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Toxicity of extracts derived from different parts of cassava plant, *Manihot esculenta* Crantz to four major coleopteran pests of stored-products

Arumughan Jayaprakas Cheruvan *, L. Ragesh

Central Tuber Crops Research Institute, Sreekariyam, 695 017, Thiruvananthapuram, Kerala, INDIA

*Corresponding author: prakashcaj@gmail.com

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Abstract

Fumigant toxicity of insecticidal principles extracted from tuber rind, fresh leaf, fresh leaf with petiole, and dried leaf of cassava (var. M4) was studied against four major stored-product insect pests *viz. Sitophilus oryzae (*L.*)*, *Rhyzopertha dominica* (F.*)*, *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* (L.) under laboratory conditions (28±2ºC, Rh. 75±5%). Mortality of the test insects varied with respect to extracts collected from different parts of the plant, and time of exposure. Extract collected from cassava rind recorded the highest toxicity. *Callosobruchus chinensis* was highly susceptible and showed immediate knockdown effect to the active principles extracted from tuber rind, fresh leaf, fresh leaf with petiole, twig and semi-dried leaf. The extract collected from various parts of plant caused 100% mortality of *R. dominica* at 1 hour after treatment (HAT), but the same collected from tuber and dried leaves did not show any toxic effect. Mortality of *S. oryzae* was 100% at 1 HAT with tuber rind extract, but no response was observed from the extract collected from semi-dried leaf, twig, and leaf with petiole. No fumigant action was observed in all the four coleopteran pests exposed to the extract collected from dried leaves. The study revealed that fresh leaf and tuber rind are good sources for the extraction of biofumigant against major coleopteran pests, however dried leaves are unfit for same purpose.

Key words: Cassava, extracts, Sitophilus oryzae, Rhyzopertha dominica, Tribolium castaneum, Callosobruchus chinensis.

Introduction

Insect pest infestation is a major problem in the storage of cereals and pulses. Realising the fact that indiscriminate application of synthetic pesticides has created major challenges to man and ecosystem, there is a global concern to contain pests using non-chemical methods, particularly to lessen the pesticide residues in food (Flinn and Hagstrum, 2001). Fumigation is an effective method to protect stored-products from insect infestation. The commonly used fumigant like aluminium phosphide, ethylene dibromide and methyl bromide are associated with health and environmental pollution, and also many of the stored- product pests have developed resistance against synthetic fumigants (Zettler, 1982). Phillips et al. (2001) opined that exposure to low or sublethal doses pose an increased risk in phosphine resistance. Opit et al. (2012) reported high levels of resistance in several strains of major storage pests to phosphine in the USA.

The use of natural compounds in place of synthetic insecticides is an alternative strategy to reduce environmental pollution, and to preserve non-target organisms. Phytochemicals have been suggested as the alternatives to synthetic insecticides as they are the storehouse of a wide range of bioactive chemicals (Wink, 1993). Plant products are inexpensive products for the management of stored-grain pests (Mishra et al., 2012b), and are potentially suitable as vital components in integrated pest management strategies (Saxena, 1989; Schmutterer, 1992). The insecticidal activity of many plant derivatives against several stored-product pests has been demonstrated (Malik and Mujtabe Naqvi, 1984; Singh, S. 2017).

Cassava (*Manihot esculenta* Crantz), originally from Amazonia, is a woody shrub extensively cultivated as an annual crop in tropical and subtropical regions that provides the staple food of an estimated 800 million people worldwide. Although the leaves of cassava are rich in proteins, minerals, and vitamins, the presence of antinutrients and cyanogenic glucosides are the major drawbacks to human consumption. However, cyanogen can play a pivotal role in pest management.

Desmarchelier and Ren (1996) has patented cyanogen as a fumigant to replace methyl bromide in a variety of applications. Being a good source of cyanogen, cassava was used for the isolation of insecticidal molecules for the development of a fumigant against the stored-product pests.

Materials and Methods

Insects

Colonies of four beetle species were reared under laboratory conditions (Temp. 28±2°C; Light and Dark photo period 12:12). *Sitophilus oryzae*, *Tribolium castaneum* and *Rhyzopertha dominica* were reared on whole grain wheat, and *Callosobruchus chinensis* was reared on cowpea seeds in plastic containers (100 g capacity).

Sample preparation

Twigs, leaves, leaves with petiole, semi-dried and dried leaves, tuber flesh and tuber rinds of cassava variety, M4, were collected from the experimental field of ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, India for the isolation of insecticidal molecules. Each sample (200 g) was macerated and distilled in 500 ml of distilled water. The distillates were collected 25 ml each, and thus four fractions were collected for bioassay on the selected four weevil species.

Bioassays

The bioassay revealed that first fraction of the extract was highly toxic to the four species of weevils exposed; hence this fraction was used for the subsequent toxicity study. Plastic tubes of 3 ml capacity were loosely impregnated with cotton and each tube was separately loaded with 1 ml of the extract. The tubes were perforated around with a needle to ensure the dissipation of biofumigant. The tubes were introduced separately into plastic containers of 250 ml capacity wherein the test insects were released. Cotton impregnated with distilled water was run as control. To retain the biofumigant within the experimental containers, these were closed airtight. All the treatments were replicated three times. Mortality of *S. oryzae*, *R. dominica* and *T. castaneum* was recorded at 1, 2 and 12 hours after treatment (HAT), whereas *C. chinensis* was observed at 2, 3 and 5 minutes after treatment as these are highly susceptible to the fumigant.

Statistical analysis

The percentage of mortality was determined and transformed to arcsine values for analysis of variance (ANOVA) using SAS (version 9.3). Treatment means were compared using least significance difference (LSD) at $p < 0.05$ level of significance. .

Results

The extracts collected from cassava tuber rind, fresh leaf, fresh leaf with petiole, twig and semi-dried leaf were highly toxic to *R. dominica* (Table 1). Mortality of *R. dominica* was 100% at 1 HAT in all the treatments, except with dried leaf. The extract collected from cassava tuber flesh was significantly less toxic to *R. dominica* than all other treatments. No mortality was observed in the control and treatment with dried leaf extract.

Mortality of *S. oryzae* at 1 HAT was 100% due to the treatment of extract collected from tuber rind, but there was no difference in the rate of mortality between the extracts of fresh leaf and semi-dried leaf. In all the treatments, except tuber flesh and dried leaf extracts, over 70% mortality of *S. oryzae* was noticed, however no mortality was observed with the treatments of extracts collected from tuber flesh and dried leaf.

Table 1. Mortality of *Rhyzopertha dominica* and *Sitophilus oryzae* due to the fumigant action of cassava extract

Treatment	*Rhyzopertha dominica* (Hours after treatment)	*Sitophilus oryzae* (Hours after treatment)

	1	2	12	1	2	12
Tuber rind	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)
Fresh leaf	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	86.7 (68.9)	86.7 (68.9)	100.0 (89.6)
Fresh leaf with petiole	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	73.3 (59.2)	78.3 (62.5)	88.3 (70.1)
Twig	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	73.3 (68.9)	86.7 (68.7)	91.7 (76.1)
Semi-dried leaf	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	86.7 (68.9)	90.0 (74.9)	96.7 (83.6)
Tuber flesh	26.7 (30.8)	26.7 (30.8)	26.7 (30.8)	0 (0.4)	0 (0.4)	0 (0.4)
Dried leaf	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)
Control	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)
(0.05)			3.3			5.3

Values in parenthesis are arc sine transformed values

The extracts collected from tuber rind and from semi-dried leaf were shown significantly higher toxicity to *T. castaneum* than the other treatments (Table 2). Mortality of *T. castaneum* was 100% at 1 HAT due to the treatment with tuber rind extract. The extract of semi-dried leaf recorded higher toxicity than fresh leaf with petiole, and twig extracts. In all the treatments, except the extracts of tuber flesh and dried leaf, 100% mortality was observed at 12 HAT

Preliminary study revealed that *C. chinensis* was highly susceptible to the bioactive components extracted from cassava, hence the mortality was observed at 2, 3 and 5 minutes after treatment. Immediate knockdown effect of the test insect was noticed due to the treatment with the extracts of tuber rind, and semi-dried leaf. Except the extract from tuber flesh and dried leaf, higher mortality was also observed in all other treatments.

Table 2. Mortality of *Tribolium castaneum* and *Callosobruchus chinensis* due to the fumigant action of cassava extract

Treatment	*Tribolium castaneum* (Hours after treatment)			*Callosobruchus chinensis* (Minutes after treatment)		
	1	2	12	2	3	5
Tuber rind	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)
Fresh leaf	50.0 (45.0)	56.7 (48.8)	100.0 (89.6)	93.3 (77.6)	100.0 (89.6)	100.0 (89.6)
Fresh leaf with petiole	78.3 (62.9)	86.7 (72.7)	100.0 (89.6)	90.0 (74.9)	93.3 (77.6)	100.0 (89.6)
Twig	61.7 (52.1)	70.0 (65.7)	100.0 (89.6)	93.3 (77.6)	96.7 (83.6)	100.0 (89.6)
Semi-dried leaf	88.3 (77.7)	93.3 (80.9)	100.0 (89.6)	100.0 (89.6)	100.0 (89.6)	100(89.6)
Tuber flesh	0 (0.4)	0 (0.3)	0 (0.3)	0 (0.4)	0 (0.4)	0 (0.4)
Dried leaf	0 (0.4)	0 (0.3)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)
Control	0 (0.4)	0 (0.3)	0 (0.4)	0 (0.4)	0 (0.4)	0 (0.4)
CD (0.05)		8.3			5.5	

Values in parenthesis are arc sine transformed

Discussion

The use of chemical fumigants for the management of stored-product pests is highly discouraged due to multiple reasons. Aulicky and Stejskal (2018) reported that although fumigation with phosphine proves satisfactory control of major stored-product pests, its effect sharply declines with the increasing distance from the fumigated spot. In the present investigation, the extracts collected from different parts of cassava was found toxic to *R. dominica*, *S. oryzae*, *T. castaneum* and the toxicity was extremely high as in the case of *C. chinensis*. Cassava tuber rind extract recorded the highest toxicity among the extracts from other parts of the plant. Burns et al. (2012) observed that concentration of cyanide in the tuber rind was much higher than that of the tuber parenchyma and leaves. Aggarwal et al. (2001) reported the fumigant action of essential oil from *Artemisia annwa* against *C. maculatus*, *R. dominica* and *S. oryzae*. Fumigant action of extracts collected from semi-dried leaf was significantly higher than that of fresh leaf; this may be due to the increase in concentration of active principles in leaf due to the loss of water during slow drying. The extract collected from fully dried leaves did not show any toxicity to all the test insects, which is a clear indication that the active principles are fumigant in nature. Plant extracts and essential oils are known to possess insecticidal, ovicidal and repellent activities against various stored-product insects (Desmarchelier, 1994: Shaaya et al., 1997). El- Nahal et al., (1989) reported that adults of *C. chinensis*, *S. granarius*, *S. oryzae* were susceptible to the essential oil from *Acorus calamus,* but *T. castaneum* and *R. dominica* were tolerant to all doses and exposure time. They also reported that the exposure period is the most important factor affecting the efficiency of the vapours than dosage. Leaf, bark and seed extracts of *Aphanamixis polystachya* have been reported to exert repellent and insecticidal effects against *C. chinensis* (Talukder and Howse, 1994). Several natural products including principles from many spices, herbs and medicinal plants are known to have a range of useful biological properties against insects (Tripathi et al., 1999a, b). Insecticidal activity of *Cinnamomum* cassia bark, *Illicium verum* fruit and *Foeniculum vulgare* fruit was reported by Kim et al., (2003).

Although phytochemicals are reported to have very good insecticidal action against stored-product pests, poor extractability and cumbersome techniques in the isolation of active principles are the bottlenecks in its commercial availability in the markets. In the present study, cassava bio-wastes, which are either underutilized or thrown as waste, are promising source for the extraction of biofumigant.

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Entomocidal, repellent, antifeedent and growth nhibition efects of different plant extracts against *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera)

Mansoor ul Hasan[1], Qurban Ali[2], Sehrish Kanwal[1], Najuf Awais Anjum[2]

[1]Department of Entomology, University of Agriculture, Faisalabad, Pakistan
[2]Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan
*Corresponding author: mansoorsahi2000@yahoo.com
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ABSTRACT

In present investigation, toxic, repellent, antifeedent and growth inhibition effects of five different plant extracts: *Melia azedarach, Pegnum hermala, Salsola baryosma, Azadirachta indica* and *Zingiber officinale*, were evaluated on different life stages of *T. castaneum*. The highest mortality (10.14%) was observed with *A. indica* and the minimum mortality (0.67%) was invoked by *Z. officinale* treatment. Similarly, *A. indica* showed highest repellent effect compared to rest of the plants. Feeding deterrence was highest (90.15%) with *S. baryosma* treatment, followed by *P. hermala* (84.85%), *M. azedarach* (80.19%), *A. indica* (73.48%) and *Z. officinale* (57.58%). The extracts inhibited the growth of *T. castaneum*. Inthe case of *A. indica,* the lowest numbers of larvae (32.67), pupae (16.33) and adults (11.33) emerged at 15% concentration, while the highest emergence of larvae (80.33), pupae (75.00) and adult (71.00) were observed for *Z. officinale.* The other three plant extracts had moderate regrowth inhibition on the beetle. Overall, *A. indica* extract was found to be the most effective while, *Z. officinale* extract was least effective against the beetle. This study can be very helpful in future when the use of plant extracts become common and available to the farmers as an alternative to synthetic pesticides.

Key words: Mortality, repellency, antifeedant effect, growth inhibition, plant extracts, *T. castaneum*

Introduction

Food security is an emerging threat to world's expanding population. Stored grain insect pests are severely damaging our valuable products like wheat. These stored grain pests are responsible for about 10% loss of cereals all over the world (Danahaye *et al*., 2007). According to Matthews, (1993) 10-40% losses per annum have been estimated globally due to the infestation of these insects. These losses can reach 9% in developed and up to 20% in the developing countries throughout the world (Phillips and Throne, 2010).

Among the stored product insect pests, *T. castaneum* is the pest of economic importance throughout the world which cause serious damage to stored products (Arbogast, 1991). It is one of the major pests of wheat flour (Howe, 1965). Both larvae and pupae are responsible for the losses. In case of severe destruction, flour may be converted into greyish and moldy produce with pungent off-smell. Thus, the commodity becomes unfit for human use (Atwal and Dhaliwal, 2002). It can cause economic loss of 40% of wheat flour (Ajayi and Rahman, 2006).

Widespread and indiscriminate use of pesticides is posing a serious threat to the environment and human health (Subramanyam and Hagstrum, 1996). Consequently, the use of biopesticide for the control of stored commodity pests has received considerable attention throughout the world (Faraz