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Resistance of *Callosobruchus maculatus* (Fab.) to Pirimiphos methyl in Three Zones in Nigeria

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Abstract

The influence of climate on the resistance of *Callosobruchus maculatus* Fab. to pirimiphos methyl from three Geographical Zones in Nigeria was investigated in the laboratory at 32 ± 2 °C and 70 ± 5 % relative humidity. Samples of cowpea seeds infested with *C. maculatus* were obtained from twelve locations spread across savannah forest (northern boundary), rain forest and mangrove forest (southern boundary). Survival rates of 20 bruchids from each location were observed for two hours using 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml of pirimiphos methyl (Actellic® 25EC). Two age groups of the bruchids (1-day old and 7-day old adults) were treated separately to study the age influence on their resistance and samples were replicated three times. Low survival rates were observed in both age groups of *C. maculatus* from all the locations when treated with 0.4 ml and 0.5 ml of pirimiphos methyl. The 7- day old adults showed resistance to 0.3 ml treatment while the 1-day old adults had low survival rate at this treatment. Resistance in 1-day old *C. maculatus* increased gradually in samples from the mangrove forest and peaked at the edge of the rain forest before gradually decreasing towards the savannah forest at 0.3 ml treatment. Seven-day-old adults treated with 0.4 ml showed some level of resistance in two central locations within the rain forest when compared to all other locations. Incidentally these two locations fall within cities where awareness for the use of insecticide might be high.

Key words: Resistance, Pirimiphos methyl, *Callosobruchus maculatus*, three geographical zones.

Introduction

Grain legumes, especially cowpea, occupy a prominent place in the nutrition of Nigerian people because their edible seeds form a cheap alternative source of protein in their diets (Ofuya, 2001). In Nigeria, *Callosobruchus maculatus* (Fabricius) is the major primary pest of stored seeds of cowpea and it causes a wide spread damage which constitutes a major constraint on the availability of this legume (Ofuya and Credland, 1995). The control of this bruchid is largely dependent on different conventional insecticides that were being used indiscriminately by local farmers and marketers, an act that can lead to the evolvement of resistance in *C. maculatus*.

Insecticide resistance has developed over the years within many groups of pesticides and several species of insects (including stored product pests) are resistant to one or more insecticides. Insecticide resistance and the consequent losses of food caused by failure to control insect pest causes economic losses of

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several billion dollars worldwide each year (Elzen and Hardee, 2003)

Geographical variation in the resistance status of different insect pest to diverse insecticide has been observed by various researchers (Jermannaud, 1994; Shelton et al. 2000; Huang et al, 2004, Leontieva et al., 2006; Pereira et al., 2006). This study was carried out in the South West of Nigeria where the vegetation ranges from savannah forest (northern boundary), rain forest and mangrove forest (Southern boundary). The objective of this study was to determine the resistance of *C. maculatus* from these different geographical zones to pirimiphos methyl, one of the major insecticides used for its control in Nigeria.

Materials and methods

Insects

Adult cowpea bruchids, *Callosobruchus maculatus* (Fab.) were collected from infested cowpea seeds obtained from retail stores in twelve locations spread across three vegetational zones, viz; savannah forest, rain forest and mangrove forest (Table 1). Being the largest and widest area out of the three zones surveyed, more sampling points were located within the rain forest. The insects were reared to F_1 generations on clean uninfested cowpea seeds ("Sokoto white"cultivar) in Kilner jars at 32 ºC and 70 % RH in the Storage Research Laboratory of Federal University of Technology, Akure, Nigeria.

Insecticide

Pirimiphos methyl (Actellic® 25EC) used for the bioassay was obtained from an agricultural chemical sales center in Akure.

Bioassays

The bioassay used was the impregnated filter paper technique (Anonymous, 1974). The base of each experimental Petri-dish (8.5 cm diameter) was lined with a filter paper (2 mm pore size). A 1m1 syringe was then used to apply pirimiphos methyl to the filter paper before covering the dish. The undiluted treatment volumes used were 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml; untreated filter papers were also set up as control. Twenty unsexed adult bruchids were then released into each treated dish and their mortality was observed after 2 hours. This was done separately for all the samples. Two age groups (1- day and 7- days old) were treated separately and each test had three replicates.

Table 1. Sampling points of *Callosobruchus maculatus* distributed across three vegetational zones in Nigeria.

Sample	Vegetational Zone
Sample A	Savannah Forest
Sample B	Savannah Forest
Sample C	Savannah Forest
Sample D	Rain Forest
Sample E	Rain Forest
Sample F	Rain Forest
Sample G	Rain Forest
Sample H	Rain Forest
Sample I	Rain Forest
Sample J	Mangrove Forest
Sample K	Mangrove Forest
Sample L	Mangrove Forest

Data analysis

The data collected were subjected to analysis of variance (ANOVA) and the treatment means were separated using the New Duncan Multiple Range Test of window SPSS version 10 at $P \leq 0.05$. The LD_{50} of the treatments was also computed.

Results

The survival of *C. maculatus* from the different geographical areas in the various volumes of pirimiphos methyl used was significantly low ($P \leq 0.05$) at high volumes (0.4 and 0.5 ml). Though not accommodated in the response charts (Figures 1 and 2), both one-day and one-week old insects had total or nearly total death (0 to 1 survived) at 0.5 ml application.

The response of one-day old adult *C. maculatus* to pirimiphos methyl is shown on Figure 1. In all the samples from the geographical zones treated, there was a progressive decrease in the rate of survival as the volume of the insecticide increased. When 0.1 ml of the insecticide was applied, above 50 % of the insects from most of the sampled areas survived. The survival rate of the samples from the savannah forest increased towards the rain forest but there was a sharp decrease at the edge of the rainforest (Sample D) which gradually increased peaking at the boundary between the rainforest and the mangrove forest (Sample I) before a gradual decline of insect survival rate into the mangrove forest. The application of 0.3ml resulted in a sharp decline in the survival rate of the insects from all the zones especially those from the savannah forest. In most of the sampling points, less than 10 % survived this volume (0.3 ml) but a gradual increase in the survival rate was observed from the mangrove forest, which peaked at the edge of the rainforest. The sample from this peak point (Sample I) had the highest survival of over 5 insects, which shows some resistance to this volume (0.3 ml) when compared to the other sampling points from within and without the rainforest. Generally, there was no significant difference ($P \geq 0.05$) in the low survival rate of the insects from all the sampling point at 0.3, 0.4 and 0.5 ml.

The response of one-week old *C. maculatus* to pirimiphos methyl (Figure 2) shows a higher survival rate at 0.1 ml. Unlike the one-day old, 0.3 ml application revealed 50 % and above survival rate of the one week old insects for many of the sampled points, although the same survival pattern was observed along the geographical zone as in one-day old insect response. Application of 0.4 ml of the insecticide had a high lethal rate on the one-week old insects with their survival

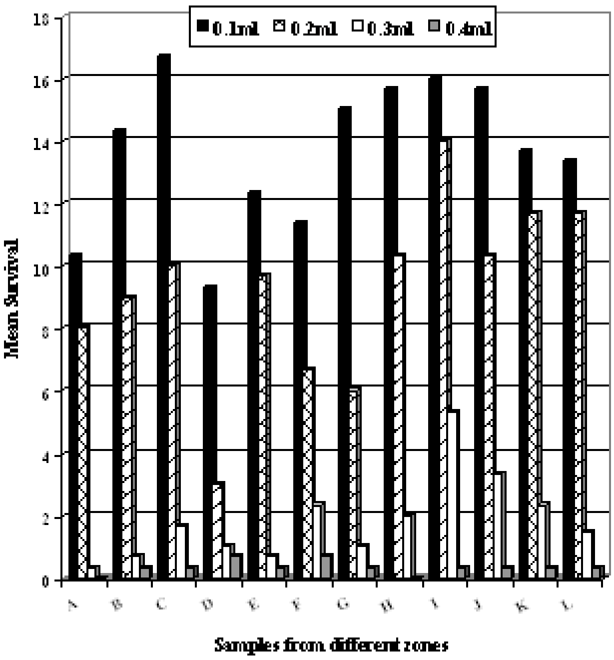

Figure 1. Response of one day old adult C. maculatus from different geographical zones to pirimiphos methyl

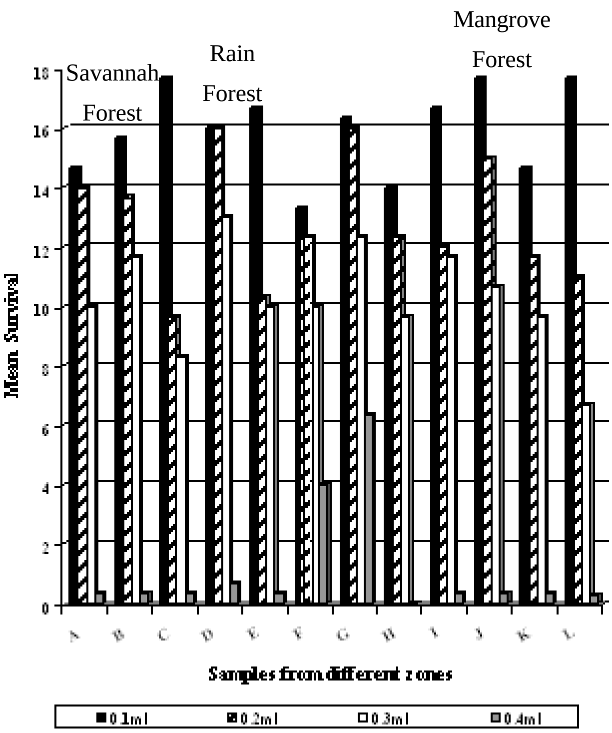

Figure 2. Response of one week old adult C. maculatus from different geographical zones to pirimiphos methyl.

rate being significantly lower than those of 0.3 ml application, but not significantly different from those of 0.5 ml application except in two samples (Samples F and G) at the middle of the rain forest. Sample G had the highest mean survival rate (6.33) followed by sample F (4.00) with those of the other samples ranging between 0 to 0.67 mean insect survival.

The LD_{50} chart (Figure 3) clearly reveals the trend in the level of susceptibility/resistance of both experimental ages of *C. maculatus* from the three zones to pirimiphos methyl. The range of the LD_{50} value for the one-day old insects was narrowest in the mangrove forest (3.17-3.52 µL/mm^2), which is higher than those of many sampling points within the savannah and rainforest. The widest range (0.88-4.23 µL/mm^2) and highest LD_{50} value falls within the rainforest. The one-week old insects have higher LD_{50} values than one-day old insects with most of the sampling points having values between 4.05 to 4.93 µL/mm^2 , which shows a narrower range

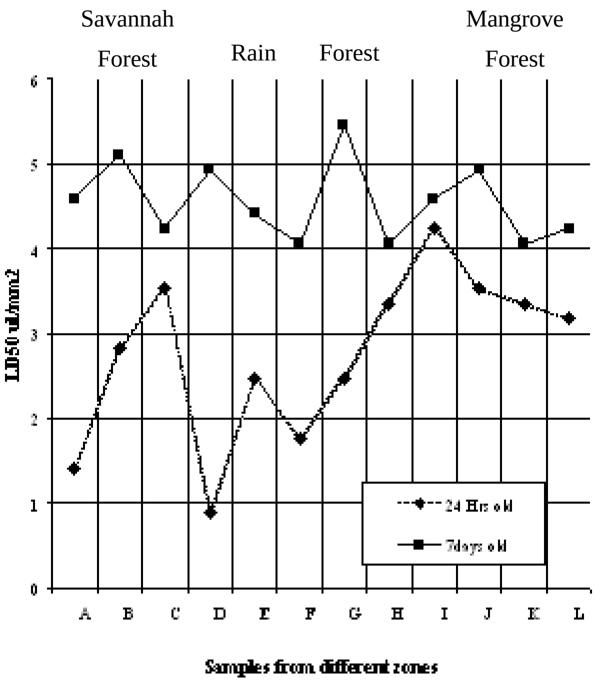

Figure 3. LD_{50} values for pirimiphos methyl used for the treatment of adult C. maculatus from three geographical zones.

* LD_{50} value is in mL/mm^2 of filter paper

than those of the one-day old. Only sample B (savannah forest) and Sample G (rainforest) have LD_{50} values higher than 5.00 µL/mm^2 which is an indication of higher tolerance of pirimiphos methyl in the insects from these two sampled points.

Discussion

The influence of insect age on resistance level in *C. maculatus* was clearly revealed in this study as the one-week old insects have higher LD_{50} values than their corresponding one-day old insects in all the sampling points. Sclerotization of insect cuticle increases with age whereby the cuticles becomes hardened and darkened having additional waxy layers, hence the integument becomes more resistant to adverse environmental stimuli such as chemicals (Richard and Davies, 1977). The one-day old group of Sample I at the edge of the rainforest and mangrove forest had a high LD_{50} (4.23 µl/mm^2) that was close to that of its corresponding one-week old group (4.58 µl/mm^2). The LD_{50} of the one-day old group of this strain was even equal or higher than those of some one-week old insect strains across the three geographical areas surveyed. This could mean that a high resistance to pirimiphos methyl is innate in this strain, which is, already conferred from the parent, because age has little effect on the insects from this sample point. This could be as a result of physiological resistance which is conferred either through modification in composition or structure of the cuticle which decreases penetration of the insecticide or by the alteration of cholinesterases so that they are not affected by the organophosphate used (pirimiphos methyl) (Pedigo and Rice, 2006).

In tropical climates, high temperatures prevail throughout the year and these high temperatures speed up biological activity of storage pests, however because the climate is more equable throughout the year in the humid zones, the rate of population growth of pests is also high throughout the year (Lale and Ofuya, 2001). But the drier zones have wide seasonal fluctuations in temperature and humidity, hence, fluctuating insect population. The above-expatiated reason could be linked to the narrow range of LD_{50} within the more (constantly) humid mangrove forest than the other two zones with lower (fluctuating) humidity. Also at high temperature and high humidity insecticides are broken down quickly (Odeyemi and Daramola, 2000) and this will constantly increase the lethal dose of pirimiphos methyl in the mangrove forest, hence reducing the susceptibility of insect strains from this zone.

The influence of geographical difference in the resistance status of *C. maculatus* to pirimiphos methyl was observed in this study. This observation is in agreement with geographical response to insecticide reported in some stored product insect pests and field crop insects. Jermannaud (1994) observed that *Sitophilus zeamais* from five different locations in Ghana show some evidence of resistance to pirimiphos methyl in one of the locations (68 % mortality) while some show no resistance (100 % mortality). Likewise, he observed that *S. zeamais* from seven different locations in Zimbabwe show high resistance to pirimiphos methyl but to varying degrees, while a location revealed as low as 2 % mortality, another have as much as 45 % weevil mortality. Leontieva et al. (2006) likewise reported a wide spread resistance by Colorado potato beetles (CPB), *Leptinotarsa decemlineata* Say, to organophosphorus insecticides in Russia. They reported that some local populations have up to 90 % resistance phenotype to decis (0.01 %) while others contain less than 10 %. Pereira et al. (2006) reported that no resistance was observed against Delfin (*B. thuringiensis* based product) in a population (Hassan) of Diamondback moth (DBM), *Plutella xylostella* L., in India, but two other Indian populations (Malur and Belgaum) of this cruciferous vegetables pest showed high levels of resistance to this insecticide.

Sample G and Sample I proved to have the highest LD_{50} values for one-week old and one-day old age groups of *C. maculatus* respectively. These two sampling points fall within the rainforest and they have higher human populace than the other sampling points surveyed. More insecticides are being used in these points mostly by (illiterate and untrained) grain storekeeper/ marketers and pesticide sellers. This is because a large tonnage of cowpea is being handled and treated indiscriminately due to its high demand within these sampling points and this could account for the high LD_{50} values from these points since virtually no prior reports are available on the resistance of *C. maculatus* to pirimiphos methyl. Fragoso et al. (2002) and Pereira et al. (2006) attributed the variations in the resistance of insects from different locations to greater use of insecticide and usage pattern in those locations. Local appearance of resistant CPB micro population was noted by Leontieva et al. (2006), this is similar to these two populations of *C. maculatus,* which falls within the wide rainforest.

The baseline data generated in this study can be used to manage the variability in resistance of *C. maculatus* to pirimiphos methyl within the savannah forest, rainforest and mangrove forest areas in Nigeria. Quick application of insecticide treatment at the onset of infestation will reduce the lethal dose required to stop the infestation by *C. maculatus* since the one-day old age groups have lower LD_{50} values than the one-week old age groups. The application rates within the rainforest should be carefully calibrated so that the lethal dose can be deposited on the grains (Huang et al., 2004) and also trained pest management personnel should be involved in order to minimize the springing up of resistant micro populations within this zone.

References

Anonymous,1974. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. FAO Method No. 15. FAO Plant Protection Bulletin 22, 127-137.

Elzen, G.W., Hardee, D.D., 2003. United States Department of Agricultural-Agricultural Research Service research on managing insect resistance to insecticides. Pest Manag. Sci. 59, 770-776.

Fragoso, D.D., Guedes, R.N., Guedes, R.N., Picanco, M.C., Zambolim, L., 2002. Insecticide use and organophosphate resistance in coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). Bull. Entomol. Res. 92, 203-212.

Huang, F., Subramanyam, B., Toews, M.D., 2004. Susceptibility of laboratory and field strains of four stored-product insect species to spinosad. J. Econ. Entomol. 97, 2154-2159.

Jarmannaud, A., 1994. Field evaluation of a test kit for monitoring insecticide resistance in stored-grain pests. Proceedings of the 6th International Working Conference on Stored-product Protection, 2, 795-797.

Lale, N.E.S., Ofuya, T.I., 2001. Overview of pest problems and control in the tropical storage environment. In: Ofuya T.I. and Lale N.E.S.(eds) Pests of Stored Cereals and Pulses in Nigeria. Dave Collins Publications, Nigeria. pp 1-23.

Leontieva,T.L., Benkovskaya, G.V, Udalov, M.B., Poscryakov, A.V., 2006. Insecticide resistance level in *Leptinotarsa decemlineata* Say population in the South Ural. Resist. Pest Mgmt. 15, 25-26.

Odeyemi, O.O., Daramola, A.M., 2000. Storage practices in the tropics. Volume 1: Food storage and pest problems (1st ed.). Dave Collins Publications, Nigeria. 253pp.

Ofuya, T.I., 2001. Biology, ecology and control of insect pests of stored food legumes in Nigeria. In: Ofuya T.I. and Lale N.E.S.(eds) Pests of Stored Cereals and Pulses in Nigeria. Dave Collins Publications, Nigeria. Pp 23-58.

Ofuya, T.I. and Credland P.F., 1995. Responses of three populations of the seed beetle, *Callosobruchus maculatus* (F.)(Coleoptera: Bruchidae) to seed resistance in selected varieties of cowpea, *Vigna unguiculata* (L.) Walpers. Journal of Stored Products Research 31, 17-27

Pedigo, L.P. and Rice, M.E., 2006. Entomology and pest management. 5th edition. Pearson Prentice hall, Upper Saddle River, New Jersey. p. 563.

Pereira, S.G., Sannaveerappanavar, V.T. and Murthy, M.S., 2006. Geographical variation in the susceptibility of Diamondback Moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) to *Bacillus thuringiensis* products and acylurea compounds. Resist. Pest. Mgmt., 15, 26-28.

Richard, O.W. and Davies, R.G., 1977. Imm's general textbook of entomology. Volume 1: Structure, physiology and development. 10th edition. Chapman and hall, London. p.12.

Shelton, A.M., Sances, F.V., Hawley, J., Tang, T.D., Boune, M., Jungers, D., Collins, H.L., Farias, J., 2000. Assessment of unsecticide resistance after the outbreak of diamondback moth (Lepidoptera: Plutellidae) in California in 1997. J. Econ. Entomol. 93, 931-936