SCREENING FOR RESISTANCE TO BOTANICAL AND SYNTHETIC INSECTICIDES BY *Sitophilus zeamais* AND *Callosobruchus maculatus* ON STORED GRAINS

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ABSTRACT

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An experiment was conducted in the Pathology Laboratory, University of Uyo, Nigeria to assess the possible development of resistance by Sitophilus zeamais and Callosobruchus maculatus to neem (Azadirachta indica) leaf powder and actellic dust (Pirimiphos methyl) tested separately on maize and cowpea grains. The experiment was laid out in randomized complete design and replicated four times. Mortality, damage assessment and progeny development were also assessed. Treatments were applied as direct admixtures at 5% and 10% to 100g of maize and cowpea, respectively while control was without insecticide application using three successive generations of S. zeamais and C. maculatus. Results revealed that actellic applied at 5g and 10g were more toxic to S. zeamais and C. maculatus, respectively and suppresses progeny development as well as reduced damaged caused by the insect pests in the three generations studied compared with neem and the untreated control. Mortality was observed to decrease with subsequent generations while progeny development was more in the control treatments in all generations of insects studied. The study has demonstrated the occurrence of resistance in the two insect strains to Pirimiphos methyl and neem leaf powder as low mortality and increased progeny development was observed in the second and third generation of insects subjected to the treatments. This calls for serious concerns in the management of insecticides against storage insect pests.

Keywords*: Insecticide resistance, actellic, neem, weevil*

INTRODUCTION

The use of insecticides on stored products has progressed in recent years from application of few inorganic materials to the use of a large number of highly effective organic compounds (Oparaeke and Kuhiep, 2006). The economic situation in a developing country like Nigeria has been adversely affected by post- harvest losses of agricultural products which are usually encountered especially during storage arising from pest and other spoilage agents. Insect pest if not controlled could be a huge hindrance to a profitable, efficient production and storage of grain. The efficient control and removal of stored grain pests from food commodities has long been the goal of entomologists throughout the world (Adedire, 2003). Synthetic pesticides are the major tools for crop protection in developing countries and their continuous usage raises a lot of concern such as insects developing resistance to pesticides, toxic residues on grains, handling hazards, health hazards to operatives and pest resurgence (Obeng-Ofori and Reichmuth, 1997). Therefore, there is the need to develop measures of tackling these problems especially that of pest resistance to pesticides which is a growing problem in stored product protection. Resistance can develop when the same pesticide or similar ones with the same mode of action are used over and over again without achieving the aim of control. It is often thought that pests change from one form to another or undergo mutation in order to become resistant, but it is not the individual pest that changes, but the population (Peng *et al*., 2003). A resistant population begins with as little as one resistant pest and overtime, the number of resistant individuals' increases until the entire population is resistant to the pesticide which hitherto was used to control them (Tabashnik, 2014). Resistance therefore is defined as a change in the sensitivity of a pest population to a pesticide, resulting in failure of a correct application of pesticides to control the pest (Schmutterer, 1990). The development by pest of acquired resistance or increased tolerance to pesticides is now a well-known pest management problem (Sharma and Meshram, 2006). Therefore, this study was conducted to screen three successive generations of *Sitophilus zeamais* and *Callosobruchus maculatus* (primary insect pest of stored maize and cowpea respectively) so as to determine whether there is resistance to pirimiphos methyl (synthetic insecticide) and neem leaf powder (botanical pesticide).

MATERIALS AND METHODS

Culturing of insects

Adults *S. zeamais* and *C. maculatus* were obtained from infested stock of maize and cowpea grains, respectively from Itam market, Akwa Ibom State, Nigeria. The insects were reared on whole maize and cowpea grains in 500 l glass jars containing 400 g of previously sterilized grains for seven days (Udo, 2000) to allow for oviposition. After this, the parent insects were sieved out using impact test sieve and the grains were allowed to stand for 28 days to enable the emergence of same age progeny representing the first filial generation which were used for the different bioassays in the first screening. Insects that survived the mortality test were then reared on whole grains again and allowed to oviposit into fresh sterilized grains for another seven days after which they were discarded. The emerging same age progeny representing the second filial generation were used to screen for mortality and progeny development as well as damage assessment (Epidi *et al*., 2009). Again, insects that survived in the mortality test were reared on whole previously sterilized grains for seven days to allow for oviposition after which the parent insects were discarded by sieving and freezing. Emerging progeny representing the third filial generation were used for the different bioassays.

Collection of plant materials

Neem (*Azadirachta indica*) leaves were collected from Uyo metropolis in Akwa Ibom State and shade dried for five days. The dry leaves were ground into fine powder using manual hand grinder and stored in black polyethylene bags in the laboratory prior to usage.

Procurement of synthetic insecticide

Actellic 2% dust which contains 2% pirimiphos methyl was purchased from Dara Investment Limited, an Agrochemical shop in Uyo, Akwa Ibom State, Nigeria.

Bioassays

Mortality test

This was conducted by measuring 100 g of maize and cowpea, respectively into white plastic cups and actellic dust and neem leaf powder added as direct admixtures at concentrations of 5% and 10% while the control was without any insecticide. Twenty adults each of *S. zeamais* and *C. maculatus* of mixed sexes, respectively were introduced into treated and control grains. The experiment was laid out using complete randomized design and replicated four times. The plastic cups (200 ml size) were covered with white muslin cloth and held in place with rubber bands. Insect mortality was recorded after 24 hours and up to 96 hours while insects were considered dead on failure to respond to three probing using a blunt dissecting probe (Obeng-Ofori and Reichmuth, 1997). The same procedure was applied in the second and third screening tests.

Damage assessment

Maize and cowpea grains were treated with 5% and 10% of both actellic dust and neem leaf powder, respectively and ten pairs of each insect species were introduced into treated and untreated grains. The untreated grains without any insecticide added served as the control. Each treatment was covered with white muslin cloth and held in place with rubber bands and left to stand undisturbed for four weeks. Samples of 100 grains were taken from each cup and the number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed using weighing balance. Percent weight loss was calculated following the method of FAO (1985) as:

$$\%\ \text{weight loss} = \frac{[\text{UaN} - (\text{U} + \text{D})]}{\text{UaN}} \times 100$$

Where:

U = weight of undamaged fraction in the sample

N = total number of grains in the sample

Ua = average weight of one undamaged grain

D = weight of damaged fraction in the sample

This process was repeated for the second and third screening.

Progeny development

One hundred grams of maize and cowpea grains respectively, were measured into 200-ml plastic cups and 5% as well as 10% of actellic dust and neem leaf powder added. Controls were without insecticides and each treatment was replicated four times. The experiment was left to run undisturbed for five weeks (Epidi *et al*., 2009). The number of insects that emerged was counted after 24 hours up to the 96 hours of the sixth week. The process was applied in the second and third screening involving the second and third filial generations.

RESULTS AND DISCUSSION

The result of insect mortality is shown in Table 1 and 2 where there was an increasing mortality throughout the duration of application with a significant mortality of over 80% and 60% was recorded by Pirimiphos methyl and neem leaf powder, respectively against the two insect pest species after 96 hours of application in the first generation. However, an increase in mortality (96%) was observed in Pirimiphos methyl against *C. maculatus* in the second generation but this dropped to 72% in the third generation. There was a declining mortality of *S. zeamais* through the generations screened with the F_1 recording a mortality of 70%, F_2 recorded a mortality of 62% and F_3 57%.

There was a declining mortality in both insects in the three generations screened when neem leaf powder was applied with mortality declining from over 50% in F_1 to 20% in F_3 for *S. zeamais* and 60% in F_1 to over 40% in F_3 for *C. maculatus* after 96 hours of exposure to treatment. The reduced mortality across the generations could be attributed to a possible development of resistance to insecticides by the insect species. This was observed by earlier researcher (Oparaeke and Kuhiep, 2006) that Pirimiphos methyl alongside other insecticides caused significant reduction in F_1 progeny at four weeks but at the F_2 stage, none of the treatments caused significant

suppression of *S. zeamais*. Applying treatment was better than the untreated control as treated grains were protected with significant mortality recorded on treated grains. The presence of active ingredients in the treatments was able to suppress insect population beyond the economic threshold level (Cassida, 1990).
There was also a reduction in damage (Table 3) recorded for the different insect species through the generations screened compared with the control. In the F_3 generation when neem was applied, 52% a percent weight loss was recorded as against 38% recorded from actellic dust. The damage observed was increasing with increasing generation as this proved reduced effectiveness of the insecticides. This probably is because of the resistance developed by the insects to the insecticides. As observed by Umoetok *et al*. (2013) insects acquiring resistance seem to be more damaging than the ones that could be controlled by insecticides.
Progeny development was more in untreated grains than the treated grains (Table 4). However, all through the three generations, progeny of the two insect species was on the increase and this is a pointer to reduced efficacy of the chemicals applied to protect the grains against successive insect generations. Because of inability of the insecticides to protect grains, there was a proliferation of the progeny developing from infested grains.

Table 1: Percent insect mortality of different generations using neem leaf powder at different times after treatment against *S. zeamais* and *C. maculatus*.

Neem (g)	Hours after treatment			
	24	48	72	96
F_1 Generation				
		S. zeamais		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	7.50 ± 0.29	22.50 ± 0.71	32.50 ± 0.41	42.50 ± 0.41
10	10.00 ± 0.71	27.50 ± 0.50	40.00 ± 0.50	53.75 ± 1.11
$LSD_{0.05}$	0.09	0.16	0.12	0.22
		C. maculatus		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	11.60 ± 0.25	24.40 ± 0.71	40.00 ± 0.50	54.60 ± 1.11
10	16.30 ± 0.71	30.20 ± 0.50	42.10 ± 0.41	60.40 ± 0.50
$LSD_{0.05}$	2.44	2.65	2.28	0.16
F_2 Generation				
		S. zeamais		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	2.50 ± 0.25	11.25 ± 0.25	16.25 ± 0.00	20.00 ± 0.48
10	7.50 ± 0.56	15.00 ± 0.41	21.25 ± 0.48	27.50 ± 0.95
$LSD_{0.05}$	0.08	0.69	0.09	0.21
		C. maculatus		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	12.80 ± 0.25	26.60 ± 0.95	40.20 ± 0.50	48.60 ± 1.11
10	14.00 ± 0.25	30.30 ± 0.48	44.60 ± 0.50	58.00 ± 0.41
$LSD_{0.05}$	2.15	2.54	2.84	3.19
F_3 Generation				
		S. zeamais		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	2.50 ± 0.29	7.50 ± 0.56	16.25 ± 0.00	10.00 ± 0.25
10	3.75 ± 0.48	11.25 ± 0.65	13.75 ± 0.29	20.00 ± 0.48
$LSD_{0.05}$	N.S.	N.S.	N.S.	N.S.
		C. maculatus		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	9.00 ± 0.25	18.30 ± 0.48	29.40 ± 0.58	38.70 ± 0.63
10	11.44 ± 0.65	22.20 ± 0.63	33.00 ± 0.41	44.40 ± 1.60
$LSD_{0.05}$	1.19	1.82	2.65	3.46

Okonkwo and Okoye (1996) reported the resistance of certain stored product pests to widely used food industry pesticide as reaching the highest level ever recorded in the USA. In another development, mutation resistance on stored product insect pests was reported from all over the world (Tyler and Boxall, 1982). The development of resistance in insects is worrisome when botanicals are considered because the resource poor farmers would have to contend with prohibitive cost of synthetic chemicals which must be applied in combination to arrest insect pest menace. This therefore calls for concerted efforts on the judicious use of pesticides both synthetic and botanical in order to slow down the development of resistance in storage insect pests.

Table 2: Insect mortality (%) of different generations using Pirimiphos methyl (actellic) at different times after treatment against *S. zeamais* and *C. maculatus*

	Hours after treatment			
Pirimiphos methyl (g)	24	48	72	96
F_1 Generation				
		S. zeamais		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	16.25 ± 0.61	45.00 ± 1.60	47.50 ± 1.90	50.00 ± 1.03
10	23.75 ± 0.63	47.50 ± 1.19	57.50 ± 1.19	70.00 ± 1.08
$LSD_{(0.05)}$	0.16	0.38	0.41	0.27
		C. maculatus		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	17.50 ± 0.75	31.80 ± 0.58	54.90 ± 1.19	77.10 ± 1.08
10	19.50 ± 0.61	36.50 ± 0.63	62.00 ± 0.50	84.50 ± 1.08
$LSD_{(0.05)}$	3.28	3.64	4.22	4.80
F_2 Generation				
		S. zeamais		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	12.50 ± 0.25	16.25 ± 0.99	21.25 ± 0.85	31.25 ± 0.58
10	22.50 ± 0.87	40.50 ± 0.50	51.75 ± 1.32	62.25 ± 0.50
$LSD_{(0.05)}$	0.16	0.18	0.29	0.14
		C. maculatus		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	20.50 ± 0.41	42.90 ± 0.50	63.90 ± 0.50	80.40 ± 1.08
10	24.30 ± 0.87	46.00 ± 0.41	74.00 ± 1.08	96.40 ± 0.41
$LSD_{(0.05)}$	5.23	4.87	3.46	2.26
F_3 Generation				
		S. zeamais		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	5.00 ± 0.41	11.25 ± 0.25	17.50 ± 0.25	22.50 ± 0.41
10	12.50 ± 1.30	27.50 ± 0.41	41.25 ± 0.75	57.50 ± 0.25
$LSD_{(0.05)}$	N.S.	0.09	0.15	0.09
		C. maculatus		
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
5	12.80 ± 1.30	26.40 ± 0.41	43.40 ± 0.75	57.70 ± 0.25
10	17.40 ± 0.25	39.10 ± 0.87	55.50 ± 0.25	72.90 ± 1.08
$LSD_{(0.05)}$	4.10	3.35	2.49	1.68

Table 3: Effect of actellic dust and neem leaf powder on damage caused by *S. zeamais* and *C. maculatus*

Treatment/ level (g)	Weight loss (%)					
	S. zeamais			*C. maculatus*		
	F_1	F_2	F_3	F_1	F_2	F_3
Neem						
0	42.00	57.23	62.41	52.00	74.30	77.98
5	38.12	40.79	44.21	51.42	54.73	58.63
10	32.71	37.20	39.48	41.15	48.67	52.16
$LSD_{0.05}$	1.31	1.07	1.25	1.81	1.36	2.43
Actellic dust						
0	47.24	52.41	60.87	66.68	72.53	78.62
5	28.40	31.37	35.23	30.75	37.84	41.34
10	20.08	22.43	28.92	29.30	34.26	38.72
$LSD_{0.05}$	0.95	1.39	2.36	2.54	1.56	7.40

CONCLUSION

The above work has demonstrated the possible development of resistance of storage insect pests to both synthetic and botanical insecticides. This trend is worrisome as stored insect pest would no longer respond to toxicants applied against them. Therefore, programmed application of insecticides should be carried out by experts where adequate government policies are in place and supported.

Table 4: Effect of neem leaf powder and actellic dust on progeny production by *S. zeamais* and *C. maculatus* in the three generations

Treatment/ level (g)	Progeny development					
	S. zeamais			*C. maculatus*		
	F_1	F_2	F_3	F_1	F_2	F_3
Neem						
0	1092.8	1105.6	2234.4	504.0	849.0	1176.0
5	781.4	844.8	1732.8	107.0	151.0	184.0
10	705.0	764.8	1682.8	101.0	148.0	180.0
$LSD_{0.05}$	25.26	N.S.	27.97	71.00	85.00	124.20
Actellic dust						
0	844.6	1076.0	2178.4	387.0	564.0	844.0
5	141.2	328.4	1002.8	116.0	138.0	174.0
10	59.0	147.6	578.6	111.0	137.0	171.0
$LSD_{0.05}$	25.35	20.37	13.91	29.00	37.26	102.99

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