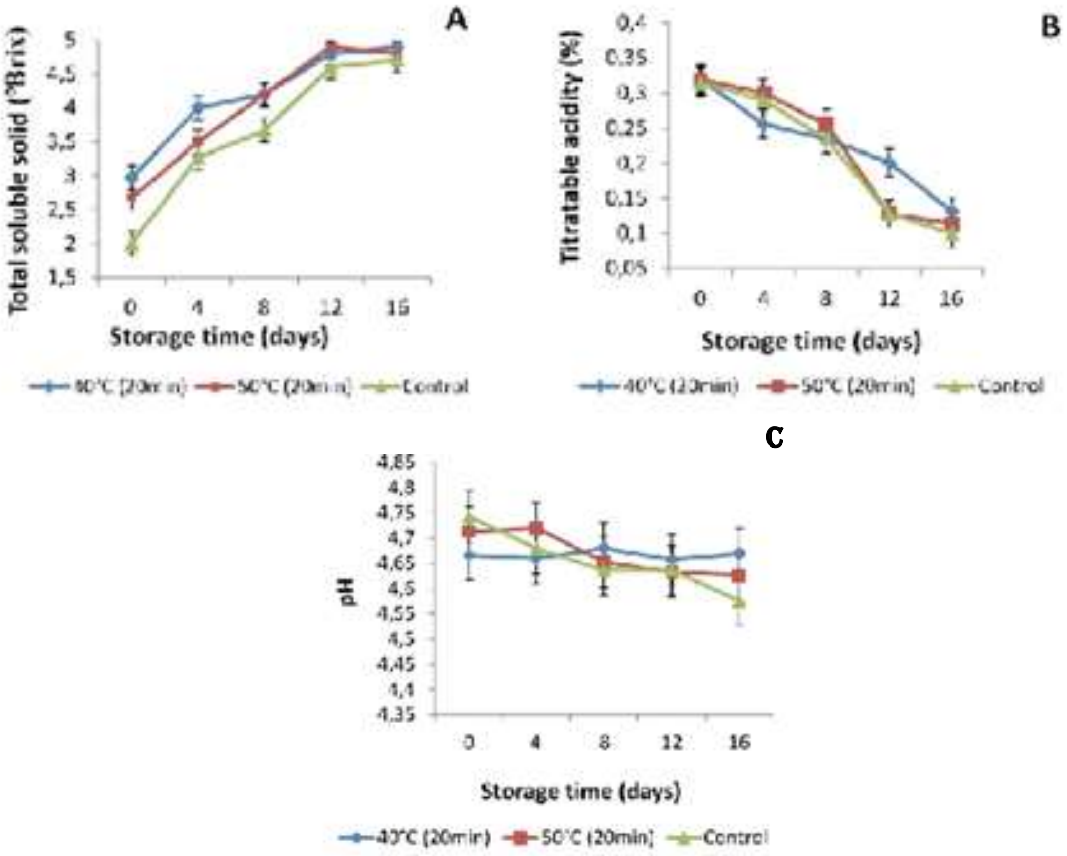

Figure 2. Effect of hot water treatments on Total soluble (A), titrable acidity (B) and pH (C) of fruit during storage at 20°C. Control ( ), 40°C (20min) ( ) and 50°C (20min) ( ). Data are means ± SE.

higher TSS in a treated tomato fruits during the gradual increase of TSS during the storage period.

There was no statistical difference observed among treated and control fruits in titratable acidity of the tomato fruits. However, the values for titratable acidity of treated and control fruit during storage decreased with storage time, with lower titratable acidity in the control treatment than fruits treated with hot water (Figure 2B). Organic acids, including malic or citric acid, are primary substrates for respiration phenomenon, therefore, a decrease of acidity is anticipated in highly respiring fruit (El-Anany et al., 2009). The pH of hot water treated and control fruits fluctuated during the storage period (Figure 2C). However, at the end of the storage period, the differences between final pH values for all samples were not statistically significant.

Effect of hot water treatment on color of tomato fruit during storage

Figure 3 summarizes the effect of hot water on color of tomato during storage. The values for hue angle of tomatoes significantly ($p < 0.05$) differed among the

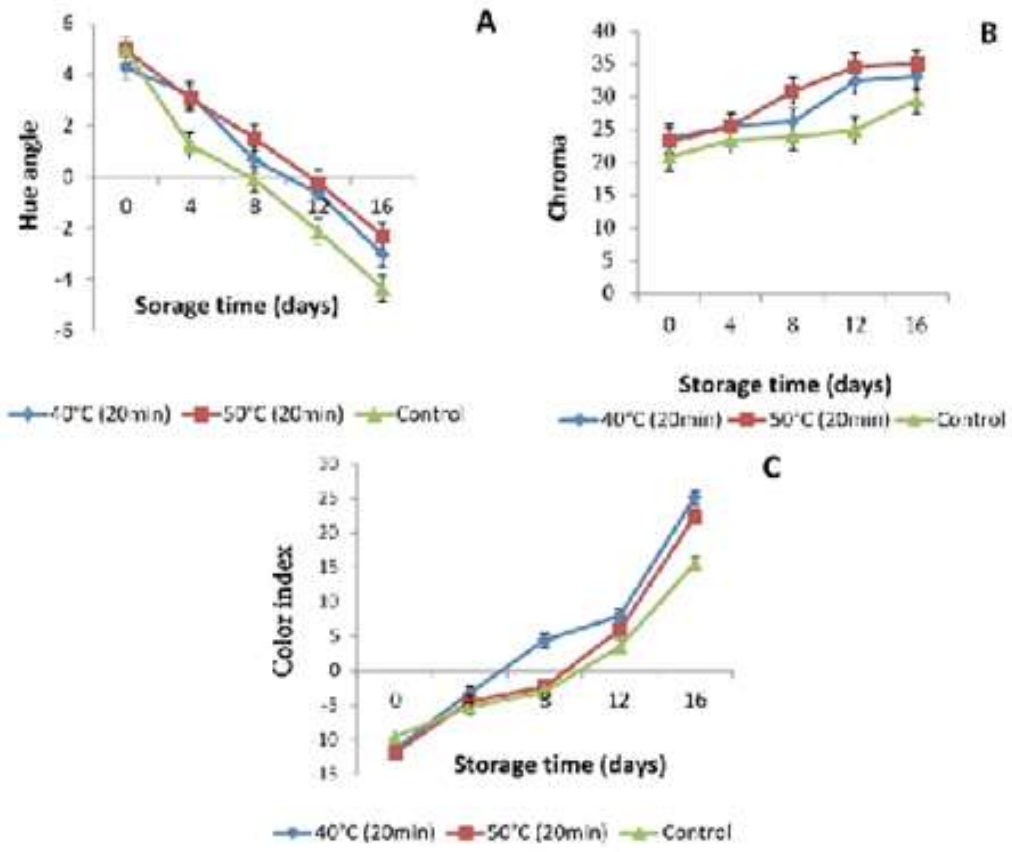

Figure 3. Effect of hot water treatments on hue angle (A), chroma (B) and total colour change (C) of the fruit during storage at 20°C. Control (—▲—), 40°C (20min)(—●—) and 50°C (20min)(—■—). Data are means ± SE.

treatments, showing a decrease with the storage time for the control and hot water treated samples. This suggested that tomato fruits gained deep red color over storage time (Figure 3A). The rate of reduction in hue angle of fruits treated by hot water was low compared with fruits in control treatment. The result indicated that hot water treatment of the fruits can retain the color of tomato fruit. Both in hot water treated and control fruits, chroma was significantly ($p < 0.05$) increasing during storage (Figure 3B). The rate of increment was significantly ($p < 0.05$) higher in hot water treated fruits than in control ones. The final values of chroma in fruits treated at 50°C for 20 min increased during storage, showing the retention of redness in tomato fruits. The color index in the control fruits was significantly lower ($p < 0.05$) than that of hot water treated fruit (Figure 3C). The significant ($p < 0.05$) increment in color index might be an indication of the development of deep red color in tomato. The result indicated that hot water treatment of tomato fruits retained the redness of the fruit even after 16 days of storage. Color is one of the important quality attributes of tomato for consumer acceptability (Lim et al., 2010). Our study suggests that some negative changes attributed to chilling injury during ripening and storage of the fruits at non-chilling temperatures may have taken place, affecting the visual quality parameters in untreated tomato fruits upon ripening and storage. Both McDonald et al. (1999) and Soto-Zamora et al. (2005) observed that hot water treatment increased respiration and ethylene evolution, and that red color development was enhanced by heat treatment and inhibited by chilling.

Effect of hot water treatment on chlorophyll, ß-carotine and lycopene content of tomato fruit during storage

Chlorophyll content of both treated and control green tomato fruits decreased during storage. The chlorophyll content in the control was higher than that of the hot water treated fruits (Figure 4A) until day eight. However, there was no difference among the treatments on their chlorophyll content of the fruits after day eight (Figure 4A). This implies that fruits without hot water treatment did show a degradation of chlorophyll, although they retained more chlorophyll than the treated fruits. In

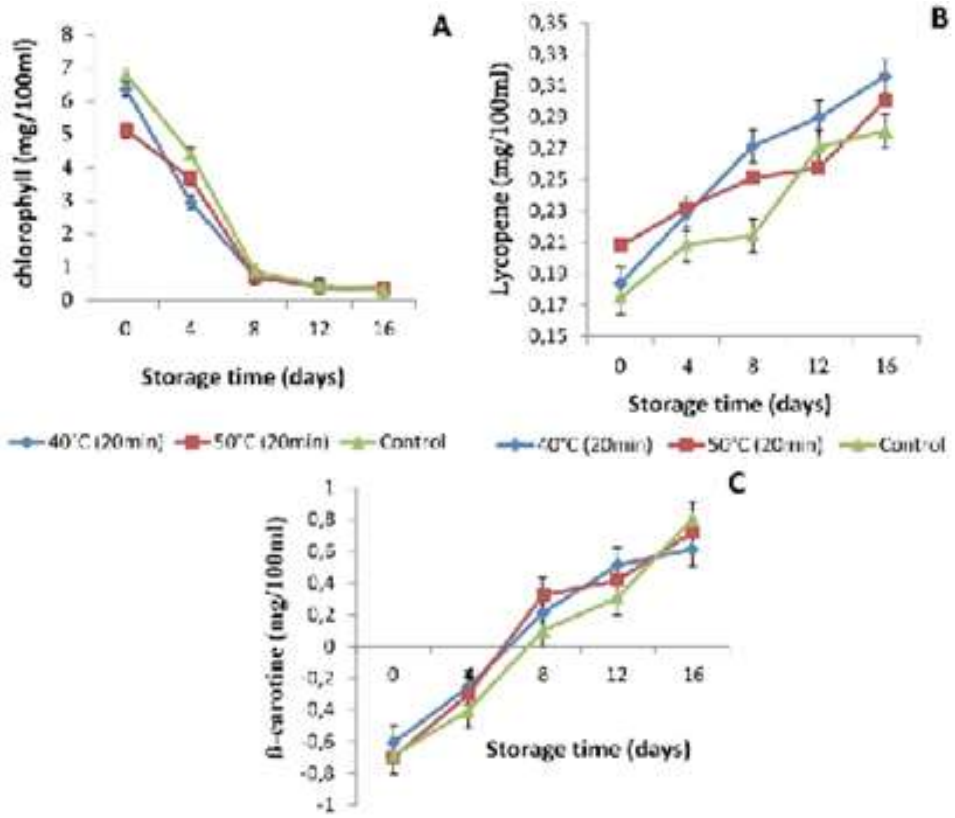

Figure 4. Effect of hot water treatments on chlorophyll (A), lycopene (B) and beta-carotene (C) content of tomato fruit during storage at 20°C. Control (—▲—), 40°C (20min)(—◆—) and 50°C (20min)(—■—). Data are means ± SE.

addition, fruits treated with 40 and 50°C for 20 min rapidly lost original chlorophyll after chilling and ripening (Figure 4A).

The accumulation rate of lycopene and ß-carotine in the control fruits was slow compared to hot-treated tomato fruits (Figure 4 B, C). The value of lycopene synthesis of tomato fruits significantly ($p < 0.05$) increased compared to control fruits while no difference was observed in ß-carotine content among all the treatments. Although there was a significant decrease in chlorophyll content in the control (Figure 4A), it was not accompanied by the generation of lycopene and carotenoids. Chlorophyll degradation and lycopene accumulation, which are the most important processes during fruit ripening and senescence, commenced in treated tomatoes after transfer from 4 to 20°C. The generation of the normal red color in ripening fruits is the result of chlorophyll destruction and accumulation of carotenoids and lycopene. The present study showed treatment at 40 and 50°C for 20 min could maintain the capability of lycopene and carotenoids synthesis which would otherwise be interrupted by chilling stress. Moreover, the failure to ripe (accumulation of lycopene and carotenoids) in control fruits could be the result of interruption in the conversion of chloroplasts to chromoplasts due to the destruction of plastids under the chilling temperature.

Conclusion

From the current study, we could conclude that hot water treatment of tomato fruit at 40 and 50°C for 20 min could significantly maintain quality of tomato fruits by enhancing physical and quality attributes. In physical quality parameters, 40°C treatment for 20 min reduces weight loss, reduces chilling injury index and increases fruit firmness during storage. In addition, it increases shelf life better than control by three and half days on average. With regard to chemical quality attributes, 50°C treatment for 20 min is better for higher lycopene content compared to other treatments.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

We thank Jimma University College of Agriculture and Veterinary Medicine for providing greenhouse facility, laboratory facility and research fund for the study. We also thank Daniel Damtew, and Wondemagegn Gebreselassie for their technical assistance in greenhouse work.

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Vol. 7(7), pp. 69-75, September 2016
DOI: 10.5897/JSPPR2015.0212
Article Number: 1BF1A6E60446
ISSN 2141-6567

http://www.academicjournals.org/JSPPR

Journal of Stored Products and Postharvest Research

Full Length Research Paper

Insecticidal activity of plant extracts and essential oils of bleed water against the bean weevil

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Received 21 April, 2016; Accepted 29 July, 2016

This study was aimed to evaluate the toxicity of semi-purified fractions (FS) (Hexane - FH, ethyl acetate - FAE, ethanol/water - FEA) and the essential oils of the bark and leaves of *Croton urucurana* against the bean weevil (*Callosobruchus maculatus*). The insects were subjected to concentration-mortality bioassays of the FS, to determine the LC_{50}. Subsequently, the effect of the FS was evaluated, through the method of vaporization and multiple choices. The fumigation action was evaluated using the essential oils, and the population growth was assessed using the FAE-leaf. All FS were toxic for *C. maculatus*, however, the lowest LC_{50}as well as a higher mortality in the vaporization method were obtained with the FAE-leaf. The essential oils from the bark caused an insect mortality greater than 80%.The least preferred rate of insects was obtained with the beans treated with FAE-bark while the beans treated with FAE-leaf reduced the population growth of the *C. maculatus*. Thus, we conclude that the semi-purified fractions and the essential oils of bark and leaves of *C. urucurana* interfere with the survival and biology of *C. maculatus*.

Key words: Euphorbiaceae, Plant extracts, repellency, bean weevil.

INTRODUCTION

The weevil *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae) is an important pest for stored grains that can cause significant damage to cowpea when left untreated (Gbaye et al., 2011). *C. maculatus* larvae feed on the inside of the grains causing weight losses of up to 80% after six months of storage, where various holes are left by the insects, thereby facilitating the mycotoxin contamination of grain and reducing the commercial value of beans (Aboua et al., 2010; Kedia et al., 2015; Kirado and Srivastava 2010). The control of this pest in storage systems depends primarily on fumigant insecticides such as deltamethrin, malathion, methyl bromide and phosphine (Erler et al., 2009; Manzoomi et al., 2010; Nyamador et al., 2010).

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However, the use of conventional insecticides and fumigant compounds has caused serious side effects such as the selection of specimens resistant to these chemical molecules, toxic waste problems and toxicity for humans and the environment (Aboua et al., 2010; Mollaei et al., 2011).

Therefore, there is a need to develop safer alternatives that can reduce the use of conventional insecticides and fumigants for stored products (Ketoh et al., 2002; Jenkins et al., 2003). There are studies in which it was discovered that products derived from plants degrade quickly in the environment and the majorities majority are less toxic to mammals, while also being more selective to non-target organisms. There are also reports that state that these products can also delay resistance development of the insect plague (Rahman and Talukder, 2006).

The Euphorbiaceae family, has several biological activities, however, it is little studied for its insecticidal activity. Numerous species of the *Croton* genus have various activities, which are anti-inflammatory, anti-bacterial, against gastrointestinal disorders (Peres et al., 1997; Suárez et al., 2003; Anazetti et al., 2004; Fisher et al., 2004; Dalbo et al., 2006; Salatino et al., 2007). Among the medicinal plants, *C. urucurana*, stands out in the treatment of gastric ulcers, rheumatism, wound healing and diarrhea (Peres, 1998). However, there are few reports for the species *C. urucurana* Baillon and its activity asinsecticide, deterrent, repellent or inhibitor to the digestive enzymes of insects. Despite numerous studies reporting on the plant's insecticide potential against Lepidoptera, there are few studies that use *C. maculatus.* Considering the advances in research related to insecticides of botanical origin, the prospects for its use in the control of insect pests, and even the lack of studies using *C. maculatus*, this research was developed in order to evaluate the insecticidal activity of semi-purified fractions (hexane, ethyl acetate and ethanol / water) and essential oils from the bark and leaves of *C. urucurana* against *C. maculatus.*

MATERIALS AND METHODS

Croton urucurana was collected in the municipality of Dourados, Mato Grosso do Sul (MS), Brazil. A voucher specimen of the species was deposited in the Herbarium of the Federal University of Mato Grosso do Sul, in Campo Grande, MS, Brazil (CGMS) under N° 5009. For the preparation of semi-purified fractions (FS) fresh bark and leaves of *C. urucurana* was first fragmented into small pieces and subjected to extraction by maceration with ethanol (w/v, 1:2). After seven days, filtering was carried out and the solid material was disposed, and subsequently the solvent evaporated (± 40°C) under vacuum in a rotary evaporator, yielding the crude ethanolic extract (CEE) from the barks and leaves of *C. urucurana*. Subsequently, the CEE was fractionated by liquid-liquid partition with increasingly polar solvents, hexane and ethyl acetate to give fractions: hexane (FH-leaf), ethyl acetate (FAE-bark and FAE-leaf) and ethanol-water (FEA-leaf). The water content of the fractions was determined from an aliquot of the same fractions, submitted to drying (100°C), until the weight was constant.

To obtain the essential oils, the fresh barks and leaves of *C. urucurana* were subjected to extraction for four hours by hydrodistillation in a modified apparatus type Clevenger, followed by distillate extraction with hexane (Simionatto et al., 2007). After removal of the solvent, the crude yield was calculated. Insects, *C. maculatus*, were collected in the Property of producers in the region of Bom Jesus (PI), Brazil, and elsewhere in southern Piauí state.These populations were conducted reared in for the laboratory, after which they were multiplied and stored in plastic jars of 19 cm in height by 12 cm in diameter, sealed with fabric of the type *voile,* which allows air circulation and prevents the escape of insects. The substrate used for the maintenance of the populations was cowpea, which was used as a food resource and for the inventory of insects. The maintenance of the populations was performed weekly to avoid the presence of mites and parasitoids.

Bioassays I – Concentration-mortality of (FS) of *C. urucurana*

To conduct For the bioassay the semi-purified fractions were diluted in absolute ethanol at concentrations of 0, 390, 781, 1552, 3125, 6250, 12,000, 25,000 and 50,0000, 100,000, 200,000 and 500,000 ppm. Thereafter, 0.3 ml of each solution was added into a cylindrical and transparent glass vials with a 20 ml capacity of 20 ml. The vials were homogenized in order for the active ingredient to be uniformly distributed over the entire inner surface of the vial, until the complete evaporation of the solvent. The experiment was conducted in a completely randomized design with five repetitions for each treatment. Twenty non-sexed adult insects were exposed to dry residues of the fractions hexane, ethyl acetate and etanol-water of *C. urucurana*. Mortality was assessed after 72 h to calculate the LC_{50} values of the extracts.

Bioassay II - Vaporization test

For this bioassay pots with a capacity of 2.5 liter were used with 250 g of beans treated with LC_{50} of each FS of *C. urucurana*. The containers had a lid that was perforated in two places with3 cm holes for the entry and exit of the steam generated by the compressor. With the aid of an adapted compressor, the FS were applied within the pots where the insects were. After 72 h the containers were opened and the number of dead insects was recorded. The experimental design was completely randomized, with five replications and 100 insects in each container in which the evidence only included beans and insects.

Bioassay III - Multiple choice test

The bioassay was performed with an arena containing five plastic flasks with a capacity of 145 ml interconnected by hoses of 5 cm in diameter to a central flask of 250 ml. The central flask received 100 non-sexed adult insects while the vials at the extremities received 50 g of beans treated with 2 ml of each FS; the concentration used was LC_{50} and zero (0). The experiment was conducted with five replications and the preference of the insects was evaluated after 1, 24 and 48 h.

Bioassay IV – Fumigant effect (Essential oils)

In this bioassay, 100 g of beans were used and were added to pots with a capacity of 150 ml. At the bottom of the lid, a filter paper was placed that was treated with 2 ml of the essential oil obtained from the leaves and bark of *C. urucurana*. Then, 20 non-sexed adult insects were released in every pot, totaling five replications in a completely randomized design. The evidence was treated with

Table 1. LC_{50} values of Semi-purified fractions of *C. urucurana* on *C. maculattus.*

Fractions	Inclination± EPM	LC_{50} (95% CI) (ppm)	X^2	*P*
FH (Sheet)	0.8002 (0,034)	357.70 (30033 - 43075)[c]	113.4232	0.3923
FAE (Shell)	0.8564 (0,034)	175.98 (15057 - 20634)[a]	98.6397	0.7931
FAE (Sheet)	0.8462(0,033)	150.00(13355-24655)[a]	97.6591	0.7888
FEA (Sheet)	0.7253 (0,036)	446.86 (41372 - 490285)[b]	95.9570	0.8447

SEM= Standard Error of Means, LC= Lethal Concentration, 95% CI = 95% Confidence interval, X^2= Chi-square, *P*= probability. Means followed by the same letter in the column do not differ significantly amongst themselves by the Tukey's test ($p < 0.05$) Draw the line after FH (Sheet).

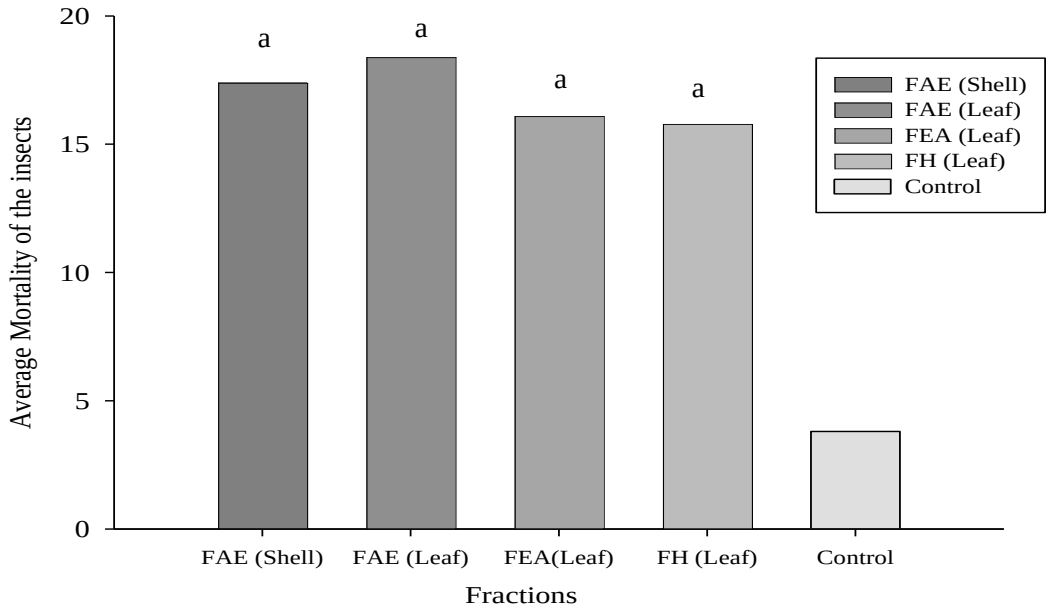

Figure 1. Average mortality of *C. maculatus* treated with semi-purified fractions of *C. urucurana* during the vaporization bioassay. Bars followed by the same letter do not differ between treatments by Tukey's test ($p < 0.05$).

distilled water. Mortality assessments were performed at 24, 48 and 72h after the start of the experiment.

Bioassay V - Instantaneous rate of population growth (FAE-Sheet)

The bioassay instantaneous rate of growth (r_g) was conducted using glass jars with a capacity of 1.5 L by adding 200 g of beans treated with LC_{50} of FAE-leaf and 50 non-sexed adult insects of the population of *C. maculattus*. The jars were sealed with a cap containing a small hole in the center which was closed with a *voile* type fabric to allow gas exchange. The experiment was conducted in a completely randomized design with four replications. After 120 days, after the start of the bioassay, the number of emerged insects, the insects living body mass and the mass of grains, were evaluated. The instantaneous rate of population growth (r_g) was calculated using the equation proposed by Walthall and Stark (1997). Where r_g = ln (Nf / No) /Δt; Nf being the final number of insects; No the initial number of insects and Δt is the variation of time. A positive value of $r_g$indicates population growth, $r_g = 0$ means that the population is stable and a negative value of r_g indicates a declining population until extinction.

Statistical analysis

The mortality results were submitted to the Probit analysis, through the PROC PROBIT procedure of the System of Statistical Analysis Program (SAS Institute, 2000), generating the concentration-mortality curves. The mortality data were corrected for the mortality that occurred in the control treatment. Details of the other bioassays were subjected to avariance analysis and Tukey's average grouping test, where appropriate (PROC GLM, SAS Institute, 2002).

RESULTS

The data from the experiments revealed that the toxicity of the treatments decreased in the following order: that is, Ethyl acetate (Leaf); Ethyl acetate (Bark); Hexane (Leaf); Ethanol Water (Leaf) in accordance with LC_{50} values (Table 1); since the ethyl acetate fractions (Leaf) and (Bark) are of greater toxicity against *C. macullatus*, with lower LC_{50} values. When it was analyzed, the mortality results in the bioassays of the vaporization (Figure 1)

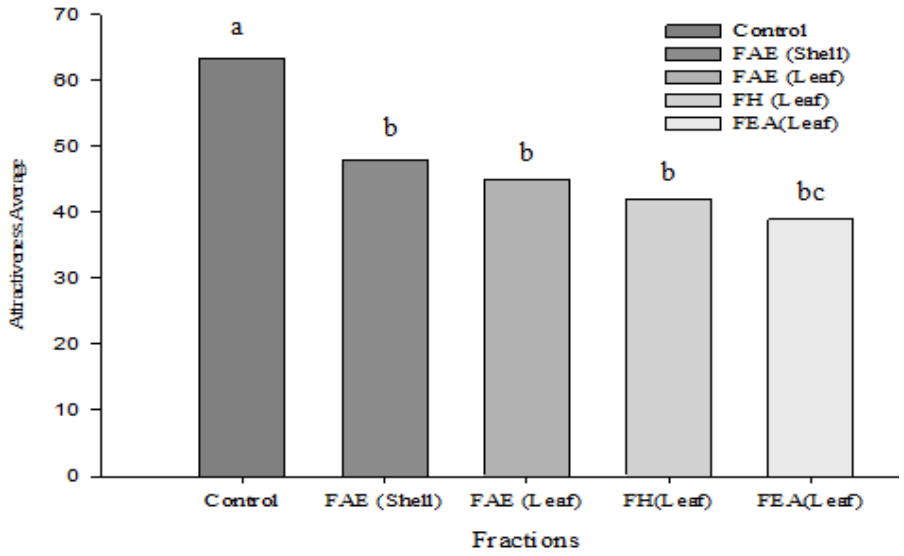

Figure 2. C. Attractiveness Average of *C. maculatus* to the grains treated with semi-purified fractions of *C. urucurana*. Means followed by the same letter do not differ significantly between treatments by Tukey's test ($p < 0.05$).

Table 2. Mortality effect of *C. maculatus* subjected to essences oils of bark and leaves from*C. urucurana*.

Treatments	Insect mortality
Control	00.8[c]
OE bark	17.1[a]
OE leaf	12.8[b]
CV%	29.26
F Average	713.6** 10.23

Means followed by the same letter in the column do not differ significantly between treatments by Tukey's test ($p < 0.05$).

revealed that all FS differ significantly from the control, however there were no significant differences between treatments. The mortality of *C. maculatus* occasioned by FS confirms the insecticide potential of *C. urucurana*.

In the multiple choice test (Figure 2), insects frequented all treatments, however, there was a difference in the repellency of FS when compared to the control. (Figure 2). The results indicate a probable existence of repellency of the tested FS, suggesting that the FEA - Leaf posses one or more allelochemical, which is able to ward off insects on contact with the beans treated with FS. The essential oils from the leaves and bark have shown promising results in the mortality of *C. maculattus*, especially for oils extracted from the bark, which shows that we must consider the plant organ in the study (Table 2). Based on the obtained results, it can be concluded that the essential oils obtained from the bark and leaf of *C. urucurana* showed as potential as a fumigant, mainly when obtained from the bark, where there was an insect mortality percentage higher than 80%. The population growth rate of insects subjected to grains treated with FAE-leaf was different when compared to the control, revealing a reduction of the multiplication of the *C. maculatus* species showing a lower growth of insects, for a period of ninety days (Table 3). The results also showed a lower intake of dry biomass of beans and a lower body mass of insects when treated with FAE-leaf. Based on the results obtained, it can be seen that the number of emerged insects was reduced when compared to the control (by 50%) when in contact with the dry residue of the FAE-leaf from the *C. urucurana* to 150,000 ppm.

DISCUSSION

The findings of this study indicated that the pesticide potential of *C. urucurana* showing that it has chemical metabolites is able to influence the tested parameters.

Table 3. Instantaneous rate of population growth, consumption of biomass of beans, body mass and number of adult insects emerged from *C. maculattus*, for 120 days, subjected to FAE-leaf of *C. urucurana*.

Treatment µg i.a.cm^2	Instantaneous rate of growth r_g	Dry biomass consumption of beans (g)[1]	Body mass (g)	Number of adults emerged for 120 days
Control	0.035766[a]	675.42[a]	3.5[a]	1308.6[a]
FAE(Leaf)	0.030454[b]	473.44[b]	1.3[b]	784.2[b]
CV%	9.0	13.86	39.14	31.18
Averages	0.03311	574.43	2.4	1046.4

Means followed by the same letter in the column do not differ significantly between treatments by Tukey's test ($p < 0.05$).

This fact may be related to the phenolic compounds with insecticidal effect present in the species of the genus *Croton*, such as tannins (Peres et al., 1997, 1998). These phenolic compounds can easily bind with the protein to form a protein-tannin complex that reduces the growth and survival of the insects, since they inactivate the digestive enzymes and hence, inhibit digestion (Mello and Silva-Filho, 2002). Furthermore, these compounds interact with proteins to render these class substances that are very toxic to insects, fungi and bacteria (Shirley 2001; Silva et al., 2009).

According to the results obtained in this study, the effect of FS from the bark and leaves of *C. urucurana* were similar to those observed for the mortality of *Anagasta kuehniella* (Silva et al., 2009) and *Dysdercus maurus* (Silva et al., 2012), confirming the need for the continuity of phytochemical studies as well as for the efficiency of molecules in the control of bean pests. When the vaporization test was re-viewed, all of FS from the *C. urucurana* showed a high percentage of mortality, a fact that highlights the toxicity of FS when applied directly on to where the insects are. This makes the investigations promising, while demonstrating the mechanism of the action of the involved metabolites in which the insect metabolic route can act. It is worth mentioning that although 100% of the FS showed satisfactory results of the *C. maculatus* mortality rates, certain criteria should be taken into account to evaluate the efficiency of the FS such as the vegetable organ and the concentration used, where in this study, in the vaporization test, the FAE-leaf proved the most efficient, considering that it caused higher mortality with lower concentrations.

The tested essential oils have a high toxicity against *C. maculatus* causing significant insect mortality when compared to the control, especially the essential oils obtained from the most active bark, with approximately 80% mortality rate. Randau et al. (2004),studying some of the *Croton* species, state that terpenoid can be associated with the insecticide activity and are found in all parts of the plant, with predominance of these substances in the leaves and roots. Therefore, the result of this study shows that the active chemical constituents may be present in FAE-leaf and essential oils of the bark. Essential oils are substances of botanical with insecticide activity (Lee et al, 2008; Chu et al., 2011). Due to its high volatility at ambient temperature, it has fumigant activity that may be important for the control of insect pests of stored products.

The results obtained for the multiple-choice test indicate that FS has a reasonable level of repellency when compared with the control, but highlights that the FEA-leaf which, in absolute terms, showed a lower number of insects that visited the grains impregnated with this fraction. The repellent effect is an important property to be considered in controlling pests in stored products, because the higher the repellency, the lower the infestation. With that, there is a reduction or suppression of the posture, and hence a smaller number of emerged insects. Therefore, natural products can successfully be used by farmers to protect stored grain from insect infestation. Many herbs have been used in developing countries to protect grains and legumes from stored product pests, such as *Lamiaceae*, which showed similar results to the present study (Ngamo et al., 2007; Li et al., 2013). The FAE-leaf reduced multiplication of insects, indicating that the present alleochemical interferes with the life cycle, in which each generation is affected negatively in their development during 90 days exposure to the residue of the FS. Similar results were obtained by (Carvalho et al., 2014), to evaluate the population growth rate of *Zabrotes subfasciatus* in beans treated with the crude extract of *C. Urucurana,* causing population reductions of 50% in 90 days. This confirms that the botanical species has allelochemicals that must be isolated and identified in order to find the bioactive molecule responsible for the negative effects on the insects as well as the site of action of these molecules. The use of plant extracts with repellent or insecticidal properties for stored grain is a traditional method common in rural areas all over the world (Regnault-Roger et al., 2012; Kedia et al., 2015). Tropical ecosystems (such as Caatinga-Cerrado) are particularly rich in plants that are used by local communities to treat diseases, thus, indicating the potential to discover new compounds (Albuquerque et al., 2008). And the species studied in this work has the potential to be used for the control of *C. maculatus.*

Conclusions

The semi-purified fractions of *C. Urucurana,* containing FAE-leaf, exhibit toxicity to *C. Maculatus,* when subjected to the lowest of LC_{50}. Through the Vaporization test, all FS exhibit significant mortality to *C. macullatus*. The essential oil obtained from the bark has increased efficiency to control *C. macullatus.* FAE-leaf reduces the multiplication of insects when evaluated for a period of ninety days. The results suggest that *C. urucurana*, has properties that cause lethal and sublethal effects on *C. maculatus*.

Conflicts of Interests

The author has not declared any conflict of interests.

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