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Full Length Research Paper

Comparative pesticidal activity of dichloromethane extracts of *Piper nigrum* against *Sitophilus zeamais* and *Callosobruchus maculatus*

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Piperine a clinically established pesticide was investigated in the seeds, fruit and leaves of *Piper nigrum*. The dichloromethane extracts obtained from the parts of the plant were analysed through Thin Layer Chromatography (TLC) using silica gel-GF 254 impregnated aluminium plate and various solvent system consisting of chloroform : methanol (8:2), (6:4), ethyl acetate : methanol (9:1), and acetone : hexane (6:4) mixtures showing Rf value of 0.95 corresponding to the standard piperine. In all parts of the plants investigated, acetone : hexane solvent system showed distinct Rf value of 0.95 corresponding to the standard piperine. 0.289 mg/ml, 0.578, 0.868, 1.16 and 1.45 mg/ml concentration of dichloromethane seed extracts were used to carry out the pesticidal activities on both pests. The results showed that mortality rate on both pests are directly proportional to each equivalent concentration of piperine in the extracts. Pesticidal potency on *Sitophilus zeamais* was significantly higher (P<0.05) relative to *Callostrobuchus maculatus* within 9000 s of exposure period.

Key words: Piperine, *Piper nigrum*, pesticidal, *Sitophilus zemais*, *Callosobruchus maculates*.

INTRODUCTION

Plants are known to compose of secondary chemical compounds which have usually been regarded as a part of the plant's defense against plant-feeding insects and other herbivores (Lupina and Cripps, 1987). There has been considerable interest among scientists to screen plants for secondary chemical compounds which could possibly be used for developing medical and pesticide compounds particularly in the tropical rain forests where plants species are numerous but threaten with extinction (Pathak and Krisna, 1991). Though, some of these compounds have been shown to be active against pests, others have proven to be toxic and harmful to the environment and ecosystem (Adgeh, 1989). Hence, there is a need to look for a suitable environmental friendly organic source that has pesticide potency.

Among the plants investigated to date, one showing enormous potential is the pepper family otherwise known as *Piperaceae* (Dodson et al., 2000). They are generally perennial climbing vine extensively grown for its berries which are used as spices. They have also been traditionally used in most parts of the world as pesticide (Sigamony et al., 1986; Wood et al., 1988; Miyakado et al., 1989; Pathak and Krishna, 1991). The species, *P. nigrum* (black pepper) under investigation is the major component of spices in the roasted meat locally referred to as "Suya" and pepper spice in pepper soup delicacies in Nigeria.

The phytochemical screening of *P. nigrum* fruit shows that it contains 4% alkaloids in the berry (Dev and Koul, 1997). Although information on the compounds responsible for the insecticidal activities is scarce, it has been documented that the amide olefinic or alkyl isobutylamides compounds such as piperine, piperettine, tricostacine, peepuloidin, piplartin and trichonine contribute in no small measure (Adgeh, 1989). These compounds have been demonstrated to be toxic to fruit flies, adzuki bean weevils, cockroaches and several other insect species (Gbenwonyo et al., 1993; Su and Hovart, 1981).

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The present study investigates the pesticidal activity of the compound in extract of *P. nigrum* on stored products insects found in maize and beans, the common staple food grains in Nigeria.

MATERIALS AND METHODS

Identification and extraction of *Piper nigrum*

The identity of *P. nigrum* was authenticated at the botany unit of the Department of Basic and Applied Sciences, Babcock University. The method of Sofowora (1982) was slightly modified in carrying out extraction of the seeds, fruits and leaves of the plants. These various parts were extracted with dichloromethane under reflux for three hours using Soxhlet apparatus (Model no 3567, Austria). Extracts collected were thereafter suction filtered with vacuum sucker (model no 7651, Indonesia). The resultant filtrate was concentrated in vacuum to remove any traces of the solvent where the viscous brown olive oil produced was allowed to cool in ice cold bath for two minutes before chromatographed. The TLC plates used were pre coated with silica gel. 1 mg extracts of leaves, seeds, fruits and piperine standard were dissolved in 0.5 ml of methanol. The respective extracts were spotted on the plates and allowed to run in the four solvent system – chloroform : methanol (8:2), chloroform : methanol (6:4), ethyl-acetate : methanol (9:1) and hexane : acetone (6:4).

Pest culture

Adult *C. maculatus* and *S. zeamais* used for the study were obtained from naturally infested bean and maize seeds collected from open market in Babcock University environment. The method of Scott and Mackibben (1976) was used to culture the pest. New generation were reared on clean uninfested respective grain variety in Kilner jars under fluctuating temperature of 32±3°C and relative humidity of 70±5%. The grains used were disinfested in deep freezer for 72 h and later dried for three hours in the laboratory before culturing.

Pesticidal effects

Scott and Mackibben (1976) method was used with minor modification. Twenty grams each of disinfested grains were weighed into separate Petri dishes (9 cm diameter) into which was added 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquots of the extracts (seeds only). Two separate Petri dishes (C_0, Ca,) were used for this assay as a control. C_o was a positive control without treatment while C_a was a negative control treated with 1 ml of acetone. The extracts were allowed to dry for about thirty minutes before introducing the weevils. Ten unsexed *C. maculatus* and *S. zeamais* were separately introduced into each of the Petri dishes concurrently. Mortality count was carried out at 15 min intervals for maize weevil and 30 min interval for bean weevil. Insects were certified dead when probed with a pin at the abdomen and there was no response.

Statistical analysis

Chi-square analysis was used for mortality test (Bailey, T.J; 1995)

RESULTS AND DISCUSSION

The percentage yields of the extract from different part of the plant subjected to soxhlet extraction are shown in Table 1. System 1, 2 and 3 gave one spot having Rf values of 0.94, 0.94 and 0.91, respectively, which were similar to the Rf of the standard piperine run alongside (Table 2). However, System 4 resulted in a more clear differentiation of spots present in the dichloromethane extracts of *P. nigrum* with a Rf value of 0.95 having moved a distance of 5.3 cm which confirmed the presence of piperine in each parts of the plant (Table 3). This indicates that solvent 4 is the most appropriate vehicle to convey piperine. The concentration of piperine in each part of the plant extracts was calculated following Grieg (1993) Hilton et .al; (1998) and Farnham (1998) as shown in Table 4 and this informs the use of the seed extract for the pesticidal test.

Table 1. Yields of the extracts from different *Piper nigrum* plant parts.

Plant parts	Weight used for extraction	Percentage yields
Seed	121.5	18.76
Fruit	73.9	18.88
Leave	24.2	20.66

Table 2. Rf values of *Piper nigrum* extracts observed using different mobile phase systems.

System	Rf Values
1. Chloroform: methanol (8:2)	0.94
2. Chloroform: methanol (6:4)	0.94
3. Ethyl acetate: methanol (9:1)	0.91

It is not very surprising from this present study that the seed extract killed adult *S. zeamais* and *C. maculatus* since similar action was found in boll weevil (Scott et al., 2003). When 0.298 mg/ml of the extract was applied on the *S. zeamias*, 100% mortality was achieved in the whole population at one hundred and thirty-five minutes (135 min) after exposure (Figure 1). However, it took just 60 min for a similar occurrence in *C. maculatus* using 1.45 mg/ml under the same condition (Figure 2). This showed that mortality rate was directly proportional to the concentration of dichloromethane seed extract on both pests under investigation. The mortality rate for the positive control experiment was drastically reduced (10%) while that of negative control gave a considerable mortality of (20%) on both pests (Figures 1 and 2). The low mortality rate observed for acetone could be as a result of evaporation which may contribute to less lethal effect.

This study clearly showed that the pests' population declined drastically relative to concentration and time of exposure (Figure 3). It is evidently that *P. nigrum* had

Table 3. Distance by different components present in the crude extract of *Piper nigrum* in mobile phase system 4, acetone : hexane (6:4).

Seed distance (cm)	Fruit distance(s) (cm)	Piperine standard (cm)	Leaves distance (cm)
2.8	2.5	5.3	2.1
3.2	3.2		3.1
3.6	3.6		3.6
4.0	4.2		4.0
4.5	4.5		5.3
5.0	4.7		
5.3	5.0		
	5.3		

Table 4. Concentration of piperine in each of the *Piper nigrum* extract(s).

Sample	Diameter (mm)	Amount (x 10^{-4} µg)	Volume spotted (µl)
Seeds	10	8.6842	6µl
Fruits	7.5	4.7368	6µ
Leaves	5.5	1.5789	6µl

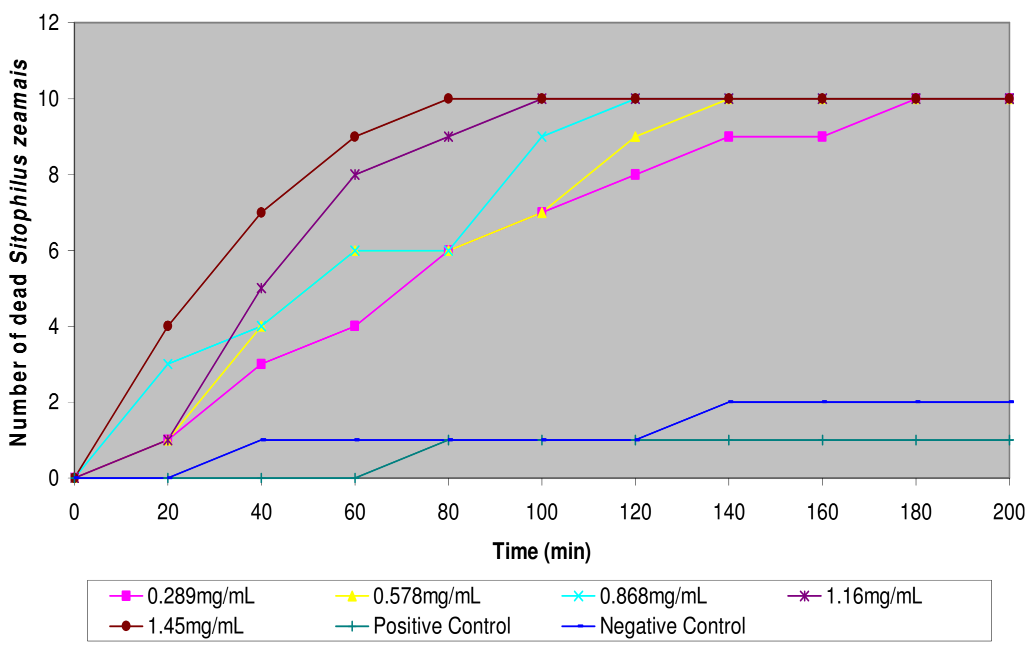

Figure 1. Comparative effects of different concentrations of *Piper nigrum* dichlorometane extract on *Sitophilus zeamais*

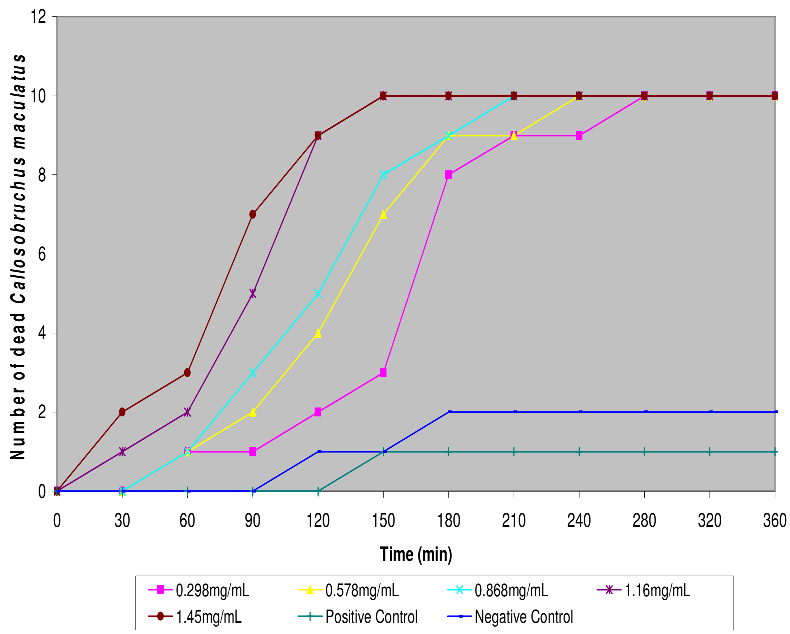

Figure 2. Comparative effects of different concentrations of *Piper nigrum* dichlorometane extract on *Callosobruchus maculatus*

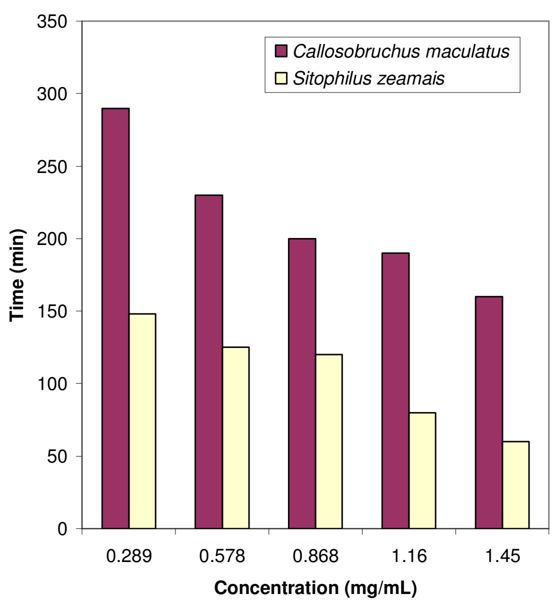

Figure 3. Comparism of the mortality rate in *Callosobruchus maculatus* and *Sitophilus zeamais* after application of *Piper nigrum* extracts.

more potency on *S. zeamais* compared to *C. maculatus*. This could probably be due to the sizes of the organisms.

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