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Biopesticidal Activity of Cassava *(Manihot esculenta* Crantz) Seed Oil Against Bihar Hairy Caterpillar (*Spilarctia obliqua*) and Cowpea Aphid (*Aphis craccivora*)

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Abstract

Cassava seed oil (CSO) from mature fruits of indigenous cassava (*Manihot esculenta* Crantz) varieties was extracted in petroleum ether (60-80ºC). Gas chromatography studies on the lipid profile of the oil showed the presence of 13 fatty acids with dominance of C_{18} component (oleic). Other major components of the oil were stearic acid (19.61%) and myristic acid (10.73%). The oil was highly unsaturated and can be used as edible oil. The biopesticide property of the CSO was tested against aphids (*Aphis craccivora*) on cowpea and ivy gourd. The oil at 1 and 0.5% concentrations gave 100% mortality of the pest within 24 and 48 hours of treatment, respectively. Bioassay of CSO at 1% concentration against fourth instar larvae of *Spilarctia* (*Spilosoma*) *obliqua* (Bihar hairy caterpillar) resulted in 93.3% mortality in the larval population.

Key words: Cassava seed oil, biopesticide property, fatty acid methyl esters, Bihar hairy caterpillar, cowpea aphid, larval population

Introduction

Tuber crops form important staple or subsidiary food for millions of people in the tropical and subtropical countries (Oselebe and Okporie, 2008). Cassava (*Manihot esculenta* Crantz) is one of the most popular tuber crop cultivated extensively in Asia, Africa, Central and South America and other parts of the world as a food or industrial crop (FAO, 2012). Many varieties of the crop profusely produce fruits. The fruits are dehiscent and each fruit usually bears three seeds embedded in a capsule. The seeds are similar in shape and size to the seeds of castor (*Ricinus communis*). Cassava is propagated vegetatively, despite true cassava seed (TCS) production appears to be promising for reducing or eliminating the diseases caused by viruses. However, problems like storage of stake, low multiplication rate and the long growth cycle are the hindrances in popularising this technology (Carlos Iglesias et al., 1994). Cassava seed is a rich source of lipids (Nartey and Møller, 1973), but it is less exploited and seeds go as a waste.

Naturally occurring and biologically active compounds from plants are considered to be less hazardous plant protection chemicals than synthetic pesticides. Jayaprakas et al. (2000) established the insecticidal activity of the bioactive principles isolated from cassava seeds against stored insect pests. The antimicrobial activity of cassava seed oil (CSO) against the clinical isolates of *Staphylococcus aureus*, *Propionibacterium acnes*, *Escherichia coli*, *Pityrosporium ovale* and *Candida albicans* was reported by Popoola et al. (2007).

Spilarctia (*Spilosoma*) *obliqua* (Lepidopera: Arctiidae) (Bihar hairy caterpillar) and *Aphis craccivora* (cowpea aphid) are polyphagous pests of field crops. *Spilarctia obliqua* attacks

a wide range of field crops, including sweet potato, pulses, oil seeds, vegetables and beans (Bhattacharya and Mandal, 2004). *Aphis craccivora* is the sucking pest of many crops and its high reproductive potential and ability to transmit viral diseases makes it the most potent and economically important pest of many crops (Liburd and Nyoike, 2008). Honey excreted by the pest is rich in sugar which promotes the growth of sooty mould (*Capnodium* spp.) on the plants, arresting photosynthetic activity. The objectives of the current investigation were to extract the bioactive principles from cassava seeds and to test their insecticidal properties against *Spilarctia obliqua and Aphis craccivora*.

Materials and Methods

Extraction of CSO and study of physico-chemical properties

Cassava fruits were collected from the experimental fields of Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, Kerala, India and sun dried in cloth bags of 5 kg capacity. The fruits ruptured while drying and the seeds were released into the bags. The seeds were crushed into fine powder and 100 g was subjected to soxhlet extraction with 300 ml of petroleum ether (60-80°C) for 6-8 hours following the methods of Moorthy (1978) and Jayaprakas et al. (2000). The extract was made moisture free with anhydrous sodium sulphate and the solvent with active principles were filtered through a muslin cloth. The solvent was completely removed by using a rotary evaporator (Buchi Labortechnik AG, Switzerland) and the oil containing the active principles was stored at 4°C in air-tight containers for the toxicity studies on the test insects.

Chemical properties of the oil, i.e., iodine value, saponification value, acid value, free fatty acid content and peroxide value were estimated according to AOAC (1975). Specific gravity was determined using a pycnometer and refractive index with Abbe Refractometer (B and S model, Bellingham + Stanley Ltd. United Kingdom). Presence of cyanide was also tested by alkaline picrate method (Indira and Sinha, 1969).

Preparation of fatty acid methyl esters (FAMES)

Fatty acid methyl esters of CSO were prepared according to the boron trifluoride method (AOAC, 2005). Cassava seed oil (50 mg) was methylated using methanolic NaOH and boron trifluoride in a 30 ml teflon lined screw-capped test tube. The methyl esters produced at the end of the reaction was extracted into n-hexane and it was concentrated by removing excess hexane using a rotary evaporator for further analysis.

Gas chromatographic analysis

Analysis of the FAMES was carried out using a gas chromatograph Varian Star # 1 (Varian Inc. USA) with FID detector. One µl of FAMES was injected into the GC at injector temperature 260°C, oven temperature 120°C for 5 min, ramp rate 3°C min^{-1}, 240°C for 10 min, detector temperature 270°C and carrier gas flow rate 1 ml min^{-1}. Run time was 55 min. A standard FAME mixture (FAME mix, SUPELCO, C_4-C_{24} unsaturates) containing methyl esters of 37 different fatty acids were also run separately and chromatograms were recorded.

Maintenance of test insects

Culture of *Aphis craccivora* was maintained on cowpea (*Vigna unguiculata*) plants raised in pots (13 cm diameter x 12 cm height) and on ivy gourds (*Coccinia grandis*) plants in experimental fields. Larvae of *S. obliqua* collected from the pea field were reared on sweet potato leaves in the laboratory at 29 ± 3°C and relative humidity 70 ± 10% in plastic trays (25 x 20 x 5 cm). Dead larvae were removed and the mother culture was cleaned every day. The fourth instar larvae were used for the experiments.

Toxicity of cassava seed oil on aphids and Bihar hairy caterpillar

Different concentrations of CSO (0.001-1.0%) were prepared with 0.1% of surfactant (teepol). The leaves of cowpea and ivy gourd infested with aphids were placed in separate petri dishes (8 cm diameter) and were sprayed with the test solutions using an atomizer. Distilled water was taken as control and treatment was replicated thrice. Mortality of aphids was observed for three consecutive days. Toxicity of CSO to aphids was tested in the field also. Intensity of infestation by aphids on the crops was categorized into 5 grades (1-20%: grade I; 21-40%: grade II; 41-60%: grade III; 61-80%: grade IV and > 80%: grade V). Infested cowpea plants were sprayed with CSO at 1.0% and 0.5% and water was run as control. Each treatment was replicated thrice and the mortality of aphids was recorded for three consecutive days.

In the case of *S. obliqua*, CSO at different concentrations ranging from 0.001-1.0% were applied onto the 4th instar larvae by using an atomizer and mortality was recorded for three days. Treatments were replicated thrice with five insects/treatment and water was used as control.

Results and Discussion

Physico-chemical properties of cassava seed oil

The oil was slightly yellow with mild odour and its physico-chemical properties are given in Table 1. The refractive index was similar to sunflower, soybean and castor oil, but specific gravity was low. The low acid value indicates that CSO has very less free fatty acids. The average iodine value of 120 indicated high unsaturation of the oil and that supports the possibility of using CSO as edible oil for health promotion. The cyanide, which was determined as HCN, was absent in the oil and there were no peroxide linkages in the oil as evidenced by the nil peroxide value.

Moorthy (1978) studied the esterification of CSO by thin layer chromatography and acid value determination and reported that 99% of the fatty acids in CSO existed as their corresponding triglycerides. Popoola and Yangomodou (2006) reported that the major fatty acids of CSO obtained after extraction with petroleum ether were palmitic, linoleic and oleic acids.

The chromatogram of the methyl esters of the fatty acids in CSO was obtained by gas chromatographic analysis. The peaks were compared with the standard peaks and 13 fatty acids were identified (Table 2). All these were long chain fatty acids with C_{14}, C_{16}, C_{17}, C_{18}, C_{20}, C_{22} and C_{24}, and oleic acid was the predominant component (63.17%), followed by stearic acid (19.61%) and myristic acid (10.73%).

Table1. Physico-chemical properties of cassava seed oil

Parameter	Content
Refractive index	1.4713
Specific gravity	0.864
Iodine value	120 mgI_2 100^{-1} g
Saponification value	123.59 mg 100^{-1} g
Acid value	4.0
Peroxide value	Nil
HCN Content	Nil

Table 2. Fatty acid profile of cassava seed oil

Peak	Fatty acid	Content (%)
C14:0	Myristic Acid	10.73
C16:0	Palmitic acid	0.15
C16:1	Palmitoleic acid	0.076
C17:1	Cis-10-heptadecenoic acid	3.46
C18:0	Stearic acid	19.61
C18:1(c + t)	Elaidic acid	0.36
C18:1(c + t)	Oleic acid	63.17
C18:2 n6t	Linolelaidic acid	1.13
C18:3 n3	Linoelenic acid	0.17
C20:0	Arachidic acid	0.23
C20:1	Cis-11-eicosenoic acid	0.083
C22:0	Behenic acid	0.17
C24:1	Nervonic acid	0.08

Toxicological study

Mortality of the aphids was directly proportional to the concentration of CSO and the time of exposure. Hundred percent mortality of aphids was observed both in cowpea and ivy gourd within 24 hours of treatment with a concentration of 1.0% CSO (Figs. 1 and 2). More than 50% mortality of aphids was also noticed in the treatment with 0.1% concentration, but mortality was low in the batches with 0.01% and these observations corroborate with the earlier reports (Sampson et al., 2000; Tunc and Sahinkaya, 1998).

In the field experiments, CSO at 0.5 and 1.0% concentrations reduced the aphid infestations on ivy gourd and cowpea from

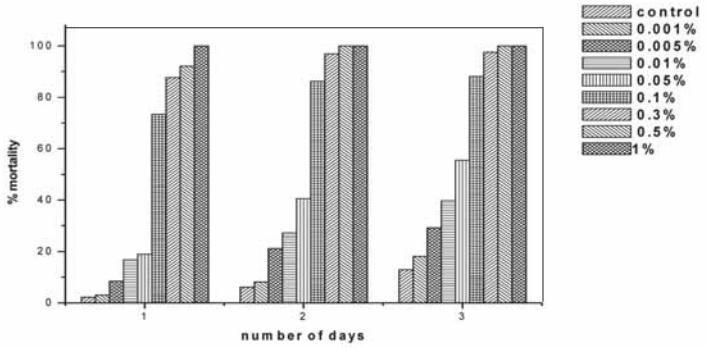

Fig. 1. Bioassay of cassava seed oil against cowpea aphids (*A. craccivora*) *in vivo*

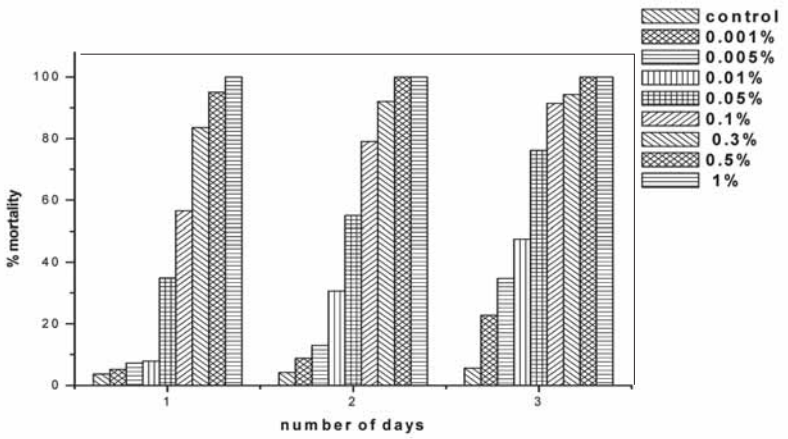

Fig.2. Bioassay of cassava seed oil against ivy gourd aphids

grade V to grade I on the third day after treatment (DAT).

Cassava seed oil at 1.0% was toxic to the fourth instar larvae of *S. obliqua* and its mortality was 93.3% (Table 3) on the third day after treatment (DAT). Data analysis showed that variation in mortality was significant among the treatments ($p < 0.01$), but below 0.05% it was on par with control.

The results revealed that cassava seed oil was highly effective against aphids and Bihar hairy cater pillar and the insecticidal activity of cassava seed oil may be attributed to the higher content of oleic acid (63.17%). The other major constituents of CSO are two saturated fatty acids, stearic acid (19.61%) and myristic acid (10.73%). According to Harada et al. (2000), saturated fatty acids are not having well established insecticidal activity. Don-Pedro (1990) reported the insecticidal activity of oleic and linoleic acids on the eggs of *Callosobruchus maculatus* on cowpea seeds and its LC_{50} value was 1.64 ml kg^{-1}, which was approximately 3 and 8 times more toxic than groundnut oil and linoleic acid respectively. It was also reported that when the ovicidal activities of lauric, oleic and linoleic acids were tested by dipping eggs of *C. maculatus* infested cowpea seeds in acetone based solutions, their toxicities were on par. Lauric, oleic and linoleic acids exhibited LC_{50} values of 40, 38 and 26 ml l^{-1} respectively and these were 2-4 times more toxic than acetone-based solutions of groundnut and traditional coconut oil on eggs of *C. maculates*. Puritch (1975) screened fatty acids for their toxicity to different life stages of the balsam woolly aphid (*Adelgespiceae* Ratz.) and found that the mortality of the pest was due to two major groups of fatty acids, one centering around capric acid, within the low-chain (4-12 C) saturated fatty acid series and the other around oleic acid, within the unsaturated 18-C fatty acids.

The aphicidal capacity of fatty acids increased when they were converted to their potassium salts or soaps. The soaps of caprylic, capric, oleic and linoleic acids were the most effective. Certain members of the aliphatic fatty acid series possess promising insecticidal properties as contact toxicity (Siegler and Popenoe, 1925). Ramsewak et al. (2001) reported that linoleic and oleic acids have

Table 3. Bioassay of cassava seed oil against *S. obliqua*

Concentration of CSO (%)	Mortality (%) (DAT)		
	1	2	3
Control	$0 \pm 0\ (1 \pm 0)^{a}$	$0 \pm 0\ (1 \pm 0)^{a}$	$0 \pm 0\ (1 \pm 0)^{a}$
1.0	$86.7 \pm 23.0^{d}(10.2 \pm 0.4)$	$86.7 \pm 23.1^{c}(10.2 \pm 0.4)$	$93.3 \pm 11.5^{d}(10.6 \pm 0.4)$
0.5	$53.3 \pm 23.0^{c}(7.8 \pm 0.5)$	$53.3 \pm 23.1^{b}(7.8 \pm 0.47)$	$66.7 \pm 11.5^{c}(9.14 \pm 0.39)$
0.3	$40 \pm 0^{c}(7.32 \pm 0)$	$40 \pm 0^{b}(7.32 \pm 0)$	$46.7 \pm 11.5^{c}(7.8 \pm 0.5)$
0.1	$46.7 \pm 11.5^{c}(7.8 \pm 0.5)$	$46.7 \pm 11.5^{b}(7.8 \pm 0.5)$	$46.7 \pm 11.5^{c}(7.8 \pm 0.5)$
0.05	$13.3 \pm 11.5^{b}(4.0 \pm 1.5)$	$26.7 \pm 11.5^{b}(7.2 \pm 0.9)$	$26.7 \pm 11.5^{b}(6.1 \pm 0.6)$
0.01	$0 \pm 0\ (1 \pm 0)^{a}$	$0 \pm 0\ (1 \pm 0)^{a}$	$0 \pm 0\ (1 \pm 0)^{a}$
0.005	$0 \pm 0\ (1 \pm 0)^{a}$	$0 \pm 0\ (1 \pm 0)^{a}$	$0 \pm 0\ (1 \pm 0)^{a}$
0.001	$0 \pm 0\ (1 \pm 0)^{a}$	$0 \pm 0\ (1 \pm 0)^{a}$	$0 \pm 0\ (1 \pm 0)^{a}$

Values in parenthesis are ($\sqrt{x+1}$) transformed values. Mean values in the same column with same letter in the superscript are not significantly different

insecticidal activity against fourth instar of *Aedes aegyptii* larvae and have potential feeding deterrent activity against neonate larvae of *Helicoverpa zea*, *Lymantria dispar, Orgyia leucostigma* and *Malacosoma disstria*. Oleic acid is a mono unsaturated fatty acid with 18 carbon atoms (IUPAN name : (9Z)-Octadec-9-enoic acid) and in CSO it occurs as its triglyceride ester (Moorthy, 1978). According to Kabara (1987), mono unsaturation and esterification with polyhydric compounds (glycerol, polyglycerol, sucrose, etc.) generally increases the insecticidal activity of a fatty acid.

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