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INSECTICIDAL ACTIVITY OF BLACK PEPPER (*PIPER NIGRUM* L.) EXTRACTS[1]

Pio A. Javier and Belen Morallo-Rejesus[2]

ABSTRACT

The insecticidal activity of the black pepper extracts was determined against nine insect species belonging to four orders.

Both the crude and the semi-purified extracts were topically more toxic than malathion against diamondback moth larvae and adult houseflies. The semi-purified extract was generally more toxic than the crude extract. The toxicity of the semi-purified extract to the test insects decreased as follows: housefly > cotton stainer > corn weevil > diamondback moth > black armyworm > red flour beetle > common cutworm > lesser grain borer, while their susceptibility to the crude extract decreased in the order: housefly > cotton stainer > corn weevil > diamondback moth > black armyworm > common cutworm > lesser grain borer > red flour beetle.

Black peper mixed with grains was more toxic than malathion against corn weevil as a residual contact and stomach poison. It also inhibited growth. If the grains were to be stored for two months, the use of ground form is preferable over the extracts.

Key words: *Piper nigrum* L., botanical insecticide, plant extract, corn weevil, stored product insects.

INTRODUCTION

Plants have been a major source of medicinal drugs and pesticidal compounds. Inspite of the current spurt of activity, majority of the plants reputed to possess insecticidal properties remain chemically obscure. In some instances, biologically active constituents have been isolated and even identified, but convincing data on toxicity to insects have not been fully documented.

The insecticidal properties of black pepper, *Piper nigrum* L. (Piperaceae) against rice weevil, *Sitophilus oryzae* (L.) (Curculionidae) and cowpea weevils, *Callosobruchus maculatus* (F.) (Bruchidae) were investigated by Su (1977). The extracts of black pepper were found to be highly toxic to the rice weevil when used to surface-treat the wheat, and to adult rice weevils and cowpea weevils by topical application. Likewise, Scott and Mckibben (1978) found the extracts of ground black pepper to be highly toxic to adult boll weevils, *Acanthonomus grandis* Boheman (Curculionidae) by topical treatment. Since the topical and residual toxicity of black pepper has not yet

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[2]Researcher I, National Crop Protection Center; and Professor, Department of Entomology, respectively, University of the Philippines at Los Baños, College, Laguna 3720.

been evaluated on other storage insects (e.g. corn weevil, lesser grain borer and red flour beetle) and agricultural pests (e.g. diamondback moth, common cutworm, black armyworm and cotton stainer), the present investigation was pursued.

The study was conducted to isolate the possible insecticidal extracts in black pepper and to determine their toxicities against major coleopterous stored grain insects and other agricultural pests. The study was conducted in the Department of Entomology, University of the Philippines at Los Baños (UPLB), College, Laguna from January 1979 to March 1981.

MATERIALS AND METHODS

Mass rearing of test insects

The test insects belonged to four different orders. The coleopterous stored grain pests were corn weevil, *Sitophilus zeamais* Motsch. (Curculionidae); red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae); lesser grain borer, *Rhizopertha dominica* (Fab.) (Bostrichidae); and bean weevil, *Callosobruchus* sp. (Bruchidae). The lepidopterous pests were common cutworm, *Spodoptera litura* (Fab.) (Noctuidae); black armyworm, *Spodoptera exempta* (Walker) (Noctuidae); and diamondback moth, *Plutella xylostella* (Linn.) (Plutellidae); while the hemipterous and dipterous insects were cotton stainer, *Dysdercus cingulatus* Fab. (Pyrrhocoridae); and housefly, *Musca domestica* Linn. (Muscidae), respectively. The extracts were evaluated against two to three week-old adult coleopterous insects, second instar larvae of common cutworm and black armyworm, second instar nymphs of cotton stainer, third instar larvae of diamondback moth and two to four-day-old adult houseflies.

Laboratory strains of corn weevil, lesser grain borer, red flour beetle and cowpea weevil were reared on corn, *Zea mays* L. 'UPCA Var 3' (Graminae), sorghum, *Sorghum bicolor* L., Moench. 'Cosor 3' (Graminae), and mungo, *Vigna radiata* L. 'CES 55' (Leguminaceae), respectively as described by Santhoy and Morallo-Rejesus (1975). Twenty-eight days after infestation, the jars were examined for adult emergence. The rearing media were sieved every three days thereafter to obtain uniformly-aged adults.

Larvae and egg masses of common cutworm and black armyworm were field-collected and reared according to the method of Morallo-Rejesus and Martinez-Aguda (1980), except that the insects were provided with sterilized sodium hypochloride-treated mulberry and sorghum leaves, respectively. The extracts were evaluated against F_1 and F_2 larvae only. Larvae and pupae of diamondback moth were reared as described by Barroga and Morallo-Rejesus (1975-76). Houseflies were collected at the Poultry Division, Animal Science Department, UPLB, and were reared as described by Baldos (1974). Likewise, mating adults of cotton stainer were field-collected from okra plants. They were allowed to oviposit on moistened fine sand in acrylic pans and were provided with fresh okra fruit every three days. Newly-hatched nymphs were transferred to other acrylic pans.

Isolation and bioassay of the extracts

Isolation of black pepper extracts. The extraction procedure of Scott and Mckibben (1978) was used with slight modifications. Three hundred grams of ground black pepper (particle size < 846 μ) was refluxed in 350 ml ethanol for 20 minutes. The mixture were filtered; the residue was extracted three more times with ethanol. An equal volume of distilled water was added to the combined filtrates; afterwards, the solution was shaken with anhydrous ethyl ether. The solvent was removed using rotary evaporator. The semi-purified extract was prepared by dissolving the crude extract in chloroform and passing the mixture through a column (585 mm long x 45 mm diam) of aluminum oxide using chloroform-methanol (10:1 v/v) solution.

Thin layer chromatography (TLC) of crude and semi-purified extracts was done using ethyl ether as carrier.

Topical toxicity. The crude and the semi-purified extracts were dissolved in acetone to obtain a stock solution of 50 mg/ml. Several concentrations ranging from 0.5 to 32 mg/ml were prepared serially from the stock solution. Each test insect was topically applied with one μl solution of the extract on the dorsum using a Burkard® microapplicator. Each concentration was replicated three times using 20 insects per replicate. The treated weevils/beetles were confined in 90 cm diam. petri dish without food while the lepidopterous, hemipterous, and dipterous insects were kept in petri dishes provided with food. The petri dishes were kept at 27 ± 1°C and 64 ± 2% RH.

Equal number of insects were treated with acetone as control and malathion (0,0-dimethyl-dithiophosphate of diethyl mercaptosuccinate) as the standard insecticide. Mortalities were recorded at 24 hours after treatment. Insects that did not move when touched gently with a small pointed object were considered dead. Likewise, moribund insects were counted dead. LD_{50} values were determined by probit analysis (Finney 1962).

Admixture treatment of the medium. Four stored grain insects were used in this study.

For the corn weevil, one hundred grams of corn previously disinfested in an oven set at 45°C for five days was placed in ball jars. Two ml of the crude or semi-purified black pepper extract was sprayed at 120, 250 and 500 ppm concentrations into the medium using an atomizer. Corn sprayed with acetone served as control, while 10 ppm malathion was used as standard (FAO/WHO 1974). The ball jars were rotated while spraying to obtain an even distribution of the extract. The treated medium was allowed to dry overnight before infesting with weevils. In addition, 600 and 1200 ppm ground black pepper (GBP) were admixed directly with the grains. All treatments, including the control, were replicated three times.

Ten pairs of three-week-old corn weevil adults were introduced into each ball jar. The adults were allowed to oviposit for one week and the jars were examined daily for adult mortality. Live insects were returned to the ball jars while dead insects were discarded. Insects that were still alive at the last count were removed from the jars. Likewise, the F_1 adult progenies were counted 28 days after removal of the parent weevils and continued for four weeks. Data on the number of offsprings were statistically analyzed using complete randomized design and differences among treatments were determined using Duncan's multiple range test. Extracts that were not promising, based on topical toxicity tests and admixture treatments of the medium, were not tested further.

The residual toxicity of the extracts at two and four months after grain application was determined by reinfesting the ball jars with batches of 20 adults per replicate. Mortality was determined daily for 10 consecutive days.

Other stored grain insects were also tested. The procedure was the same as mentioned above except that sorghum instead of corn was used as the rearing medium for the lesser grain borer, milled rice for red flour beetle, and mungo for cowpea weevil. The one-month residual toxicity of the extract against these insects was low; consequently, the extract was not tested further.

RESULTS AND DISCUSSION

Isolation and bioassay of the extracts

Isolation of black pepper extract. Results of the TLC analysis of the crude and semi-purified black pepper extracts indicated possible presence of seven and eight components, respectively. All the seven components in the crude extract were also present in the semi-purified extract as indicated by their Rf values. No attempt was made to separate the different components either for identification or for individual bioassay against the test insects. Su (1977) in her purification of black pepper extract through TLC, identified five components under UV. Two of the components (Rf values of 0.31 and 0.33) were identical to the two components of piperine which have very low toxicity to rice and cowpea weevils. Other components such as piperonal, piperic acid, piperedic acid, α-phellandrene and capsaicin were not toxic at 50 μg/insect. Su (1977) attributed the toxicity of black pepper to either one or more of the three components with Rf values of 0.42, 0.47, and 0.54, perhaps in conjunction or synergism with piperine.

Topical toxicity. The toxicity of the extracts varied among different insect species as shown by the LD_{50} values (Table 1). Susceptibility of insects to the semi-purified extract decreased as follows: housefly > cotton stainer > corn weevil > diamondback moth > black armyworm > red flour beetle > common cutworm > lesser grain borer, while their susceptibility to the crude extract decreased in the order: housefly > cotton stainer > corn weevil > diamondback moth > black armyworm > common cutworm > lesser grain borer > red flour beetle. In comparison, insect susceptibility to malathion was as follows: cotton stainer = common cutworm = black armyworm > corn weevil > red flour beetle > housefly > lesser grain borer > diamondback moth.

Table 1. Insect toxicity caused by topical application of crude (CE) or semi-purified (SPE) black pepper extract to eight insect species; malathion served as standard treatment.

Treatment	LD_{50}[1] mg/g body weight	Confidence Limit (p = 0.05) Lower limit	Upper limit
		Corn weevil	
SPE	0.735	0.719	0.752
CE	1.261	1.184	1.345
Malathion	0.007	0.006	0.008
		Lesser grain borer	
SPE	10.200	9.052	11.489
CE	7.119	6.326	8.000
Malathion	0.437	0.393	0.474
		Red flour beetle	
SPE	5.629	4.743	6.448
CE	21.514	18.824	24.590
Malathion	0.081	0.063	0.095
		Diamondback moth	
SPE	1.181	1.042	1.361
CE	1.819	1.583	2.083
Malathion	11.319	10.292	12.458
		Common cutworm	
SPE	7.169	3.008	16.664
CE	5.008	3.320	7.745
Malathion	0.002	0.001	0.003
		Black armyworm	
SPE	2.289	1.818	2.880
CE	2.112	1.773	2.521
Malathion	0.003	0.001	0.004
		Housefly	
SPE	0.183	0.170	0.198
CE	0.215	0.198	0.234
Malathion	0.334	0.229	0.373
		Cotton stainer	
SPE	0.390	0.332	0.460
CE	0.819	0.610	0.914
Malathion	0.001	0.001	0.001

[1] Recorded at 24 h after treatment.

The semi-purified extract was 3.89 to 1.17 times more toxic than the crude extract to red flour beetle, cotton stainer, corn weevil, diamondback moth, and housefly in decreasing order. In contrast, the crude extract was 1.43 times more toxic than the semi-purified extract to the lesser grain borer, while both extracts were equally effective against the black armyworm and the cutworm. Compared with malathion, the semi-purified and the crude extracts were 9.64 and 6.22 times more toxic to the diamondback moth and 1.83 and 1.55 times more toxic to housefly, respectively. The diamondback moth has already developed resistance to malathion and cross-resistance to diazinon and dichlorvos (Barroga and Morallo-Rejesus 1975-76). Barroga and Morallo-Rejesus (1982) reported a 320- and 720-fold malathion resistance in some populations of *Plutella* in College, Laguna, and in Trinidad, Benguet, respectively.

Recently, Cayabyab and Magallona (1982) reported that among the six insecticides they tested against housefly, malathion was the least toxic to Calamba population since the highest dose of 8.7 mg/g hardly caused 50% mortality. They added that selection in houseflies with carbaryl led to cross-resistance to DDT, N-acetylcarbaryl, and malathion. Malathion has been used continuously for housefly control since Atienza (1969) reported that some housefly populations have already developed resistance to previously used chlorinated insecticides.

Table 2. Corn weevil toxicity caused by mixing corn with ground black pepper or its extracts.[1]

Dosage (ppm)	% Mortality at various days after insect introduction[2]		
	2	4	6
Semi-purified extract			
120	3 f	53 de	87 b
250	32 e	79 cd	96 a
500	86 b	97 ab	97 a
Crude extract			
120	55 de	85 bc	95 ab
250	67 cd	95 ab	98 a
500	100 a	100 a	100 a
Ground black pepper			
600	62 d	98 a	100 a
1200	82 bc	98 a	100 a
Control			
Acetone	0 f	0 f	0 d
Malathion (10 ppm)	0 f	30 e	56 c

[1] Cumulative average of three replications; 20 adults per replicate.

[2] Computation based on transformed values, Angles = Arcsin decimal mortality. In a column, means followed by the same letter are not significantly different at 5% level of probability.

Although malathion was topically more toxic than black pepper extracts to the cotton stainer, the black armyworm and the corn weevil, these insects were also very sensitive to the extracts as indicated by the very low LD_{50} values. Hence, there is a need to further evaluate the performance of the extracts on these particular insects.

Admixture treatment of the medium. Corn weevil mortality in all ground black-pepper-treated grains was significantly higher than in acetone-treated and malathion-treated grains (Table 2). The crude extract was more toxic than the semi-purified extract since 100% corn weevil mortality (500 ppm) occurred within two days after insect introduction in the former, and four days to cause comparable mortality with the semi-purified extract. With the exception of 120 ppm semi-purified extract, all the black pepper treatments were equally toxic (69 to 100% mortality) at 6 days after infestation.

The residual toxicity of black pepper on the same grains used for the above was also determined at two and four months after treatment (Table 3). Toxicity of the extracts and ground black pepper to corn weevil did not

Table 3. Residual toxicity to corn weevils at two and four months after mixing ground black pepper or its extracts with corn.[1]

Dosage (ppm)	% Mortality at various days after insect introduction[2]							
	2 months				4 months			
	4	6	8	10	4	6	8	10
Semi-purified extract								
120	20 c	50 d	67 d	88 b	0 a	0 d	0 d	0 d
250	42 bc	78 c	92 c	100 a	0 a	7 a	13 c	15 c
500	60 b	95 ab	100 a	100 a	17 a	67 a	80 a	83 a
Crude extract								
120	68 ab	78 c	97 b	100 a	0 a	0 d	0 d	0 d
250	56 b	85 bc	97 b	100 a	10 a	35 b	52 b	63 ab
500	52 b	85 bc	92 c	100 a	13 a	42 ab	62 b	78 ab
Ground black pepper								
600	52 b	78 c	97 b	100 a	0 a	0 d	0 d	0 d
1200	87 a	100 a	100 a	100 a	15 a	40 b	50 b	57 b
Control								
Acetone	0 d	0 e	0 e	0 c	0 a	0 d	0 d	0 d
Malathion (10 ppm)	0 d	0 e	0 e	0 c	0 a	0 d	0 d	0 d

[1] Cumulative average of three replications; 20 adults per replicate.

[2] Computation based on transformed values, Angles = Arcsin decimal mortality. In a column, means followed by the same letter are not significantly different at 5% level of probability.

decrease two months after treatments of the medium; moreover, mortality increased correspondingly with days of exposure to the treated medium. Except for 120 ppm semi-purified extract, 100% mortality occurred on black-pepper-treated grains at 10 days after infestation.

On the other hand, toxicity decreased in different black pepper treatments after four months but all treatments, except at the lowest concentration of the extracts and ground black pepper, were significantly better (57-83% mortality) than the acetone and malathion treatments. If the grains were to be stored only for two months, any of the black pepper treatments (except 120 ppm crude extract) would be sufficient to protect corn grains against the corn weevils.

Ground black pepper or its extracts significantly reduced the F_1 progenies of corn weevils as compared with control (Table 4). This indicates that in addition to the direct effect on adult survival, black pepper application also affects the development of immature stages.

The application of 10 ppm malathion and all concentrations of black pepper (except 120 ppm crude extract) significantly reduced the F_1 progenies of the lesser grain borer. The semi-purified extract at 250 ppm and all concentrations of the crude extract were comparable to 10 ppm malathion, but the lowest borer emergence was noted on ground black pepper. In con-

Table 4. The number of F_1 progenies of corn weevil, lesser grain borer, red flour beetle, and bean weevil on stored grains mixed with ground black pepper or its extracts[1].

Dosage (ppm)	Corn weevil (corn)	Lesser grain borer (sorghum)	Red flour beetle (milled rice)	Bean weevil (mungo)
Semi-purified extract				
120	5 c	4 d	7 cd	38 ab
250	0 d	27 cd	3 e	8 cd
500	0 d	17 d	5 de	3 de
Crude extract				
120	2 cd	112 ab	19 a	34 ab
250	1 d	35 cd	11 ab	49 a
500	1 d	37 cd	5 de	58 a
Ground black pepper				
600	2 cd	23 d	—	16 bc
1200	0 d	23 d	—	0 e
Control				
Acetone	46 a	145 a	10 c	53 a
Malathion (10 ppm)	23 b	71 bc	5 de	39 a

[1]Average of three replications. Computation based on transformed values, $\sqrt{X + 0.50}$. In a column, means followed by the same letter are not significantly different at 5% level of probability.

trast, ground black pepper and semi-purified extract were more effective than 10 ppm malathion against bean weevil, while semi-purified extracts and 500 ppm crude extract were as effective as 10 ppm malathion.

The use of ground black pepper is preferable over the extracts for the control of stored grain insects because the former can be easily prepared without requiring several expensive solvents. Six hundred ppm ground black pepper is 13.7 times more expensive than 10 ppm malathion; however, it is 11.5 times more effective in reducing the F_1 progenies of the corn weevil. In addition, black pepper gave 100% weevil mortality two months after application while malathion was already ineffective. With the present problems of environmental contamination, black pepper could be a good substitute for malathion and other pesticides. Because black pepper is widely grown in the Philippines and is used for food seasoning, the prospect of using this locally available material as a potential source of insecticide deserves careful consideration.

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