Resistance of strains of rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) to pirimiphos methyl

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Abstract

Insecticidal resistance of five strains of *Sitophilus oryzae* (L.) to pirimiphos methyl (Actellic®) was investigated in the laboratory at 28 ± 2oC and 89 ± 5% relative humidity. Rice infested by *S. oryzae* was sampled from Nigerian stores in Akure (Ondo state), Ibadan (Oyo state), Ikotun (Lagos state) and Ilesha (Osun state) and a laboratory culture (in Akure). Twenty adult weevils from each location were exposed to filter papers impregnated with liquid pirimiphos methyl at one of seven concentrations ranging from 0.0 to 5.0%. Mortality rates of the rice weevils were observed at 3, 6 and 24 h after treatment. The mortality of weevils increased with increasing concentration and period of exposure. Irrespective of the strain, 100% mortality was observed in all the treatments at 24 h after exposure to pirimiphos methyl. Weevils sampled from Ibadan were the most resistant, while laboratory strains were the least resistant at 2 and 5% concentrations.

Keywords: Strains, insecticide, Pirimiphos methyl, Resistance, Sitophilus oryzae.

1. Introduction

Rice is an essential component of the diet of many people. It has been estimated that half the world's population subsists wholly or partly on rice (Grist, 1986). The bran of rice can be mixed with skimmed milk and used for feeding domestic animals. Broken rice grains are used for distillation of alcohols and in the production of laundry starch. Traditionally, rice grains are stored in granaries either in the threshed or unthreshed forms. These traditional methods expose stored grains to pest infestation.

Pest infestation in the field and during storage has greatly reduced the production of rice. In Nigeria, pest infestation plays a key role in keeping cereal production below the qualities and quantities demanded by an increasing human population and rapidly expanding livestock industry (Ogunwolu and Nwosu, 1987). The rice weevil, *Sitophilus oryzae* (L) (Coleloptera: Curculionidae) is a major cosmopolitan pest affecting stored rice in the world. A survey on the incidence of major pests of rice in different ecological areas of Nigeria showed, in general, that the amount of damage caused by *S. oryzae* was high (Soto et al., 1976). Both the larval and adult stages of this insect devour the kernel, causing weight losses.

Different methods (orthodox and traditional) have been developed and used to control infestation by rice weevil. Planting of resistant varieties have been reported to be effective in the control of this insect pest (Stoll, 1986). Enobakhare and Wey (1996) reported that resistant rice varieties ITA 306 and American Lamount inhibited to S. oryzae infestation. Treatment of grains with wood ash or rice husk ash have been suggested and used with some degrees of success (Stoll, 1986). Also, oils from plant sources have been reported to be toxic to S. oryzae, in addition to adversely affecting oviposition and weevil development (Su et al., 1972, Sighamony, 1986).

The most important and commonly used control measure is chemical control. This involves the use of synthetic chemicals such as pirimiphos methyl (Actellic®), permethrin (Coopex®), etc. which have been found effective in controlling S. oryzae (Wilkin et al., 1999). Although the use of synthetic chemical insecticides is a powerful tool against insect pests, indiscriminate use of different conventional insecticides by farmers and marketers has led to the development of resistance and a resurgence in some insect pests. For example, it was documented that S. oryzae showed resistance to deltamethrin (Ceruti and Lazzari, 2003). Ecological variations in the resistance status of different insect pests to diverse insecticides have been observed by various researchers (Jermannaud, 1994; Shelton et al., 2000; Pereira et al., 2006). Insecticide resistance and the consequent losses of food arising from failure of chemicals to control pests, have caused economic losses of several billion dollars worldwide each year (Elzen and Hardee, 2003).

This study was carried out to determine how geographical variation has influenced the resistance of *S. oryzae* to liquid pirimiphos methyl (Actellic®) in south western Nigeria.

2. Materials and methods

2.1. Insect culture

Sitophilus oryzae was originally obtained from naturally infested rice sampled from retail stores in different locations such as Akure (Ondo state), Ibadan (Oyo state), Ikotun, (Lagos state) and Ilesha (Osun state), Nigeria. All insects used were reared and studied in the laboratory at 28 ± 2oC, 70-75% relative humidity and a photoperiod of 14h:10h (L:D).those used as a standard were also obtained from established laboratory cultures. The laboratory cultures of S. oryzae had been maintained for more than nine generations on whole rice grain and these weevils were not exposed to insecticides in the laboratory. The weevils were reared to F1 generation on clean disinfested rice grain. The rice was disinfested by placing it inside a deep freezer for 72 h, then warming to ambient temperature before use. The insects were reared inside plastic containers (0.5 L) covered with muslin cloth and kept inside insect cages.

2.2. Synthetic chemical insecticide

The insecticide, pirimiphos methyl (Actellic® 25EC) used for the study was bought from a chemical store in Arakale, Akure. Graded acetone was used as diluent for the insecticide. Six different concentrations of pirimiphos methyl were prepared: 0.1, 0.2, 0.5, 1.0, 2.0 and 5.0% while acetone only (0.0%) served as the control treatment. To prevent evaporation of acetone, each concentration was prepared in a tightly covered reagent bottle.

2.3. Experimental procedure

An impregnated filter paper technique was used for the bioassay. Filter papers of 11-cm diameter were treated with varying concentrations of the insecticides described earlier and placed in the middle of a 9 cm-diameter Petri dish. For each treatment, there were three replicates. 0.5 mL of each concentration was applied to a filter paper and allowed to air-dry for 4 min. Twenty unsexed adult *S. oryzae* were released onto the treated filter paper and the Petri dish was covered. Weevil mortality was observed at 3, 6 and 24 h after treatment. Weevils were considered dead when they did not move after their abdomen was pricked with a sharp object.

2.4. Morphometrics of S. oryzae

Adult *S. oryzae* obtained from different locations were identified following Halstead (1963). Ten pairs of adult weevils were randomly selected from each location together with those from the laboratory and their length was measured under a microscope with a graticule in one eyepiece. A further 10 female weevils was obtained as described above and weighed to two decimal places. This was replicated three times.

2.5. Statistical analysis

Data obtained from morphometric evaluations were subjected to one-way analysis of variance (ANOVA) while mortality was subjected to two-way ANOVA using dose and location as factors to test for differences of mortality rates within and between locations. Where ANOVA results were significant means were separated using the Tukey's test.

3. Results

3.1. Response of S. oryzae obtained from different locations to different concentrations of pirimiphos methyl

Tables 1-3 show that the percentage mortality of S. oryzae obtained from the study varied with respect to the concentrations of pirimiphos methyl and the duration of exposure. At 3 h after treatment, there was no significant difference ($F_{6, 14} = 2.047$, $P = 0.073$) in the mortality of S. oryzae at the various insecticide concentrations. At 1.0% concentration there was significant difference ($F_{6, 14} = 167.72$, $P < 0.001$) from the control in the mortality of *S. oryzae* from Ikotun and laboratory cultures (Table 1). And at highest concentration (5.0%) of insecticide after 3 h of exposure, mortality of *S. oryzae* from laboratory cultures was highest (51.65%), while the lowest mortality (26.65%) at this concentration and exposure period was observed in weevils from Ibadan.

Table 1 Effect of pirimiphos methyl at 3 h after treatment on the percentage mortality (Mean ± S.E.) of *Sitophilus oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures.

Conc (%)	Akure	Ibadan	Ikotun	Ilesha	Lab. culture
0.0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
0.1	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
0.2	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	1.65 ± 0.33^{aA}
0.5	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	6.65 ± 0.33^{aB}
1.0	1.65 ± 0.33^{aA}	5.00 ± 0.58^{aA}	18.35 ± 0.33^{bB}	1.65 ± 0.33^{aA}	25.00 ± 0.58^{bB}
2.0	35.00 ± 0.58^{bB}	25.00 ± 0.58^{bA}	21.65 ± 0.33^{bA}	35.00 ± 0.58^{bB}	35.00 ± 0.58^{cB}
5.0	41.65 ± 0.33^{cC}	26.65 ± 0.33^{bA}	31.65 ± 0.33^{cA}	40.00±0.58^{bBC}	51.65 ± 0.58^{dD}

Each value is a percentage mean of triplicate samples ± standard error of the mean. Mean values followed by the same lower-case letter within a column are not significantly different at $P<0.05$ by Tukey's multiple range test. Mean values followed by the same upper-case letter within a row are not significantly different at $P<0.05$ by Tukey's multiple range test.

Pirimiphos methyl on *S. oryzae* mortality after 6 h after exposure is shown in Table 2. Except for weevils from laboratory cultures with 16.65% mortality at 0.1% concentration, the mortality of all weevils at this concentration was not significantly different (F6, 14 =2.194, P=0.169) from the control. However, at 1.0% concentration, 100% and 70% mortality was observed in weevils from laboratory cultures and Ibadan, respectively. There was no significant difference between the mortality of *S. oryzae* treated with 2.0% and 5.0% insecticide concentration.

Table 2 Effect of pirimiphos methyl at 6 h after treatment on the percentage mortality (Mean ± S.E.) of *Sitophilus oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures.

Conc (%)	Akure	Ibadan	Ikotun	Ilesha	Lab. culture
0.0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
0.1	6.65 ± 0.88^{aA}	3.35 ± 0.33^{aA}	1.65 ± 0.33^{aA}	1.65 ± 0.33^{aA}	16.65± 0.33^{bB}
0.2	33.35± 0.33^{bAB}	26.65± 0.88^{bA}	36.65± 0.67^{bB}	31.65±0.33^{bAB}	45.35± 0.33^{cC}
0.5	66.65 ± 0.67^{cB}	51.65± 0.67^{cA}	75.00± 0.58^{cC}	66.65 ± 0.58^{cB}	81.65± 0.33^{dC}
1.0	75.00 ± 1.00^{cB}	70.00± 0.88^{dA}	90.00± 0.58^{dC}	76.65 ± 0.33^{dB}	100.00±0.00^{eD}
2.0	98.35 ± 0.33^{dB}	83.35± 0.67^{dA}	100.00±0.00^{eB}	95.00 ± 0.58^{eB}	100.00±0.00^{eB}
5.0	100.00± 0.00^{dB}	86.65± 0.33^{dA}	100.00±0.00^{eB}	100.00±0.00^{eB}	100.00±0.00^{eB}

Each value is a percentage mean of triplicate samples ± standard error of the mean. Mean values followed by the same lower-case letter within a column are not significantly different at $P<0.05$ by Tukey's test. Mean values followed by the same upper-case letter within a row are not significantly different at $P<0.05$ by Tukey's test.

After 24-h exposure periods to pirimiphos methyl at the varying concentrations tested, there was 100% mortality of S. oryzae irrespective of their source and this was significantly different (P>0.05) from the control where no mortality was recorded (Table 3).

Table 3 Effect of pirimiphos methyl at 24 h after treatment on the percentage mortality (Mean ± S.E.) of *Sitophilus oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures.

Conc (%)	Akure	Ibadan	Ikotun	Ilesha	Lab. culture
0.0	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}	0.00 ± 0.00^{aA}
0.1	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}
0.2	100.00± 0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}
0.5	100.00± 0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}
1.0	100.00± 0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}
2.0	100.00± 0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}
5.0	100.00± 0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}	100.00±0.00^{bA}

Each value is a percentage mean of triplicate samples ± standard error of the mean. Mean values followed by the same lower-case letter within a column are not significantly different at $P<0.05$ by Tukey's test. Mean values followed by the same upper-case letter within a row are not significantly different at $P<0.05$ by Tukey's test.

3.2. Comparison of the mortality of S. oryzae from different locations

There was no mortality of *S. oryzae* obtained from all locations under study (except laboratory samples) after 3 h of exposure to pirimiphos methyl at 0.1-0.5% concentration. However, at the highest concentration (5%) tested, the mortality rate of S. oryzae from Ibadan was the lowest and significantly different ($F_{5,\ 10}$= 62.89, P<0.001) from that of other locations (Table 1). After 6 h of exposure to 0.1% pirimiphos methyl, mortality rate of S oryzae from laboratory cultures was significantly different from other locations. And at 2 and 5% concentration, mortality rates (83.35 ± 0.67 and 86.65 ± 0.33, respectively) observed in S. oryzae from Ibadan was significantly different ($F_{5,\ 10}$ =114.38, P<0.001) from other locations (Table 2). At 24 h after treatment, 100% mortality rate was observed in weevils across all locations (Table 3).

3.3. Morphometrics of S. oryzae obtained from four geographical locations

The morphometrics of *S. oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures is shown in Table 4. Result showed that there was no significant difference ($F_{4,10}$ = 2.874, P=0.251) in the length and weight of weevils from all locations sampled. Although both male and female weevils from the laboratory cultures appeared to be slightly longer, this apparent difference was not statistically significant.

Table 4 The morphometrics of *Sitophilus oryzae* obtained from four geographical locations in Nigeria and from laboratory cultures.

Source of weevil	Length (mm)		Weight (g)	
	Male	Female	Male	Female
Akure	2.88 ± 0.34	3.20 ± 0.35	0.01 ± 0.00	0.02 ± 0.00
Ibadan	2.75 ± 0.35	3.15 ± 0.41	0.01 ± 0.00	0.02 ± 0.00
Ikotun	2.85 ± 0.41	3.20 ± 0.48	0.01 ± 0.00	0.02 ± 0.00
Ilesha	2.80 ± 0.35	3.20 ± 0.42	0.01 ± 0.00	0.02 ± 0.00
Lab. culture	2.90 ± 0.39	3.25 ± 0.26	0.01 ± 0.00	0.02 ± 0.00

Each value is a percentage mean of triplicate samples ± standard error of the mean.

4. Discussion

The results from this study showed that adult *S. oryzae* mortality was modulated by various concentrations of pirimiphos methyl and by the period of exposure. Rice weevils from Ibadan are the most resistant to pirimiphos methyl at higher concentration and longer exposure time. Those from Akure and Ilesha are the least resistant while weevils from the laboratory cultures are the most susceptible.

Insecticide resistance of some storage pests to certain synthetic chemical insecticides has been reported. Rahim and Ong (1991), from a survey on resistance carried out in Malaysia in 1985 reported that *Sitophilus* spp., *Tribolium castaneum* (Herbst) (Coleloptera: Tenebrionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) had shown resistance to malathion and methyl bromide. In addition, it was reported by Suleiman et al (1994) that four major grain beetles: *S. oryzae*, *S. zeamais* (Motschulsky), *T. castaneum*, and *R. dominica* collected from 30 locations in Malaysia showed some levels of resistance to phosphine and methyl bromide, with resistance more pronounced in *S. oryzae* and *T. castaneum* than other insects evaluated. Ceruti and Lazzari (2003) when using molecular markers to detect insecticide resistance in stored-product beetles reported that *S. oryzae* was more resistant compared to other beetles tested.

The differences in the resistant status of *S. oryzae* are influenced by different factors. Ecological differences in the resistance status of *S. oryzae* to pirimiphos methyl in this study was in agreement with the ecological response reported for some field and stored-product insect pests to insecticides. Jermannaud (1994) observed that different levels of resistance exist among *S. zeamais* obtained from five different locations in Ghana. He also reported that S. zeamais from seven different locations in Zimbabwe show high resistance to pirimiphos methyl. Fragoso et al. (2002) also reported that insect population of the coffee leaf miner *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) obtained from 10 sites show different levels of resistance to chemical insecticides. Perez et al. (2000) tested the resistance of field population of *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), *Helicoverpa* spp. (Lepidoptera: Novtuidae), and *Bemisia tabaci* (Gemadius) (Homoptera: Aleyrodidae) obtained from six different sites from Nicaragua to different insecticides and observed that these insects show different levels of resistance to different insecticides. Perez-Mendoza (1999) observed that *S. zeamais* collected from field had moderate resistance to DDT, moderate to high level of resistance to lindane, low to high level of resistance to primiphos methyl.

Rice weevils obtained from Ibadan proved to have the highest level of insecticide resistance to pirimiphos methyl. Ibadan has a tropical climate and falls within savannah forest, which is characterized by high temperature throughout the year. Temperature affects the activity of insects, therefore, the population growth rate of the pest is always high throughout the year (Lale and Ofuya, 2001). Ibadan also has a large human population and there are many farms and markets within and around the metropolis. More insecticides might be used in the region to treat produce. Although pirimiphos methyl is not normally used directly on rice, the walls and storage pallets in rice stores are treated. It is also used to treat produce like wheat, cowpea and maize which are marketed together with rice and S. oryzae can cross infest from treated produce and develop resistance to pirimiphos methyl. Fragoso et al. (2002) and Pereira et al. (2006) attributed the variation in the resistance of insect from different locations to greater use of insecticides and usage pattern in those locations.

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