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Insecticidal Toxicities of Glucosinolate-containing Extracts from Crambe Seeds[1]

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ABSTRACT   Glucosinolate components were extracted from defatted crambe (*Crambe abyssinica* Hochst ex. R. E. Fries) seed meal by using four different extraction solvents. These extracts containing naturally occurring glucosinolates were tested in bioassays against some selected agricultural and public health insect pests. The results showed that continuous aqueous exposure to dilutions of the extracts caused acute mortality to mosquito [*Aedes aegypti* (L.)] larvae. Crambe seed meal (containing intact glucosinolates) showed toxicity against house fly larvae (*Musca domestica* L.) when incorporated into the regular fly diet. Neither extracts nor seed meal were acutely toxic to the red flour beetle [*Tribolium castaneum* (Herbst)] or the sawtoothed grain beetle [*Oryzeaphilus surinamensis* (L.)] within 24 h; however, longer observation demonstrated a high mortality and antifeedant effect toward these insects. The two species of grain beetles began dying 10 d after exposure. The glucosinolate extracts also were effective on western corn rootworm [*Diabrotica virgifera virgifera* (LeConte)] larvae. No observable behavioral changes were found on German cockroaches [*Blattella germanica* (L.)]. The results showed that crambe glucosinolates have potential as a possible control agent for certain agricultural and public health insect pests.

KEY WORDS Natural product, glucosinolates, crambe, insecticidal activity, pest control

Glucosinolates are an important and unique class of secondary plant metabolites that occur in only 11 families of dicotyledonous plants, mostly in the family Cruciferae. These naturally occurring products are considered to serve as chemical defenses against insect pests (Dawson et al. 1993, Duncan 1991, Fenwick et al. 1983, Hedin 1986), and it was reported that the volatile aglucone is the actual active compound rather than the nonvolatile glucosinolate (Borek et al. 1994). The volatiles were considered to be a result of myrosinase/substrate interaction *in vivo* (Fenwick et al. 1993).

Although glucosinolates and their breakdown products have been regularly included in discussions on naturally occurring toxicants (Tookey et al. 1980, VanEtten & Wolff 1973), until recently there has been little definitive evidence to support this contention. Most of the studies on the physiological properties of glucosinolates and their breakdown products have been concluded in conjunction with mammalian and avian feeding experiments using rapeseed (Fenwick & Curtis 1980, Bell et al. 1972) and crambe (*Crambe abyssinica* Hochst ex. R. E. Fries) (VanEtten et al. 1969). These studies showed considerable enlargement of thyroid, adrenal gland, kidney, and liver in animals, especially livestock, and liver hemorrhage in poultry (VanEtten et al. 1969). The toxicological effects of glucosinolates and their breakdown products on insects have been of much less concern and have scarcely been investigated (Fenwick et al. 1983) compared to the intensive studies on livestock.

Furthermore, effects of glucosinolates on insect pests have been emphasized mainly on chemical ecological functions rather than acute toxicity. Glucosinolates have been found to play important roles in aiding certain insect species to identify their proper host plants. Experiments have shown that while too low a concentration of glucosinolates leads to ineffective larval attraction, too great a concentration may actually exert a repellent effect to the cabbage root fly (*Delia brassicae* L.) (Finch 1978). Nayar & Thornsteinson (1963) and David & Gardiner (1966) have demonstrated that several glucosinolates have a feeding stimulation effect on the diamond-back moth (*Plutella maculipennis* Curtis) and the larvae of *Pieris brassicae* L. Blau et al. (1978) indicated that allylglucosinolate was acutely toxic to larvae of the black swallowtail butterfly (*Papilio polyxenes* Stoll), which do not normally attack crucifers. In contrast, larval growth of the imported cabbageworm [*Pieris rapae* (L.)], a crucifer specialist, is not affected even by artificially high concentrations of allylglucosinolate. Larval growth of southern armyworm [*Spodoptera eridania* (Cramer)], a generalist feeder, is inhibited by high but not low concentrations of the compound. Bodnaryk (1991) reported that *p*-hydroxybenzyl glucosinolate plays a significant role in the insect-feeding-resistance mechanism of mustard seedlings. There are several reports on the oviposition-stimulating effect of the glucosinolates and their breakdown products, especially by the volatile breakdown products (Nair & McEwen 1976, Nair et al. 1976, Traynier 1965). Wolfson (1982) found that developmental responses of some insects to *Brassica nigra* L. were due to glucosinolate compounds. Wadleigh & Yu (1988) demonstrated that glutathione transferase is associated with the detoxification of glucosinolate breakdown products in three lepidopterous species, and that this enzyme played an important role in food-plant adaptation in phytophagous insects. More recently, Lazzeri et al. (1993) reported that some glucosinolates and their breakdown products were toxic toward a population of nematodes.

Crambe is a cruciferous plant that is a potentially important crop in the Midwest of the United States. The seed oil of crambe can be used as an industrial oil or for human nutrition. The remaining defatted seed meal can be used as a protein source in livestock diets. However, because the high concentration of glucosinolates and their breakdown products in the meal is toxic to the livestock, the glucosinolates must be removed or extracted from the

meal before use. Thus any use of these extracts would be of great advantage in terms of efficient use of our natural resources.

This study was conducted to determine the utility of this particular resource against selected pest insects. This practice would foster a highly effective use of a natural resource, i.e., exploiting this plant toxin against harmful insects, and at the same time, would minimize environmental hazards by using the more biodegradable natural products. We have evaluated the insecticidal spectrum of activity of glucosinolate-containing extracts of crambe seed meal by using different insect pest species of agricultural and public health concern and different routes of exposure in the bioassays. We also have compared the potency of extracts that have been obtained by using different extraction techniques.

Materials and Methods

Extraction of glucosinolates.   Polar solvents were selected for the extraction of glucosinolates because of the water solubility of these compounds. In our study, 100 g of defatted crambe seed meal (National Sun, Inc., Enderland, North Dakota) was soaked in 500 ml of the extraction solvent at 4°C for 2 d. Four different solvents were used for extraction: pure distilled water, 50% methanol (HPLC grade), 50% ethanol (HPLC grade), and 50% acetone (Optima grade). All organic solvents were purchased from Fisher Chemical Company (Fair Lawn, New Jersey) and diluted to 50% with distilled water. The slurry was filtered through a glass fritted funnel (Pyrex®, pore size 10–15 μm) and rinsed twice with 20 ml of the corresponding solvent. The filtrates then were evaporated to approximately 100 ml with a rotary evaporator at temperature lower than 40°C. At this point, the organic solvents were considered completely evaporated. The aqueous residues then were made up to 500 ml with distilled water and stored at 4°C as a stock solution of the crude extract. These solutions were analyzed for total glucosinolate and used in various bioassays with or without further dilution. Samples for total glucosinolate analysis were sent to and analyzed by POS Pilot Plant Corp., Canada.

Bioassays.   Defatted crambe seed meal and the four extracts were tested on adult German cockroaches [*Blattella germanica* (L.)], lab-reared feral strain; house fly [*Musca domestica* (L.)] larvae, lab-reared Orlando Regular strain; yellow fever mosquito (*Aedes aegypti* L.) larvae, Bangkok strain; red flour beetle [*Tribolium castaneum* (Herbst)] adults, field collected, Minnesota; sawtoothed grain beetle [*Oryzeaphilus surinamensis* (L.)] adults, USDA, Savannah, Georgia; and the western corn rootworm [*Diabrotica virgifera virgifera* (LeConte)] larvae, from non-diapausing eggs purchased from French Agricultural Research, Inc., Lamberton, Minnesota.   All insects except the mosquito and the western corn rootworm were reared in a room with temperature at 25 ± 2°C, relative humidity 45 ± 5%, and a photoperiod of 10:14 (L:D) h.

Bioassays of mosquito larvae.   Ten mosquito larvae (early 2nd instar) were exposed to 20 ml of differently diluted extracts (with distilled water) in a 50-ml jar. The larvae were transferred to the jar carefully with an eye-dropper. The

treated jars were kept in an incubator at 25 ± 1°C in the dark and with relative humidity of 45 ± 5%. The larvae were given a small amount (approximately 0.1 g) of food (ground Wardley, Tropical Flakes fish food, Wardley Laboratories, Inc., Secaucus, New Jersey). The test was done in three replicates. Mortality was recorded 24 h posttreatment. Larvae that were not able to swim were regarded as dead.

Bioassays of house fly larva. A water solution of each extract (11 ml) was mixed with 9 g regular house fly media (Purina®, Labchow, Ralston Purina Company, St. Louis, Missouri plus 1% of Red Star active dry yeast, Universal Food Co., Milwaukee, Wisconsin) in a 50-ml jar. Ten house fly larvae (2nd instar) were then placed in each jar. In addition to the extract, crambe seed meal also was used directly, being mixed with different percentages of the regular media. Every treatment had three replicates. The numbers of each stage (larval, pupal, and adult) were recorded on day 15.

Bioassays of adult German cockroaches. The aqueous solutions of each of the crude extracts (0.5 ml, 10%, 20%, 50%, and 100%) were mixed with 1 g of ground cat food (Purina® cat chow, Ralston Purina Company, St. Louis, Missouri) and fed with water to 10 roaches in a Petri dish (10-cm diam). The males and females were tested separately, and mixed-sex trials were conducted as well. Water was supplied with a moistened cotton wick and added *ad libitum*. Data were collected 1-, 2-, and 14-d posttreatment for any acute and relatively long-term toxicities. Every treatment had two replicates, and a control with food only was conducted.

Bioassays of the western corn rootworm. Fifty grams of autoclaved soil (sandy clay loam soil, collected in a pesticide-free field, Ames, Iowa, with 50% sand, 26% silt, 22% clay, 2.3% organic matter, pH 5.3) was placed in a Petri dish (10-cm diam) and moistened with 12 ml of water for control and aqueous solutions of crambe extracts for the treatments. Five corn seedlings (3–4 cm long) were arranged in a circle on top of the soil, followed by the introduction of 10 western corn rootworm larvae (3rd instar, aged 10–13 d) in the center of the dish. The treated Petri dishes then were put in an incubator at 25 ± 1°C, relative humidity 45 ± 5%, and a photoperiod of 12:12 (L:D) h. The experiment was carried out in duplicate. Mortalities were recorded after 24 h and 48 h. Larvae that were not able to move were regarded as dead.

Bioassays of red flour beetle. Each crude extract (20 ml) was mixed with 50 g of the regular media (whole wheat flour + 5% dry yeast) in a Petri dish. The media was allowed to dry in a dark fume hood overnight. Each treated medium then was transferred to a 200-ml mason jar. Two hundred adult beetles were introduced into each jar. The crambe seed meal also was tested directly against the beetles. Every test was duplicated. Data were collected on days 2, 5, 10, 13, and 15.

Bioassays of sawtoothed grain beetle. The assays on the sawtoothed grain beetle followed the same procedures as that for the red flour beetles, except that cracked corn was used as the medium. Fifty grams of corn kernels was cracked roughly and mixed with 20 ml of each extract. After drying in a dark fume hood overnight, the treated corn was transferred into a 200-ml jar into which 200 adult sawtoothed grain beetles were introduced. The same observations were taken to record the mortalities.

Data analysis. Probit analysis was used to determine LC_{50}, 95% FL, and slopes according to methods outlined in Finney (1971). Means were separated using least significant differences (LSD) ($P \leq 0.05$) (SAS Institute 1991).

Results and Discussion

Crude extracts from crambe seed meal demonstrated some interesting biological activity against certain insect pests. Relatively high insecticidal activities were found to several different insect species, although effects of these natural toxins on insects were generally slow and mild. There was no quick knockdown effect observed on any of the insects tested.

Total glucosinolate contents in crambe seed extracts are shown in Table 1. There were only slight differences in total glucosinolates among the extracts. All extracts had the same major glucosinolate component, 2-hydroxy-3-butenylglucosinolate. However, different degrees of activities shown by different extracts against the same insect have been observed in the present study. This may be attributed to some unknown factors in the extracts that can influence their toxicity.

Mosquito larvae were very susceptible to crambe extracts. The continuous exposure of 2nd instar mosquitoes to aqueous solutions of glucosinolate-containing extracts resulted in high toxicity. The mosquito larvae were intoxicated within 2–3 h after treatment with the ethanol extract, which was the most toxic extract. The LC_{50}s of the various extracts to the mosquito larvae are shown in Table 2.

The fresh extracts (kept at 4°C and used within 1 wk) are compared with long-stored and heated samples. The duration of storage and the processing and storage temperature seemed to play very important roles in the exertion of the bioactivities of glucosinolates (Table 2). Reduction of toxicity in a heated sample "acetone/heat" was observed and was considered to be caused by overheating when evaporating the solvents. This heating process may decompose the glucosinolate components (Fenwick et al. 1983). The extract "water/long" was stored at lower temperature (4°C) but for a relatively long time (> 45 d). It also showed lower toxicity against the mosquitoes compared to fresh extracts (Table 2). This reduced insecticidal activity is attributed to possible hydrolysis and further degradation of glucosinolate during the storage period or heating during the extraction.

Rearing of house fly larvae in extract-treated regular house fly diet failed due to a mold problem. However, the use of seed meal (containing intact glucosinolates) itself showed relatively strong toxicity (Table 3). The larvae were tested in 100% seed meal or a mixture with 50% regular house fly diet. The total mortality was 87 ± 3% and 77 ± 3%, respectively, whereas only 7 ± 3% of the flies died in the control. Most of the deaths in treated media occurred at the larval and pupal stages. Those flies that successfully emerged were still alive at day 15.

However, feeding German cockroaches with extract-treated diets did not cause any acute toxicity. A longer period of exposure (15 d) also did not cause any significant insecticidal effects. All males, females, or their mixtures treated with either water extract or ethanol extract showed a mortality range

Table 1. Total glucosinolate concentrations in different extracts.

Glucosinolate	Sample (µmol/g) [a]			
	H_2O	50% EtOH	50% MeOH	50% Acetone
Allyl	0.06 ± 0.00b	0.06 ± 0.00b	0.05 ± 0.00bc	0.06 ± 0.00b
3-Butenyl	0.06 ± 0.01b	0.05 ± 0.00b	0.05 ± 0.00bc	0.05 ± 0.00b
4-Pentenyl	0.06 ± 0.02b	0.06 ± 0.00b	0.08 ± 0.01b	0.08 ± 0.01b
2-Hydroxy-3-butenyl	5.34 ± 0.02a	5.24 ± 0.01a	5.03 ± 0.02a	5.54 ± 0.12a
4-Hydroxybenzyl	0.06 ± 0.00b	0.06 ± 0.01b	0.04 ± 0.00c	0.05 ± 0.01b
Total[b]	5.57 ± 0.03AB	5.46 ± 0.06BC	5.24 ± 0.02C	5.78 ± 0.14A

[a] Mean ± SEM of two replicates. Means within a column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$ [SAS Institute 1991]).
[b] Means within a row followed by the same capital letter are not significantly different (LSD, $\alpha = 0.05$ [SAS Institute 1991]).

Table 2. Toxicities of different extracts from crambe seed meal against larvae of *Aedes aegypti* 24 h posttreatment.

Extract[a]	n	Slope ± SE	LC_{50} (µmol/ml)[b]	95% FL
Acetone[a]	120	3.21 ± 0.16	0.76	0.45–1.08
Ethanol[a]	120	2.24 ± 0.68	0.64	0.38–0.89
Methanol[a]	120	3.47 ± 0.85	2.17	1.59–2.77
Water	120	2.46 ± 0.71	1.20	0.85–1.56
Acetone/heat[a,c]	120	4.69 ± 1.19	5.84	3.88–7.80
Water/long[a,d]	120	1.64 ± 0.25	3.60	3.16–4.03

[a] All solvents used in extraction were 50% in distilled water.
[b] µmol of total glucosinolates per ml of water.
[c] Heated at about 70°C and kept at room temperature for 3 mo.
[d] Stored at 4°C for more than 45 d.

Table 3. Effect of crambe seed meal on house fly larvae (15-d observation).[a]

% meal in diet	Emergence (%)	Dead pupae (%)	Dead larvae (%)	Total mortality (%)
100	13 ± 3b	43 ± 3a	43 ± 7a	87 ± 3a
50	23 ± 3b	20 ± 0b	57 ± 3a	77 ± 3a
0	93 ± 3a	0 ± 0c	7 ± 3b	7 ± 3b

[a] Mean ± SEM of three replicates. Means within a column followed by the same letter are not significantly different (LSD, α = 0.05 [SAS Institute 1991]).

from 0% ± 0% to 20% ± 10%, while control mortality fell into the same range. There was no significant relationship observed between dose and mortality. It is believed that death of the treated roaches was from natural causes rather than toxic components in the diets.

The toxicities of different extracts on western corn rootworm larvae were significantly dissimilar from each other. Among the four extracts, the ethanol and methanol extracts were more toxic. These crude extracts killed 100% of the larvae, and even a 50% dilution of these two crude extracts showed relatively high insecticidal activity with 24-h mortality of 27 ± 2% and 80 ± 10%, respectively (Table 4). Rearing adult red flour beetles and sawtoothed grain beetles with the extract-treated regular media did not show any acute toxicity within 24 h; however, these beetles died gradually as the trials continued. One hundred percent of the sawtoothed grain beetles reared in the crambe seed meal died 10 d after exposure whereas only 11.8 ± 3.3% of the beetles died in a 50% mixture of the meal and regular medium on day 10. Although it is difficult to compare the extracts with the meal because of their possible different levels of glucosinolate, mortalities caused by the extracts were still significantly higher than the control after day 10 (Table 5). In the case of the red flour beetles, the mortalities on days 10, 13, and 15 were counted as 33 ± 3.0%, 74 ± 5.0%, and 98 ± 2.0%, respectively, while no substantial mortality occurred in the control. A 50% mixture of crambe meal and medium caused significantly lower mortalities as did the medium treated with the extracts (Table 6). The antifeedant tests on the above two storage insects also revealed that either the extracts or intact glucosinolates in the crambe seed meal have high antifeedant or repellent activity on the two grain beetles. The beetles tended to move from the extract-treated medium or the crambe seed meal to the regular medium through Tygon tubing connecting the two boxes (Tsao et al. 1993).

Glucosinolate components must be removed before the seed meal of crambe is used in feeding livestock. Our results show that the crude extracts of crambe seed meal have potential as natural insect control agents. Glucosinolates can be easily hydrolyzed to smaller breakdown products in the environment or *in vivo*, and these more volatile breakdown products are generally considered to be

Table 4. Toxicities of different extracts from crambe seed meal against western corn rootworm larvae.

| | Mortality (%) | | | |
| | 24 h | | 48 h | |
Extract[a]	100%[b]	50%	100%	50%
Control	0 ± 0b	0 ± 0c	15 ± 5d	15 ± 5c
H$_2$O extract	5 ± 5b	-	35 ± 5c	-
MeOH extract	100 ± 0a	27 ± 2b	100 ± 0a	47 ± 4b
EtOH extract	100 ± 0a	80 ± 10a	100 ± 0a	85 ± 5a
Acetone extract	10 ± 10b	-	65 ± 5b	-

[a] Mean ± SEM of two replicates. Means within a column followed by the same letter are not significantly different (LSD, α = 0.05 [SAS Institute 1991]).

[b] percent concentration of the crude extract.

the toxic components (Brown et al. 1994). Activities such as toxicity against mosquito and house fly larvae imply that these crude extracts or their more purified forms could be useful for controlling insects in such places as livestock barns or small ponds as an economical and biodegradable control agent. Studies on the environmental effects and fate would be required to determine the safety or hazards of using glucosinolates as insecticides. The crude extracts or the meal itself can generate the toxic breakdown products *in situ* in the environment, such as in the soil (Brown et al. 1994). Although the modes of action of acute toxicity of glucosinolates are unknown, the pungent smell and taste of glucosinolate compounds are probably the key factors being associated with the antifeedant activity of the extracts against grain beetles. These smells and tastes are considered to be caused by volatile breakdown products, which showed similar and higher bioactivities against mosquito and house fly larvae (Tsao et al. 1993). The specific characteristic of volatilization probably also helped the expression of toxicity of crambe seed extracts against the western corn rootworm, one of the most damaging agricultural insects in corn fields (Tsao et al. 1993).

The overall goal of this research is to explore possible ways to utilize the glucosinolate-containing extracts of crambe in the control of agricultural and public health insect pests and to initiate studies on the mode of action of the glucosinolates. A wider insecticidal spectrum of activity of the crambe seed meal extracts is also being examined using other pest arthropods.

Table 5. Toxicities of crambe seed meal and its extracts against the sawtoothed grain beetle.

Sample[a]	Mortality (%)				
	Day 2	Day 5	Day 10	Day 13	Day 15
Control (medium)	0.5 ± 0.5b	0.5 ± 0.5b	0.5 ± 0.5c	0.8 ± 0.3c	0.8 ± 0.3c
H_2O extract + medium	3.0 ± 0.0b	6.5 ± 2.5b	12.3 ± 3.3b	16.0 ± 3.0b	19.3 ± 2.3b
MeOH extract + medium	4.0 ± 1.0ab	8.3 ± 3.3b	14.8 ± 5.8b	19.0 ± 5.0b	23.5 ± 6.0b
EtOH extract + medium	4.0 ± 3.0ab	7.0 ± 2.0b	12.3 ± 3.3b	16.0 ± 5.0b	23.0 ± 6.0b
Acetone extract + medium	1.5 ± 1.5b	5.0 ± 2.0b	11.0 ± 2.0b	15.3 ± 1.3b	20.5 ± 1.0b
Seed meal only	8.3 ± 0.8a	23.3 ± 8.3a	100.0 ± 0.0a	-	-
Medium + meal[b]	5.0 ± 2.0ab	7.5 ± 3.5b	11.8 ± 3.3b	15.0 ± 6.0b	16.8 ± 5.8b

[a] Mean ± SEM of two replicates. Means within a column followed by the same letter are not significantly different (LSD, α = 0.05 [SAS Institute 1991]).
[b] 50% seed meal and 50% regular medium.

Table 6. Toxicities of crambe seed meal and its extracts against the red flour beetle.

Sample[a]	Mortality (%)				
	Day 2	Day 5	Day 10	Day 13	Day 15
Control (medium)	0.0 ± 0.0d	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	1.3 ± 0.8d
H_2O extract + medium	0.3 ± 0.3cd	3.5 ± 1.5bc	7.8 ± 2.8b	14.3 ± 4.8bc	20.3 ± 2.3c
MeOH extract + medium	2.8 ± 0.8bcd	7.0 ± 2.0b	12.0 ± 1.0b	24.3 ± 2.8b	34.0 ± 3.0b
EtOH extract + medium	3.0 ± 0.0bcd	8.0 ± 1.5b	12.8 ± 3.8b	19.8 ± 4.3bc	38.8 ± 2.8b
Acetone extract + medium	3.5 ± 1.5bc	7.5 ± 1.5b	11.5 ± 0.5b	22.8 ± 2.3b	36.3 ± 4.8b
Seed meal only	11.0 ± 1.0a	20.5 ± 0.5a	33.0 ± 3.0a	74.0 ± 5.0a	98.0 ± 2.0a
Medium + meal [b]	3.8 ± 1.8b	5.5 ± 2.5b	7.8 ± 2.3b	11.3 ± 1.3cd	16.8 ± 1.8c

[a] Mean ± SEM of two replicates. Means within a column followed by the same letter are not significantly different (LSD, $\alpha = 0.05$ [SAS Institute 1991]).
[b] 50% seed meal and 50% regular medium.

Acknowledgment

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