Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.4, 2013


# Evaluation of SpinTor™ Dust in the Protection of dried *Tilapia niloticus* against *Dermestes maculatus* (De geer) (Coleoptera: Dermestidae)

*K. A. Kemabonta, W.A. Makanjuola and O. A. Omogunloye
Department of Zoology, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria.
* E mail of corresponding author: kennykemabonta@yahoo.com

**Abstract**
**Introduction**: *Dermestes maculatus* is a major pest of stored fish in Nigeria causing as high as 50% weight loss. Fishermen spray insecticides injudiciously which include Gamallin 20 which constitute danger to human health. SpinTor dust (Spinosad) is a commercially reduced-risk pesticide that is naturally derived from the fermentation from a soil bacterium, *Saccharopolyspora spinosa*. **Objectives:** No reference data on its efficacy in suppressing major insect pest of stored fish have been published. This paper therefore evaluated the efficacy and residual effect of SpinTor dust against *Dermestes maculatus* on dried Tilapia fish, *Tilapia nilotica*. **Methods:** Disinfested *Tilapia* was treated with 0.125, 0.25, and 0.5 percent Spintor dust. *D. maculatus* was introduced into containers holding 50g of untreated and treated fish. Residual effect of Spintor was evaluated at 30 and 60 days after treatment (DAT). **Results:** SpinTor dust was more toxic on adults *D. maculatus* with $LD_{50}$ of 2.338 than on the larvae with $LD_{50}$ of 2.693. Adult mortality was highest in the dried *Tilapia niloticus* treated 0.5% SpinTor dust and least in the control. No larva developed in 0.5% concentration while 629 larvae developed in the control. A significant higher number of F1 adults that emerged from (0.5% concentration) treated adults died when compared with all the other treatments and control. Histopathological test on the liver of mice showed no significant weight gain in mice fed on treated fish and the control after three months. The histopathological test of the liver of the control treated mice had no alterations in their hepatic lobes. **Conclusion:** Spintor dust can be used to protect dried fish against *D. maculatus*
**Key words** Toxicity, Spinosad, Dried fish, Mortality, $LD_{50}$, Residue, liver and Mice.

## Introduction

Fish is one of the most important staple foods on the planet. It is a rich source of proteins, amino-acids, vitamins, minerals and poly-unsaturated fatty acids not found in other sources of fat from the aquatic environment. Moreover, fish protein is known to be the best and cheapest source of animal protein (Olayide, 1973). In Nigeria, it constitutes 40% of animal protein intake by man. It's harvesting, handling, processing, storage and distribution provide livelihood for millions of people as well as providing valuable foreign exchange earnings (Al-Jubaili and Opara, 2006). Fish is a perishable commodity, especially in the tropics where ambient temperature is high. This brings about spoilage within 24hours of landing leading to post harvest losses. The need for effective processing of fish by drying is pertinent to avoid enormous losses and allow the fish get to the consumers in an acceptable condition (Okonta and Ekelemu, 2005).

Ayuba and Omeji (2006) reported that insect infestation is the cause of most prominent losses in quality and quantity of stored dried fish. *Dermestes maculatus* of the family dermestidae is a major pest of stored fish in Nigeria. Both larva and adult stages feed on dry animal tissue. The extent and value of quantitative losses caused by *Dermestes* spp. have been assessed by various authors (Azeza, 1976 and FAO, 1981). Osuji (1995) estimated a range from negligible amount, to 50% weight loss depending on length of storage, salt content, moisture content, climatic condition and general hygiene during processing. A lot of protectants have been used during drying, storage and transportation (Proctor1972, Osuji, 1973 and Khan and Khan 2001). During heavy infestation, the fishermen spray insecticides injudiciously which may be hazardous to human's health. Eyo (2001) reported Gamallin 20 and 'Otapiapia' as some of the highly toxic insecticides commonly used by fish folk to prevent insect infestation in Nigeria. Khan and Khan (2001) also reported that curers apply different types of insecticides such as dichlorvos, malathion, gamaxine, endrine and DDT on dried fishes to protect the dried fish from infestation, thus disobeying the recommendations made by Codex Alimentaris or FAO/ WHO Joint Meeting Pesticide Residue Committee (JMPRC). Since these insecticides constitute danger to human health, their use should be discouraged. It is therefore of necessity to search for a viable and environmentally safe protectant.

A naturally derived insecticide, SpinTor (Spinosad) (Dow Agrosciences LLC) belongs to a new generation of biorational products developed for agricultural industry that has a reduced spectrum of toxicity

compared with the synthetic insecticides that were previously developed. The active ingredient is derived from a naturally occurring soil dwelling bacterium called *Saccharopolyspora spinosa* (Bret et al; 1997; Sparks et al; 1998). SpinTor causes excitation of the insect nervous system, leading to involuntary muscle contractions, prostration with tremors, and finally paralysis. It has an extremely low toxicity for mammals and is therefore classified by U.S. Environmental Protection Agency as a reduced-risk material (Thomson et al; 2000). SpinTor (Spinosad) dust has been used to effectively control insect pests ( Bret et al., 1997, Crousse et al., 2001, Fang et al; 2002, Bond *et al.*, 2004 and Nayak et al., 2005). It was introduced into Nigeria in 2005 by Dow AgroSciences through SaroAgrosciences. Despite the effectiveness of SpinTor dust and its safety to man and beneficial organisms as reported by FIPS (2009) and salgado(1997, 1998), this insecticide has not been widely used in Nigeria.

Apart from the research effort on SpinTor dust by Anikwe *et al.*, 2009 against the kolanut weevil in storage, the authors are not aware of any evaluation of Spinosad on dried fish in Nigeria. It is against this background that this study was conducted to determine the efficacy and residual action of SpinTor dust against the dried fish insect pest, *Dermestes maculatus* De Geer (Fab). Mice feeding studies were also conducted to determine the magnitude of spinosad residues in animal products that would result from the consumption of fish containing residues of Spinosad.

## Materials and Methods

### Fish processing

Fresh Tilapia fish from Makoko market in Lagos State, Nigeria were properly washed in clean water, drained and then dried using local smoking kiln for six hours. The weights of the dried fish were determined immediately after drying and ranged from 9 -12g.

### Culture of *D. maculatus*

Forty copulating adult *Dermestes maculatus* collected from fish traders in Oyingbo market, Lagos were introduced into clean kilner jars containing disinfested dried Tilapia fish. A chunk of cotton wool damped in water was also introduced into each of the jars to serve as source of humidity, drinking water and as substratum for oviposition. The kilner jars were covered with muslin cloth and were secured with rubber bands to prevent escape of insects. The culture was observed for eggs laid by female adults and emergence of larvae. The larvae were separated from stock culture into new kilner jars containing disinfested dried fish where they emerged into adults. The 1$^{st}$ Filial generation was put into another set of kilner jars containing disinfested dried fish which was used to culture subsequent generations.

### The efficacy of SpinTor dust on *D. maculatus* infestation on dried fish

Seventy two (72) lots containing 50g of dried *T. niloticus* were weighed using Binatone weighing balance (Model ks-7020). These lots were further divided into 4 sets containing 18 lots each. Three of the 4 sets were treated separately with 0.125, 0.25, and 0.5% concentration of SpinTor dust (SD) respectively while the untreated 4$^{th}$ set served as the control. Six lots from each treatment were used at One-day-after treatment (1DAT), another six at 30DAT and the last six lots were used at 60DAT.

### Toxicity tests

### 1$^{st}$ Day after Treatment (1DAT)

Ten copulating *D. maculatus* adults (ages of 0-7 days, male to female ratio: 1:1 ) were introduced into the first lot and replicated three times. Ten fourth larval instars were also introduced into another lot and replicated three times. Each lot in a vial was covered with muslin cloth, strapped with rubber band to prevent escape of insect. The experiment was set up in a complete randomized block design. Mortality of adults and larvae of *D. maculatus* in the vials were noted at 24hours and thereafter, daily for 5days to determine the $LD_{50}$ values of SpinTor dust (SD) on *D. maculatus*. Mortality of larvae and adult *D. maculatus* were recorded after 35 days. The moisture content of the fish was determined at the onset of the experiment and at the end of the experiment. Weight loss of dried fish and weight of frass produced due to insect infestation were assessed after 35days. Number of larvae that emerged from treated *D. maculatus* adults were counted from the first lot and their replicates and the number of ($F_1$) adults - both dead and alive- that emerged from treated larvae. Also counted were the adults that emerged from eggs laid by the $F_1$ adults.

### Residual effect of SpinTor dust

Same procedure was repeated at 30 and 60 days after treatment (DAT). Fresh dried fish treated at the

beginning of the experiment, kept in Ziploc bags were used for these experiments. Each treatment including the control was replicated three times for adult and larvae of *D. maculatus.* All data were subjected to analysis of variance.

**Effect of SpinTor dust on Mice**

Twelve mice (*Mus musculus*), 3months of age were used for the experiment. Dried fish were weighed and treated with SpinTor dust at concentration of 0.125, 0.25 and 0.5%. A batch of untreated dried fish were also weighed to serve as control. The treated dried fish were stored in cartons.

Three mice in four sets were weighed separately into 4 different cages. The weight ranged from 52-58g. The first 3rd set of mice were fed daily with 20g of treated dried fish at 0.125, 0.25, and 0.5 % while the 4th set was fed with 20g of untreated fish and served as control. The mice were provided with 20cl of water daily. The weight of the three mice in each of the four separate cages was taken every 3 weeks for 3 months after which the experiment was terminated.

A mouse representing each concentration and the control was then placed in kilner jar containing cotton wool damped in chloroform in order to kill the mouse. Each mouse was placed on a board and pinned on the limbs for dissection. The livers of the mice were collected and placed in EDT bottles containing bouin's fluid and the samples were taken to the University of Ibadan Oyo State Nigeria for histopathological studies. The bouin's fluid was used to preserve the organs.

**Results**

**Bioassay determination of $LD_{50}$ of SpinTor dust on adults and larvae of *D. maculatus* in treated dried *Tilapia niloticus***

The $LD_{50}$ for adult *Dermestes maculatus* on dried fish was 2.338 while that for the larva was 2.693 (Table 1). Highest mortality (67%) of *Dermestes maculatus* was observed in fish treated with highest concentration (0.5%) while the least mortality (33%) was observed on fish treated with the least concentration (0.125%) of SD (Table2). The larvae that emerged from eggs laid by the adults at 1DAT increased significantly with decrease in concentration of SD. No larvae emerged and apparently, no eggs were laid by adults treated at 0.5% concentration as compared to 629 larvae on the control and 17 and 71 larvae that developed on 0.25 and 0.125% of SD treatment respectively. Weight loss and weight of frass produced by *D. maculatus* increased significantly with decrease in concentration of SD on dried fish (Table 2). The weight loss in fish and weight of frass produced on dried fish by *Dermestes maculatus* at 0.5% was significantly lower than at 0.25 and 0.125% SD.

At 1DAT, irrespective of the concentration used, over 80% of the treated larvae emerged into $F_1$ adults while all the larvae in the control, emerged into adults. There was no significant difference between the control and all treatments in the ($F_1$) adults that emerged from treated larvae (Table3). However, mortality of the emerged $F_1$ adults increased significantly as concentration of SD on dried fish increased and the $F_1$ adults in treated fish did not produce any progeny. Percentage weight loss in treated dried fish and mean weight of frass produced due to insect infestation increased significantly with decrease in concentration of SD. Moreover, the treated fish had direct contact with adult cuticle which absorbed more of the insecticide on contact. The larvae that developed however were significantly higher in the 0.25 and 0.125% concentration than 0.5% concentration where there was no larval development.

Same trend in mortality of adults, number of larvae that emerged, weight loss and weight of frass produced at 1DAT were also observed at 30 and 60 DAT. In all (1, 30, 60 DAT), the higher the level of infestation the more the weight loss and weight of frass produced by *D. maculatus* (Tables 4, - 7 )

**Effect on mice**

Table 8 shows the weight of mice fed with SpinTor treated *Tilapia* at concentrations of 0.5%, 0.25% and 0,125% respectively and the control. There was no obvious increase in weight in the mice that were fed with fish treated at 0.5%, while for the other concentrations; there was gradual increase in their weights. There was decrease in weight with increase in concentration of SpinTor treated fish. By the 12th week, 0.5, 0.25 and 0.125% and control mice had increased by 2, 7, 9 and 10g respectively.

**Discussion**

The results in the study showed that SD was effective in protecting dried *Tilapia niloticus* from *D.*

*maculatus* infestation at the 0.5% treatment while the 0.25 and 0.125% treatments were not as effective as 0.5% in preventing infestation of *D. maculatus* on dried *T. niloticus*. SD did not affect emergence of the larvae since over 90% of the introduced larvae in the treated fish successfully emerged into $F_1$ adults. However mortality of emerged $F_1$ adults was noted in all treatments and ranged from 67 to 33%. This might be due to the hairness of the larvae which prevented contact of the insecticide on the body surface of the larvae as compared to the adult with smooth cuticle. This finding is similar to that of Athanassiou et al; (2008) who found that adult *T. confusum* were more tolerant to Spinosad (SpinTor dust) than the larvae after 7days exposure on wheat treated with 150ppm SD. Mortality of the adult *T. confusum* was 81% while that for the larvae at same concentration was 40%. The effect of SD at 0.5% on *D. maculatus* was consistent through the months. It provided complete or near complete suppression of progeny production after 95days. The insecticide acts more as a contact insecticide than a stomach poison. In all cases, the higher the level of infestation, the higher the weight loss and the frass produced. This result is similar to the of Ames (1988). He reported that if *D. maculatus* are left undisturbed, they can consume all the flesh and soft tissue of dried fish until only bones and some hard tissues remain. This study also supported the report of Ayuba and Omeji (2006) that insect infestation is the cause of most prominent losses in quality and quantity of fish in Nigeria.

In laboratory studies, Spintor was reported to be highly stable and capable of causing a high prevalence of mortality for 1month after being applied to foliage or artificial surfaces (Bernardo and Viggiani, 2000). In the field, however, residues generally showed little toxicity at 3-7 days post application indicating that photolysis and rainfall quickly degrade or dilute Spinosad residues (Boyd and Boethel 1998, Crousse et al., 2001). Bond et al; (2004) in their study found Spinosad to be persistent in semi-natural field conditions for periods of 8 to >22wk depending on concentration. Moreover, applications of 1 and 5ppm Spinosad resulted in complete control of the mosquito, Ae*des aegypti*, development for 6 and 8wk respectively (Perez et al; 2007). In our study, Spintor (Spinosad) dust at 0.5% concentration suppressed adult *D. maculatus* emergence at 95DAT.

Histopathological tests showed that there was no lesion on the liver of the mice fed with SpinTor treated fish at the 0.125, 0.25% and control while 0.5% SpinTor dust brought about slight diffuse change in the liver which probably was the cause of the significant reduced weight.

Most studies earlier carried out on SpinTor dust on stored products have been on stored grains. This is indeed the first time SpinTor dust was being used on dried fish. Various authors have reported the safety of the insecticide on man. Rutherford et al; (2000) dosed dairy cows for 28 days with spinosad at rates equivalent to 0, 1, 3, and 10 µg/g in the diet. They also dosed chicken hens for 42 days with spinosad at rates equivalent to 0, 0.1, 0.3, 1, and 5 µg/g in the diet. Milk, eggs, and tissue samples were analyzed by high-performance liquid chromatography and/or immunoassay methods. Spinosad residues occurred in all of the sample types but were lowest in eggs, skim milk, and lean meat and were highest in the fat. Moreover, residues of Spinosad were highest in fat, lowest in muscle and intermediate in liver and kidney when sheep were treated with Spinosad at 14 DAT. The maximum residue of 0.2 mg/kg in peri-renal fat detected by Gao et al; (2007) was 20% of the Australian maximum residue limit. Muscle, liver and kidney residues of spinosad were also below the Australian maximum residue limits at all times tested. The residue level in this study was however not measured. Spintor dust can be a potential dried fish protectant for *D. maculatus* in an Integrated Pest Management of dried fish

**Acknowledgement**

Our acknowledgement goes to the management of Nigeria Stored Products of Research Institute, Abule –Oja, Lagos. Nigeria for the permission granted to carry out this research in their Institute.

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**Table 1.** $LD_{50}$ values for SpinTor dust on adults and larvae of *D. maculatus*

| | | | Upper limit | Lower limit |
|---|---|---|---|---|
| Adult | LD 95 | 14.007 | 46.495 | 6.692 |
| | LD 50 | 2.338 | 3.765 | 1.5 |
| | LD 5 | 0.39 | 0.761 | 0.135 |
| Larvae | LD 95 | 14.916 | 47.013 | 7.039 |
| | LD 50 | 2.693 | 4.284 | 1.744 |
| | LD 5 | 0.486 | 0.954 | 0.177 |

**Table 2.** Numbers of dead adults and developed larvae of *Dermestes maculatus* , % weight loss and frass weight of dried Tilapia treated at various concentrations with SpinTor dust for 30days.

| Treatments | Mean Adult mortality | Mean no. of larvae | Mean % weight loss | Mean weight of frass |
|---|---|---|---|---|
| 0.5% | 67c | 0.0a | 12.66a | 2.0a |
| 0.25% | 33b | 17.7b | 20.66b | 4.2b |
| 0.125% | 33b | 71.3c | 26.00b | 5.0b |
| Control | 27a | 629.0d | 62.00c | 11.0c |
| F cal. | *10.26 | *25.04 | *73.33 | |

Mean followed by the same letter along the vertical column are not significantly different at $p<0.05$
The mean difference is significant at the 0.05.

 

**Table 3.** Numbers of emerged adults and larvae of *Dermestes maculatus*, % weight loss and frass weight of dried Tilapia treated at 1DAT at different concentrations of SpinTor dust after 35days.

| Treatment | Mean of Emerged adults | % mortality of emerged $F_1$ adults | Mean number of larvae | % weight loss | |
|---|---|---|---|---|---|
| 0.5% | 83a | 67 | 0.0±0.0a | 14.00a | 0.3 |
| 0.25% | 87a | 33 | 0.0±0.0a | 16.74a | 0.5 |
| 0.125% | 93a | 33 | 0.0±0.0a | 21.34b | 1.0 |
| Control | 100a | 23 | 44.7b | 48.74c | 2.3 |

Mean followed by the same letter along the vertical column are not significantly different at $p<0.05$
The mean difference is significant at the 0.05.

**Table 4.** Numbers of dead adults and developed larvae of *Dermestes maculatus* , % weight loss and frass weight of treated dried Tilapia infested with adult *D. maculatus* 30DAT.

| Treatments | Mean adult mortality | Mean number of larvae | % weight loss | Mean weight of frass |
|---|---|---|---|---|
| 0.5% | 5.6c | 2.3a | 10.3 | 1.0 |
| 0.25% | 5.0c | 43.7b | 10.6a | 1.7 |
| 0.125% | 4.3b | 81.7c | 16.74b | 2.3 |
| Control | 3.3a | 497d | 50.0c | 5.3 |
| F cal. | *8.111 | *26.55 | *69.05 | |

Mean followed by the same letter along the vertical column are not significantly different at $p<0.05$
The mean difference is significant at the 0.05.

 

**Table 5.** Numbers of emerged adults and larvae of *Dermestes maculatus* , % weight loss and frass weight of treated dried Tilapia infested with larvae of *D. maculatus* 30DAT.

| Treatment | Mean of emerged Adults | Mean Mortality | Mean number of larvae | Mean % weight loss | Mean weight of frass |
|---|---|---|---|---|---|
| 0.5% | 5.0±0.82 | 3.3±0.87 | 0.0a | 16.7a | 1.0 |
| 0.25% | 6.0±0.82 | 2.9±0.94 | 0.0a | 21.0b | 2.0 |
| 0.125% | 6.3±1.25 | 2.7±0.47 | 16.3b | 26.7b | 2.3 |
| Control | 7.7±0.47 | 2.0±0.82 | 31.30c | 36.6c | 3.0 |
| F cal. | | | *6.862 | *12.70 | |

Mean followed by the same letter along the vertical column are not significantly different at $p<0.05$
The mean difference is significant at the 0.05.

**Table 6.** Numbers of dead adults and developed larvae of *Dermestes maculatus* , % weight loss and frass weight of treated dried Tilapia infested with *D. maculatus* 60days after storage

| Treatments | Mean adult mortality | Mean number of larvae | % weight loss | Mean weight of frass |
|---|---|---|---|---|
| 0.5% | 6.0±2.16c | 4.0±2.58a | 12.3a | 2 |
| 0.25% | 5.3±0.47b | 9.7±3.86b | 15.6a | 2.67 |
| 0.125% | 5.3±0.47b | 15.3±2.30c | 31.1b | 5.67 |
| Control | 2.7±1.70a | 154.0±9.89c | 54.4c | 8.67 |
| F cal. | 3.179 | *26.33 | *12.70 | |

Mean followed by the same letter along the vertical column are not significantly different at $p<0.05$
The mean difference is significant at the 0.05.

 

**Table 7.** Numbers of emerged adults and larvae of *Dermestes maculatus*, % weight loss and frass weight of treated dried Tilapia infested with larvae of *D. maculatus* 60days after storage.

| Treatment | Mean of emerged Adults | Mean mortality | Mean number of larvae | % weight loss | Mean weight of frass |
|---|---|---|---|---|---|
| 0.5% | 3.3±.25 | 0.0±0.00 | 2±1.41a | 6.7c | 1.0 |
| 0.25% | 4.7±0.59 | 1.3±1.25 | 4.0±2.94a | 10.3c | 1.2 |
| 0.125% | 8.3±0.94 | 2.7±1.25 | 15.3±4.5b | 23.3b | 1.8 |
| Control | 9.3±0.47 | 5.3±1.25 | 16±9.42b | 33.3a | 3.0 |
| F cal. | | | *26..33 | 13.65 | |

Mean followed by the same letter along the vertical column are not significantly different at $p<0.05$
The mean difference is significant at the 0.05.

**Table 8** Weight of mice at 3weeks interval fed with different concentrations of SpinTor treated Tilapia.

| Treatment- SpinTor dust | Average Initial weight (g) | Weight at 3wks (g) | Weight at 6wks (g) | Weight at 9wks (g) | Weight at 12wks (g) |
|---|---|---|---|---|---|
| 0.5% | 52 | 51 | 52 | 53 | 54 |
| 0.25% | 58 | 60 | 62 | 63 | 64 |
| 0.125% | 54 | 57 | 60 | 61 | 63 |
| Control | 53 | 56 | 60 | 61 | 63 |

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