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Phytochemical Examination of *Carica Papaya* L. against *Callosobruchus Maculatus* F. in Stored Bean Seeds

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Abstract

The Phytochemical Examination of *Carica papaya* against *Callosobruchus maculatus* in stored bean seed was examined in the laboratory, Department of Biology, Federal University of Technology, Owerri from June to August, 2018. The temperature and relative humidity were not controlled. The experimental design used was completely randomized design (CRD) with five treatments and four replications. The treatments used in this study were pawpaw leaf powder (50g), pawpaw bark powder (50g), pawpaw unripe fruit peel powder (50g), pawpaw root powder (50g) and control (0g). The results showed that pawpaw leaves powder gave the best performance in reducing the mean number of holes (33.2) with average weight loss of bean seeds (7.6g), followed by unripe fruit peel powder (82.3 and 65.3g weight loss), bark powder (91.5 and 76.9g) and root powder (119.1 and 94.4g) respectively at P= 0.05 level. The phytochemical constituents of *Carica papaya* showed the presence of alkaloid, tannins, saponins, glycosides and papain. The papain was present only in the pawpaw leaves and, absent in other pawpaw plant parts used in this study. These compounds have inhibitory activity and mortality effects against stored product pests and have no inhibition action in human. This study offers a sustainable, environmentally friendly and safer alternative to synthetic insecticides.

Keywords: Phytochemical examination, *Carica papaya, Bio-insecticide, Callosobruchus maculatus,* Bean seeds.

1. Introduction

Bean, (*Phaseolus vulgaris*), is an important edible legume crop in many parts of the world especially in tropical and subtropical regions. Human consume it as food, due to its high protein content and used as livestock feed to make silage and hay (Singh & Van Emden, 2009). Beans are one of the longest cultivated plants with Myanmar (Burma) being the world leader in production followed by India and Brazil. In Africa, the most important producer is Tanzania. Beans are an annual herbaceous legume with trifoliate leaves. It is a drought tolerant and short warm weather crop well adapted to the drier regions where other food legumes do not perform well. Bean production is affected by insect pests and disease infestations which lead to economic losses. Insect damage is the major constraint to bean grain production in most bean producing nations (Oluwafemi, 2005). The major insect pests that can cause economic loss are leafhoppers *(Empoasca spp)*, thrips (*Megaluro thripssjostedti*—Synonym: *Taenio thripssjostedti*), flower eating beetles *(Mylabris spp. and Coryna spp.)*, blister beetles (*Hycleus lugens*), green stink bugs (*Nezara viridula*) and weevil *(Callosobruchus maculatus)* by Machacha, Obopile, Tshegofatso, Tiroesele, Gwafila & Ramokapane (2012) . The bean weevil is a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of bean in the tropics. It causes substantial quantitative and qualitative losses manifested by seed perforation and reductions in weight, market value and germination ability of seeds (Chavez, 2011). In order to reduce serious losses experienced during storage, various techniques and control methods have been developed and more are still being developed. Management of bean seed storage pests relies heavily on the use of chemical insecticides. However, most of the small scale farmers have not adopted these new techniques due to some financial and technical reasons. Insecticides also have negative impact on the environment, humans and non-target organisms. Therefore, there is a need to develop cheap, safe and easy methods of protecting stored bean against bean weevil. Resource-poor farmers in Africa employ a range of traditional methods such as use of ash, sand, dry pepper and botanical extracts (Kang, Pittendrigh & Onstad, 2013). Naturally occurring plant products have been used to protect agricultural products against pests for many years in some parts of the world; many authors have reported insecticidal effects of plant products against a broad range of pests. Some of the techniques that can be explored include the use of plant products such as garlic, peppermint and chilies (Suleiman, 2011). Aromatic plants have both medicinal and aromatic properties and contain a variety of volatile oils which have insecticidal, anti-feedant and repellent effects on insect pests. The chemical repellency hypothesis states that non-host plant odors repel herbivores by disrupting their ability to locate or feed on the host plan (Bongoni, Steenbekkers, Verkerk, Van Boekel & Dekker, 2013). The plant products used for this study produce odors that are believed to repel weevils, thereby preventing them from attacking bean seeds. There is limited information on the use of the plant products as an alternative control method for controlling weevils in storage. The use of plant products may offer a sustainable, environmentally friendly and safer alternative to synthetic insecticides. Around the world, at least thirty five thousand (35,000) plant species are used for medical purpose (Kong, Gah, Chia & Ohia, 2003) and virtually all plant parts are usually consumed as food for efficient supply of energy. Most important industrial medicines are being synthesized from about 90 species of herbs and in developing countries like Nigeria, traditional medicines are usually based on herb mixtures collected from the wild (Molyneux, Lee, Gardner, Panter, & James, 2007). However, attention has been given to the medicinal values of plants and plants remedy in safety, efficiency, economy, and its suitability as food for efficient of energy. Most medicaments especially African, traditional medicines are prepared often from combination of two or more plant product such as *Carica papaya* which belongs to the family of Caricaceae, and several species of Caricaceae have been used as remedy against a variety of diseases (Mello, Gomes, Lemos, Delfino, Andrade, Lopes & Salas, 2008). Papaya offers not only the luscious taste but is a rich source of antioxidant nutrients such as carotenes, vitamin C and flavonoids; the B vitamins, folate and pantothenic acid; and the minerals, potassium and magnesium; and fiber (Suleiman, 2011). Together, these nutrients promote the health of the cardiovascular system and also provide protection against colon cancer.

The fruit is valued for its proteolysis enzymes including papain, which is used like brome lain, a similar enzyme found in pineapple, to treat sports injuries, other causes of trauma, and allergies (Rivera-Pastrana, Yahia, & Gonzilez-Aguilar, 2010). The fruits, leaves, seeds and stem of *Carica papaya* contain novel biological active compounds, which are potent as therapeutics or useful in industrial processes (Ravishankar, Rajora, Greco & Osborn, 2013). The present study was undertaken to examine the phytochemicals constituents of *Carica papaya* parts in eradicating weevil infestation in bean seeds

The incessant infestation of insect pest on food products has been a problem to the agricultural sector, to a large extent played a significant role in the reduction of farm product yield (Diouf, 2011 & Qiu, Sun, Zhang, Xu, Yan, Han & Wang, 2014). A drastic decline in farmer's income, increased expenditure in methods of pest controls (chemical, physical and biological), seed perforation, spread of diseases, loss of viable seeds for cultivation etc. With the high rate of insect infestation on farm products, there is need to research into viable means that are environmental friendly in decimating their hostile effect thus the reason for carrying out the phytochemical examination of *Carica papaya* against *Callosobruchus maculatus* (bean weevil) on stored bean seeds.

2. Materials and Methods

2.1. Study Area

This research was investigated in the Laboratory, Department of Biology, Federal University of Technology, Owerri. The mean temperature of the laboratory ranges from 27^{o}C- 30^{o}C and the mean relative humidity ranges from 81% - 90%. The federal University of Technology, Owerri lies between latitude 05^{o} 21^{1} and 05^{o} 42^{1}N and longitude 07^{o} 48^{1} and 06^{o} 53^{1}E. The region consists of tropical rainforest zone with average annual rainfall distribution of 2,250mm -2,800mm. This zone produces many agricultural products like maize, melon, bean, corn and palm products.

2.2. Experimental design used

The experimental design used was Complete Randomized Design (CRD), with five treatments replicated four times in the laboratory giving a total of twenty plates. The treatments include the following;

T_0 = Control (no application of pawpaw powder)

T_1= Pawpaw leaves powder (50g)

T_2 = pawpaw bark powder (50g)

T_3 = pawpaw unripe fruit powder (50g)

T_4 = pawpaw root powder (50g)

2.3. Treatment preparation and application procedure

The freshly pawpaw parts were procured locally from Ehime Mbano L.G.A Imo state. The pawpaw plant parts (leaves, roots, bark, unripe fruit peels) were washed and air dried for 30days and grinded with the electric blender into 50g of pawpaw plants parts each and were used to store bean seeds of which 50g bean weevils (*Callosobruchus maculatus*) were introduced in plastic plates covered with the net and held with rubber band for three months in the laboratory. Observations were made and recorded

2.4. Experimental parameters investigated

The mortality rate of *Callosobruchus maculatus* was recorded at 4 weeks, 8 weeks, and 12 weeks intervals by counting the number of dead *Callosobruchus maculatus*, as well as mean number of holes on bean seed caused by *Callosobruchus maculatus.* The numbers of holes on the bean seeds were determined by counting and the holes 0n each seed in plates were recorded. The weight loss of the bean seed was evaluated by difference between the initial weight of the seeds before infestation and the final weight of the bean seed after infestation by *Callosobruchus maculatus.*

2.5. Phytochemical analysis of pawpaw plant parts

The phytochemical analysis for alkaloid, tannins, saponins, glycosides and papain were carried out according to standard methods of Trease & Evans (1999) and Edeoga, Okwu & Mbaebie (2006).

2.6. Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) procedures using Statistical Package for Social Sciences (SPSS) version 20 and treatment mean were separated using Duncan multiple range test (DMRT) at 0.05% probability level.

3. Results and Discussion

3.1. Death rate of *Callosobruchus maculatus*

The control treatment shows no significant effect in the mortality in all weeks of observations (2weeks to 8 weeks). The pawpaw plant parts powder showed significant mortality effect in the control of *Callosobruchus maculatus* in all weeks (Table 1). The leave powder killed all *Callosobruchus maculatus* within 8 weeks after introduction (WAI). The fruit peel powder, bark powder and root powders had mortality effects and were not able to kill all the *Callosobruchus maculatus* within 8 weeks after introduction.

Table 1: Effect of Pawpaw Plant Parts Powder on Bean Weevil Mortality

		Mean number of dead weevils			
Pawpaw plants parts powder (g)	Mean number of weevil introduced	4WAI	8WAI	12WAI	Mean number alive
Control (0)	$50^{a} \pm 0.01$	$0^{d} \pm 0.00$	$0^{d} \pm 0.00$	$0^{c} \pm 0.00$	$50^{a} \pm 0.01$
Leaves powder (50)	$50^{a} \pm 0.01$	$26^{a} \pm 1.70$	$24^{a} \pm 1.63$	$0^{c} \pm 0.00$	$0^{e} \pm 0.00$
Bark powder (50)	$50^{a} \pm 0.01$	$12^{bc} \pm 1.15$	$18^{b} \pm 1.41$	$10^{ab} \pm 1.05$	$10^{c} \pm 1.05$
Unripe fruit peel powder(50)	$50^{a} \pm 0.01$	$14^{b} \pm .1.23$	$19^{b} \pm 1.45$	$12^{a} \pm 1.15$	$5^{d} \pm 0.75$
Root powder(50)	$50^{a} \pm 0.01$	$10^{c} \pm 1.05$	$13^{c} \pm 1.20$	$8^{b} \pm 0.94$	$19^{b} \pm 1.45$

WAI = Weeks after introduction
Mean along the column having different letters of superscript differ significantly at P =0.05 level (DMRT)

3.2. Number of holes and weight loss of bean seeds treated with pawpaw plant parts powders

Pawpaw leave powder (50g) gave the lowest mean number of holes on bean seed (33.2) with percentage mortality of 100%, followed by pawpaw fruit peel powder (50g) with mean number of holes (82.3) and percentage mortality of 90% (Table 2). The highest mean number of holes on bean seeds was in the control treatment (171.4), where there was no application of pawpaw plant parts powder. The average weight loss was recorded high in control treatment (159.3g) and was significantly high than the bean seeds treated with pawpaw root powder (94.4g), Pawpaw bark powder (76.9g), Pawpaw fruit powder (65.3g) and pawpaw leave powder (7.6g) respectively.

Table 2: Effect of Pawpaw Plants Parts Powder on Number Of Holes And Weight Loss Of Bean Seed.

Pawpaw plant parts powder (g)	Average initial weight of seeds (g)	Average final weight of seeds(g)	mean number of holes	Average weight loss (g)	mean number of dead weevil	Mortality (%)
Control (0)	$300^{a} \pm 0.01$	$141.7^{d} \pm 3.97$	$171.4^{a}\pm4.36$	$159.3^{a}\pm4.21$	$0^{d}\pm0.00$	$0^{d}\pm0.00$
Leaves powder (50)	$300^{a} \pm 0.01$	$292.4^{a} \pm 5.70$	$33.2^{d}\pm1.92$	$7.6^{d}\pm0.92$	$50^{a}\pm2.36$	$100^{a}\pm3.33$
Bark powder(50)	$300^{a} \pm 0.01$	$223.1^{b} \pm 4.98$	$91.5^{c}\pm3.19$	$76.9^{c}\pm2.92$	$40^{b}\pm2.11$	$80^{b}\pm2.98$
Unripe fruit peel powder (50)	$300^{a} \pm 0.01$	$234.7^{b} \pm 5.11$	$82.3^{c}\pm3.02$	$65.3^{c}\pm2.69$	$45^{ab}\pm2.24$	$90^{ab}\pm3.16$
Root powder(50)	$300^{a} \pm 0.01$	$205.6^{c} \pm 4.78$	$119.1^{b}\pm3.64$	$94.4^{b}\pm3.24$	$31^{c}\pm1.86$	$62^{c}\pm2.62$

Mean along the column having different letters of superscript differ significantly at P =0.05 level (DMRT)

3.3. Nutrient proximate and phytochemical of pawpaw plant parts

Table 3 and Table 4 shows results on nutrient proximate and phytochemical constituents of pawpaw plant parts. The pawpaw plant parts contained the entire nutrient proximate tested like crude protein, fat, total ash, carbohydrate and fibre per 100 grams. The phytochemical found were alkaloid, tannin, saponins and glycosides in all the pawpaw plant parts (Table 4). Papain was only present in the pawpaw leaves powder.

Table 3: Nutrient Proximate of *Carica papaya* Plant Parts

Constituents	Values per 100 grams Leaf	Bark	Unripe Fruit Peel	Root
Crude protein(g)	0.63	0.43	0.39.	0.35
Fat (Total lipid) (g)	0.29	0.22	0.11	0.09
Total ash (g)	0.69	0.62	0.27	0.32
Carbohydrate(g)	9.91	9.65	9.26	8.37
Fibre (g)	1.88	1.66	1.32	1.58

Table 4: Phytochemical Analysis of *Carica papaya* Plant Parts

Constituents	Leaf	Bark	Unripe Fruit Peel	Root
Alkaloid	+	+	+	+
Tannins	+	+	+	+
Saponins	+	+	+	+
Glycosides	+	+	+	+
Papain	+	-	-	-

\+ = Present
\- = Absent

This study showed that plant parts of pawpaw were effective in the reduction of *Callosobruchus maculatus*. This study also indicated that pawpaw leaves powder possess toxicity, feeding and oviposition deterred effect on *Callosobruchus maculatus* and reduced weight loss and number of holes on bean seeds. This possessed contact toxicity on *Callosobruchus maculatus.* The toxicity of the pawpaw leave powder may be attributed to papain compound found only in the leaves in this study. This work is in agreement with the work of Belmain and Stevenson (2001) that reported effective use of *C.sinensis* powder against legume pests. Adebiyi, Adaikan & Prasad. (2002) assayed that the pharmaceuticals of unripe pulp of pawpaw reported only the presence of saponins which agrees to this study, that reveals the treatments caused significant reduction in control of *Callosobruchus maculatus* when compared to control treatment. Therefore this study indicated that pawpaw plant parts could be used as potential storage grain protectants. Development of pest resistance to pesticides has forced the farmers to use extensive doses of insecticides, which is worldwide concern in term of pesticide residues, pest resistance and problems that synthetic insecticides have caused to the environment, well as human have resulted in using plant extracts and plant natural products for insect control (Boxall, Brice, Taylor & Bancrofti, 2002 & Nwachukwu, Okoro, Ahumibe, Nkwocha & Nwachukwu, 2014).

Pesticides are notorious for hazardous impact on human health and environment, therefore the health concerns demand food free from synthetic insecticides with alternative to insecticides by using pest and disease resistant varieties, bio pesticides and cultural practices.

4. Conclusion

The papaw leaves powder gave the most effective result in the control of *Callosobruchus maculatus* in this present study. The unripe fruit peel powder and the bark powders followed in reducing the number of *Callosobruchus maculatus* in the storage.

The greatest infestation and damage to storage bean seeds was observed in control treatment with no mortality and having high mean number of holes and mean number of weight loss on the bean seeds.

5. Recommendations

There is need for more protective measures and researchers to develop biopesticides for stored or agricultural products targeting on isolated bioactive compound of botanical against the fertility gemone or genetic makeup of storage pests. In addition, to seek out botanicals for anti-oviposition and larvicidal efficacy to help break the life cycle and hinder development of pest metamorphic stages.

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