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Can We Use the Moringa Oil as Botanical Insecticide Against *Spodoptera frugiperda*?

A.M Kamel

Department of Medicinal and Aromatic Plants, Pharmaceutical Sciences Division
National Research Centre, Giza, Egypt. The present address. Post Doctor in Laval University,
Quebec City, Canada

Abstract: In the research for botanical products to control *Spodoptera frugiperda* insect, moringa oils besides its fractions were assayed as anti-feedant and as a toxic material on target insect. Chemical analysis of moringa oil showed that the saponifiable matter comprises oleic acid as major (74.21%) followed by palmitic acid (7.16%). Arachidic and behenic acids were determined with the same quantity (4.11%). The major sterols were found to be β-sitosterol (43.45%), followed by stigmasterol (25.10%). Cholesterol was found as a low quantity (0.23). On the other hand, the higher concentration of moringa oil induced the lower feeding ratio of *S. frugiperda* to 0.01 followed by unsaponifiable and saponifiable matters (0.08 and 0.11), respectively. In the same time, the highest total corrected mortality percentage was recorded with the highest concentration of moringa oil (100%) and unsaponifiable matter (80.7%). It was concluded that moringa oils at 10% concentration could be applied as botanical insecticide to prevent the plants from *S. frugiperda* attack.

Key words: Moringa oil · Fatty acids · Sterols · Spodoptera frugiperda · Toxicity · Anti-feedant activity

INTRODUCTION

Continuous researches for new insecticidal compounds from plant origin are still carried out against the armyworm (*Spodoptera frugiperda*).

Moringa oleifera belongs with the Moringaceae family and Moringa genus, the best known and most widely distributed species [1]. It is a typical multipurpose tree of significant economic importance because there are several industrial and medicinal applications and various products to be used as food and feed [2]. For example, this oil is applied externally for skin diseases, in Haiti and elsewhere, the oil has been used as general culinary and salad oil because it's pleasant tasting, highly edible and resembles olive oil in its tasting [2,3].

Spodoptera frugiperda, it is one of the most economically important insect pests in many countries. This pest may become serious during the seedling stage [4].

In recent years, the use of synthetic organic insecticides in crop pest control programs around the world has resulted in damage to the environment, pest resurgence, pest resistance to insecticides and lethal affects on non-target organisms [5]. Management of agricultural pests over the past half century has been largely depending on the use of synthetic chemical pesticides for field and post-harvest protection of crops. Potential problems associated with continued long-term use of toxic insecticides include pest resistance and negative impact on natural enemies. Extensive surveys and report on plants used as insecticides can be found in the literature. More than 2,000 species of plants are known to possess some insecticidal properties [6].

The objective of the present study was, therefore, to determine (i) the chemical composition of the moringa oil, saponifiable and unsaponifiable matters, (ii) the antifeedant activity of the oil towards *S. frugiperda* and (iii) the toxicological effect of the oils on the target insect.

MATERIALS AND METHODS

Materials: The oil of moringa was obtained from Montréal super market, Quebec province, Canada. The oil was obtained by cold mechanical pressing.

Corresponding Author: Ayman M. Kamel, Department of Medicinal and Aromatic Plants, Pharmaceutical Sciences Div. National Research Centre, Postal code:,12311, Dokki , Giza, Egypt. And Laval University, Quebec City, Canada, E-mail: kamelayman2000@yahoo.com

Separation and Identification of Sterols and Fatty Acids in Moringa Oil:

Separation of Sterols and Fatty Acids: About 15 ml of moringa oil was saponified by 10% methanolic KOH (140 ml) at 80 °C for three hrs under reflux. The unsaponifiable matter was extracted with ether (10 × 100 ml), washed several times with distilled water, dried over anhydrous sodium sulphate. Then, the solvent was evaporated and unsaponifiable matter was weighed and kept for further analysis.

The soap solution was acidified by HCl (10%), the liberated fatty acids were extracted with ether (3 × 150 ml), washed several times with distilled water till acid free, dried over anhydrous sodium sulphate. The solvent was evaporated [7,8].

Identification of Sterols Composition: The determination of sterols was made following the official method of the Association of Official Analytical Chemists [9]. Analyses were carried out on a SHIMADZU gas chromatograph model 17-A, equipped with BPX5 (SGE Japan) methylphenyl polysiloxane-coated capillary column (30m x 0.25 mm, 0.20 im film thickness) and FID. The column was isothermally maintained at 265°C. The injector and FID temperatures were set at 280 and 300°C, respectively. Oxygen-free pure N_2 at a flow rate of 3.5 mL/min was used as a carrier gas. The internal standard used was α-cholestanol. Sterols were identified and quantified by comparing the retention times and peak areas of the unknown components with those of known sterol standard mixture. Samples were prepared and measured separately in triplicate.

Preparation of Fatty Acid Methyl Esters: Methyl esters of fatty acids were prepared by refluxing 20 mg of the liberated fatty acids with 20ml (2%) of H_2SO_4 in anhydrous methanol for 5 hr on a water bath at 90°C. The fatty acid methyl esters were extracted with petroleum ether (20 ml each). The petroleum ether extract was treated with diluted sodium bicarbonate solution to remove the acidity, washed several times with distilled water, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure [10].

Identification and Quantitative Determination of Fatty Acids by GLC: The fatty acid methyl esters were identified by using GLC under the following conditions: SHIMADZU gas chromatograph model 17-A, equipped with BPx70, cyanopropyl polysilphenylene-siloxane and flame ionization detector. Nitrogen is a carrier gas, hydrogen and air gasses flow rates at 30, 30 and 300 ml/min. Oven temperature was programmed from 70°C to 240°C increased by 10°C min. Temperature of detector and injector were 300 and 250°C, respectively. Fatty acids were identified by comparing the relative retention time of each peak with those of standard fatty acid methyl esters. These analyses were carried out in Central Services Laboratory, Laval University, (Faculty of Agriculture and Food Sciences), Quebec, Canada. Samples were prepared and measured separately in triplicate.

Insecticidal Activity:

Insect Rearing: Armyworm (*Spodoptera frugiperda)* (F.) was cultured and maintained on sweet corn leaves (*Zea mays* L.). Rearing conditions were a 12 hrs photo regime at 28±2°C and 75±5 % relative humidity. Diet-fed larvae were reared from eggs and maintained in plastic sandwich boxes containing an artificial diet based on Navy bean seed [11]. Instars number was determined from the size of the head capsule.

Antifeedant Activity: The technique used was a choice test [12] using sweet corn leaves disk of 1 cm^2 (31.33 ± 0.04 mg fresh weight). The tested materials (10 µl) were uniformly distributed over the surface of the disk by application of five concentrations (1.25, 2.5, 5, 7.5 and 10%, w/v) in distilled water using tween 80 served as an emulsifier. Disks were allowed to evaporate to give the treated disks (TD). Control disks (CD) were prepared in the same manner but without test materials. For each bioassay, four TD and four CD was placed alternately in a Petri dish with five larvae (weight 40-50 mg) and kept in a growth chamber in the dark at 30°C, 70% relative humidity. There were six replicates for each material tested. The consumed area of the treated disks (CTD) and those of the controls (CCD) was visually estimated by planimeter at regular 30 min intervals for 4-5 h to calculate the feeding ratio (FR = CTD/CCD). FR_{50}, the feeding ratio when the CD had been 50% consumed, was used as a measure of antifeedant activity.

Larval and Pupal Mortalities: Larvae were reared on an artificial diet as described by Hirai [13]. Six concentrations were tested: 0% (control), 1.25, 2.5, 5, 7.5 and 10%, (w/v). The tested materials were prepared in distilled water using tween-80 as an emulsifier (total volume is 750 ml to obtain 1000 g diet). The diet was changed every 2 days. Surviving larvae received the diet until the pupal stage.

Mortality was recorded for both larvae and pupae. Each experiment was performed on 25 larvae with five replications. Percentage mortalities were corrected according to Abbott's formula [14].

Statistical Analysis: The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90} and slope regression lines using the software developed by Reddy *et al.* [15].

Statistical analysis of the antifeedant activity was carried out also by using a computer software package, "Costat", a product of Cohort Software Inc. Barkeley, California, USA. Duncan's multiple range test [16] was used to differentiate among means.

RESULTS AND DISCUSSION

GC Analyses of Moringa Oil: Table (1) represents the fatty acids constituents of moringa oil. The saponifiable matter of the oil contains different fatty acids. For instance, oleic acid as an unsaturated fatty acid was found as major (74.21%) followed by palmitic acid as a saturated fatty acid (7.16%). Arachidic acid and behenic acid as saturated fatty acids were detected with the same quantity (4.11%).

The determination of sterol components in moringa oil by GC was summarized in table 2. The major sterols were found to be β-sitosterol (43.45%) followed by stigmasterol (25.10%). On the other hand, cholesterol was found as a low quantity (0.23).

From tables 1 and 2, it can be concluded that the unsaturated fatty acids represent 78.71% from the whole moringa oil. In the same time, the ratio between saturated and unsaturated fatty acids is 1: 4.27. Moringa oil is characterized by a high content of oleic acid (74.21%) and belongs with the oleic acid oil category [17].

On the other hand, the contents of stigmasterol, β-sitosterol and campesterol in the present analysis of moringa oil were rather comparable with the values for moringa oil reported by Anwar and Rashid, [18] and Lalas and Tsaknis [19].

Antifeedant Activity (Feeding Ratio) Against *S. frugiperda*: The obtained data in table 3 showed that the higher concentration of moringa oil reduced the feeding ratio of *S. frugiperda* to 0.01 followed by unsaponifiable and saponifiable matters (0.08 and 0.11), respectively. Generally, feeding ratio was decreased by increasing the concentration in all treatments. The presence of fatty acids and sterols in moringa oil play a synergistic effect as antifeedant on the tested insect

Table 1: Fatty acids profile of moringa oil.

Name	Retention time (R_t)	Fatty acid (%)
Caprylic acid (8:0)	15.9	0.32
Myristic acid (14:0)	21.1	0.11
Palmitic acid (16:0)	26.6	7.16
Palmitoleic (C16:1)	27.7	2.15
Stearic acid (18:0)	29.0	4.10
Oleic acid (18:1)	30.7	74.21
Linoleic (C18:2)	31.9	1.23
Linolenic acid (18:3)	34.3	1.00
Arachidic acid (20:0)	35.0	4.11
Cis-11-eicosenoic acid (C20:1)	35.5	2.7 3
Behenic acid (22:0)	37.0	4 .11
Erucic acid (22:1)	37.9	0.12
Lignoceric (C24:0)	44.8	1.32
Cerotic (26:0)	48.0	1.32
Others	-------	2.85
Unsaturated fatty acids	-------	78.71
Saturated fatty acids	-------	18.44

Table 2: Sterols profile of moringa oil.

Name	Sterol (%)
Stigmasterol	25.10
Campestanol	0.38
Campesterol	14.40
Ergostadienol	0.50
β-sitosterol	43.45
Stigmastanol	0.64
Δ^5 avenasterol	9.44
Δ^7 avenasterol	0.62
28- isoavenasterol	1.14
Cholesterol	0.23
Clerosterol	1.70
Others	2.4

where the whole oil is more effective than its fractions (saponifiable and unsaponifiable matters). Generally, sterols were more effective than fatty acids as antifeedant activity where the feeding ratio of the insects treated with unsaponifiable matters is less than treated with saponifiable matters.

El-Gengaihi *et al.* [20] confirmed the role of volatile oil as antifeedant and growth inhibiting effect in their study of the effect of *Virex agnus costus* volatile oil on *Spodoptera littoralis* larvae. Nathan and Kalaivani [21] studied the effect of azadirachtin and nucleopolyhedrovirus on *Spodoptera litura* growth (nutritional indices). They found that there were significantly greater mean larval weight losses in larvae fed on combined treatment of azadirachtin and

Table 3: Feeding ratio of *S. frugiperda* treated with moringa oil, saponifiable and unsaponifiable matters.

Antifeedant activity	Conc. (%, W / V)	Moringa oil	Saponifiable matter	Unsaponifiable matter
Feeding Ratio (FR_{50}) ± SEM	1.25	0.36 ^{a} ± 0.03	0.66 ^{a} ± 0.03	0.54 ^{a} ± 0.05
	2.5	0.25 ^{b} ± 0.02	0.55 ^{b} ± 0.04	0.35 ^{b} ± 0.03
	5	0.12 ^{c} ± 0.012	0.41 ^{c} ± 0.04	0.31 ^{c} ± 0.03
	7.5	0.04 ^{d} ± 0.003	0.21 ^{d} ± 0.009	0.11 ^{d} ± 0.01
	10	0.01 ^{e} ± 0.0001	0.11 ^{e} ± 0.007	0.08 ^{e} ± 0.001
L.S.D. (5%)		0.0094	0.0123	0.0105

Each value was following by standard error; the upper case letters indicate significant differences between concentrations

Table 4: LC_{50} and LC_{90} of moringa oil, saponifiable and unsaponifiable matters on *S. frugiperda* larvae after three days of exposure.

Treatments	Moringa oil (%)	Saponifiable matter (%)	Unsaponifiable matter (%)
LC_{50}	1.9	7.6	3.4
LC_{90}	9.5	25.8	14.8
Slope ± SE	1.4 ± 0.1	1.3 ± 0.0.07	1.9 ± 0.09

Table 5: Corrected mortality of *S. frugiperda* larvae and pupae exposed to moringa oil, saponifiable and unsaponifiable matters in an artificial diet.

		Corrected mortality (%)		
Treatments	Concentration(%, w/v)	Larvae	Pupae	Total mortality
Moringa oil	1.25	42.2	18.8	61.0
	2.5	52.1	28.9	81.0
	5	82.2	5.6	87.8
	7.5	85.0	10.4	95.4
	10	100	0.0	100.0
Saponifiable matter	1.25	28.9	5.5	34.4
	2.5	35.5	10.1	45.6
	5	44.5	3.3	47.8
	7.5	51.1	5.5	56.6
	10	62.2	5.5	67.7
Unsaponifiable matter	1.25	31.1	28.8	59.9
	2.5	42.2	20.1	62.3
	5	57.8	12.6	70.4
	7.5	64.5	15.3	79.8
	10	64.4	16.3	80.7
L.S.D. (5%)				0.652

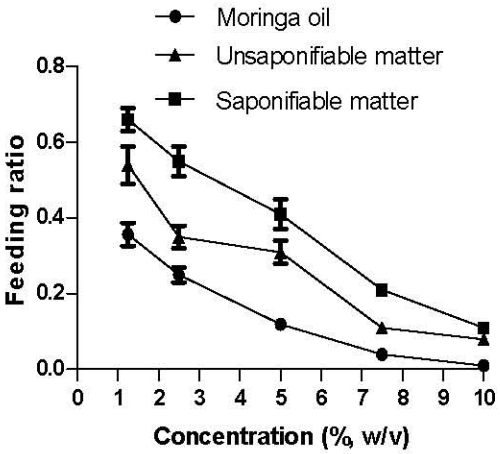

Fig. 1: Feeding ratio of *S. frugiperda* treated with moringa oil, saponifiable and unsaponifiable matters

nucleopolyhedrovirus as compared to control larvae. Food consumption was reduced perhaps by reducing growth and this could secondarily influence the food uptake, inhibition of the enzyme activities and interference with protein metabolism. In the same subject, Baskar *et al.* [22] studied the phagodeterrent effect of all crude extracts and fractions from *Atalantia monophylla* plant against *Helicoverpa armigera*. Ninth fraction showed maximum antifeedant activity than the crude extract. These results corroborate with the findings of Susurluk *et al.* [23] who reported that the phagodeterrency of fraction of the methanol extract of *Tanacetum cadmeum* was higher than the crude extract on *Spodoptera littoralis*.

Toxicological Effect Against *S. frugiperda*: The corrected mortality of moringa oil and their fractions were studied and summarized in tables 4 and 5. The obtained data

showed that LC_{50} of moringa oil, unsaponifiable and saponifiable matters were 1.9, 3.4 and 7.6% , respectively. These results revealed that moringa oil and the unsaponifiable matter were more effective than saponifiable matter on *S. frugiperda* larvae. Table 5 shows that pupae were more resistance than larvae where the corrected mortality of larvae was more than pupae in all treatments and concentrations used. The highest total mortality percentage was recorded at 10% with moringa oil (100%) and unsaponifiable matter (80.7%).

The mortality of larvae and pupae caused by oil of moringa may be due to the effect of sterols and fatty acids on the cuticle and/or may be due to the disturbance of the hormonal regulation caused by sterols. The mortality was increased with exist of the fatty acids. This result may be due to the fact that the whole components found in the moringa oil potentiated the effect on *S. frugiperda*. The other constituents might cause synergistic action as a toxicological agent.

Kamel and El-Ggengaihi [24] studied the insecticidal activity of globe cucumber extracts on cotton aphid. They found that the mortality of insect can be described on the basis that some fractions were more toxic than others due to its content in various secondary metabolites which are more toxic than other secondary metabolites on the cotton aphid.

Céspedes *et al.* [25] mentioned that the death of treated insects caused by plant extracts may be due to the inability of the moulting bodies to swallow sufficient volumes of air to split the old cuticle and expand the new one during ecdysis, or to a metamorphosis inhibiting effect of the plant extract ,especially sterols, which is possibly based on the disturbance of the hormonal regulation. All these factors may be causing a disturbance in hormonal balance which influencing on development of treated insect.

It was concluded that moringa oil is characterized by a high content of oleic acid (74.21%) and belongs with the oleic acid oil category. In the same time, the major sterols were found to be β-sitosterol (43.45%) followed by stigmasterol (25.10%). *S. frugiperda* insect affected by feeding on the diet treated with moringa oil and its fractions. Feeding ratio decreased with the higher concentration of all treatments. In the same time, moringa oil and the unsaponifiable matter were more toxic than saponifiable matter on *S. frugiperda* larvae where the highest total mortality percentage was recorded at 10% with moringa oil (100%) and unsaponifiable matter (80.7%). From the previous results, it is clearly confirmed that we can use moringa oil as a pest control agent against *Spodoptera frugiperda* insect.

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