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# The influence of post-exposure temperature on the toxicity of Spinosad against adults of *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae)

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## Abstract

Spinosad is a metabolite product of fermentation of soil actinomycete *Saccharopolyspora spinosa* with a mixture of macroliodes molecules spinosyn A and D. This biocontrol insecticide has a unique mode of action with a very low toxicity to mammalian. Cowpea Weevil, *Callosobruchus maculatus* (F.) is an important pest of legumes in the field and during storage period. In current research, effect of post-treatment temperature on the toxicity of a commercial formulation of Spinosad (Tracer24®) was determined against adults of *C. maculatus*. The experiments were conducted at four different temperatures vis. 20, 24, 28 and 32 °C and 65 ± 5 % RH under laboratory conditions. The adults were exposed to different dosages of Spinosad with 4 replications on filter paper inside Petri dishes. A control group was included in each experiment. Mortality was recorded 24,48 and 72 hours post-treatment. The $LC_{50}$ value was estimated using probit analysis with SPSS software. The $LC_{50}$ values at 20, 24, 28 and 32 °C at 24 hours post-treatment were 176, 149, 72 and 40 ppm, respectively. This criterion at 48 hours after treatment was 176, 149, 72 and 40 ppm in the same order. The $LC_{50}$ values at 72 hours post-treatment decreased at each temperature. A direct relationship between Spinosad dosages and post-treatment temperatures was detected. A field test using a similar design is essential to confirm the laboratory findings.

*Key words*: spinosad, pest control, temperature.

## Introduction

The Cowpea Weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae), is a world wide pest of *Vigna unguiculata* (Walp.), that infests both pods in the field and seeds in storage. Infestation level of Cowpea is very low at harvest and may sometimes be undetectable (Huignard et al., 1985). The Cowpea Weevil multiplies very fast in storage, giving rise to a new generation every month (Ouedraogo et al., 1996). These insects can damage 100 % of stored seeds, causing weight losses of up to 60 % (Kèita et al., 2000). Therefore, it is necessary to reduce such losses by controlling pests on stored grain (Tapondjou et al., 2002).

Spinosad is the first member of Dow Agro science's naturalyte class of insecticide and it is a mixture of tetra cyclic macrolide neurotoxins, spinosyn A and D. This compound is produced during the fermentation of the soil actinomycete *Saccaropolyspora spinosa*. It may be considered as a bioinsecticide (Copping and Menn, 2000). That is registered for use on › 100 crops in 24

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countries (Thompson et al., 2000). Spinosad is highly toxic to insects belonging to Lepidoptera, Diptera and some species of Coleoptera. This compound has a unique mode of action involving the postsynaptic nicotinic acetylcholine and GABA receptor (Watson, 2001). This microbial insecticide acts as a stomach and contact poison and degrades rapidly in the environment (Cisneros et al., 2002; Crouse et al., 2001). Spinosad has considerable contact toxicity against pest of stored-products (Toews and Subramanyan, 2003).

There are a number of factors that affects the toxicity of insecticide such as, temperature, exposure time, humidity and formulation. Temperature is one of the most important factors affecting biological processes in all living organisms (Amarasekare and Edelson, 2004) and it is also a major factor affecting insecticide toxicity (DeVrise and Georghiou, 1979). The effects of temperature on efficacy can be either positive or negative. The response relationship between temperature and efficacy has been found to vary depending on the mode of an insecticide, target species, method of application and quantity of insecticide ingested or contacted (Johnson, 1990). Musser and Shelton (2005) recorded negative temperature coefficients for spinosad against *Ostrinia nubilalis* but other research showed that toxicity of spinosad increased with increasing temperature at 15, 25 and 35 °C (Amarasekare and Edelson, 2004). The present study was conducted to determine the effects of exposure time and temperature on toxicity of spinosad against adults of *C. maculatus*.

## Materials and methods

### Insect rearing

The Cowpea Weevil colony used in this study was collected from infested store in Kashan. Fifty pairs of *C. maculatus* adults were reared in 1.5 liter wide-mouth glass jars containing 200g seeds of Cowpea. The insects were maintained at 30 ± 1 °C and 65 ± 5 % RH in dark. To provide 1-day-old adults, seeds with pupa window were separated and after day, adults that emerged were collected.

### Insecticide

Commercial formulation of Spinosad (Tracer 24SC) was used in this assay, obtained from Dow Agro science's. It is a liquid mixture of ingredients comprising Spinosyn A and D (22.8 %) and inert ingredients of 77.2 %. Stock solution and dilution of Spinosad were prepared with distilled water for treatments.

### Bioassay

The 1-day-old adults *C. maculatus* were exposed to different dosages of Spinosad. Bioassays were performed on filter paper inside Petri dishes. The inside surface of 8-cm diameter Petri dishes (bottom and lid) were treated with 2 ml each of distilled water or Spinosad solution (Toews and Subramanyan, 2003). Treating the inside surface of dish lids was necessary to ensure exposure of adults because of the Cowpea Weevil could fly upside on Petri dish surface. Fifteen 1-day-old adults were separated from cultures and introduced into each untreated or treated Petri dish. Each deposit level was replicated 4 times. A control group was included in each experiment. The experiments were conducted at four different temperatures vis. 20, 24, 28 and 32 °C and 65 ± 5 % RH. Mortality percentage was recorded 24, 48 and 72 hours post-treatment.

### Data analysis

Mortality of insects (x) transformed to arcsin $\sqrt{x}$ to normalize heteroscedastic treatment variances. $LC_{50}$ values were calculated using probit analysis with SPSS software (SPSS, 1993). All data were subjected to ANOVA analysis and percentage of mortalities was compared. Mean were compared using Duncan's Multiple Range Test ($P<0.05$).

## Results

### Effect of temperature

Results indicated that there was a diresct significant

relationship between temperature and toxicity of Spinosad. No mortality of insects was observed on dishes treated with distilled water (control group) at any temperature. Comparison of $LC_{50}$ values at four different temperatures showed an inverse relationship between temperature and $LC_{50}$ values was observed (Table 1). At 32 °C $LC_{50}$ value was lesser than other temperature. Therefore, Spinosad had positive temperature coefficient for adults of *C. maculatus*. Comparison of means showed that at 20, 24, 28 and 32 °C, the concentrations of Spinosad at the rate of 400, 300, 185 and 150 ppm of inflects 75-100 % mortalities to the tested insects, respectively.

## Effect of exposure time

At each temperature the highest dosage of Spinosad inflect 98-100 % mortality to insects 72 hours post-treatment. Duncan's means squaration tests showed that 72 hours after treatment with different dosages of Spinosad occurred significantly more mortality than 24 and 48 hours post-treatment at any temperature ($F = 61.47 - 114.56$, $df = 2$, $P < 0.05$) (Figures 1-4). Results revealed that exposure time affected on efficacy of Spinosad against adults of *C. maculatus* and a direct relationship between Spinosad dosages of exposure time was detected.

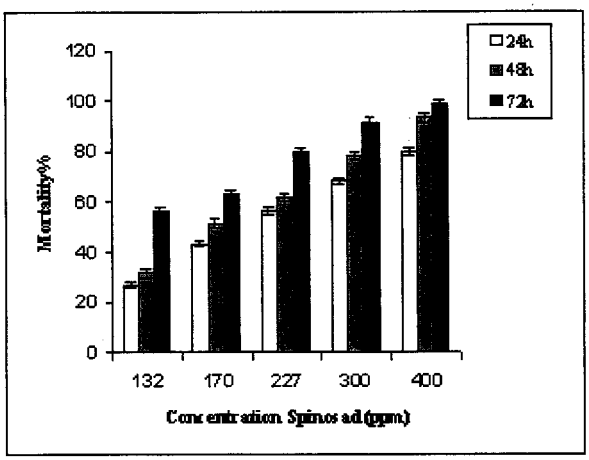


**Figure 1.** Percentage of mortality of *C. maculatus* to Spinosad for 24, 48 and 72 hours post-treatment at 20 °C.

**Table 1.** Probit regression estimates (Mean ± SE) for adults *C. maculatus* exposed for 24, 48 and 72 hours to Spinosad at four different temperature levels.

| | Hours post-treatment | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 24h | | | 48h | | | 72h | |
| Tem (°C) | $LC_{50}$(95%CL) | Slope (±SE) | $X^2$ (df) | $LC_{50}$(95%CL) | Slope (±SE) | $X^2$ (df) | $LC_{50}$(95%CL) | Slope (±SE) | $X^2$ (df) |
| 20 | 203.56 (177.66-228.86) | 2.93 (0.45) | 0.22 (3) | 176.02 (155.15-194.34) | 3.71 (0.49) | 1.39 (3) | 127.15 (101.35-146.02) | 3.67 (0.57) | 2.15 (3) |
| 24 | 183.35 (164.52-202.86) | 3.45 (0.50) | 0.36 (3) | 149.35 (132.32-163.90) | 4.00 (0.54) | 3.19 (3) | 112.11 (18.44-146.92) | 4.17 (0.65) | 7.99 (3)* |
| 28 | 96.98 (85.14-110.56) | 2.75 (0.39) | 0.99 (3) | 72.57 (64.37-80.17) | 3.75 (0.45) | 3.68 (3) | 61.38 (53.94-67.76) | 4.29 (0.53) | 1.01 (3) |
| 32 | 61.26 (51.67-71.73) | 2.21 (0.32) | 0.16 (3) | 40.89 (32.62-48.05) | 2.41 (0.34) | 0.07 (3) | 32.98 (26.84-38.02) | 3.37 (0.46) | 3.73 (3) |

*Chi-square value for goodness-of-fit of data to the probit model was significant ($P < 0.05$).

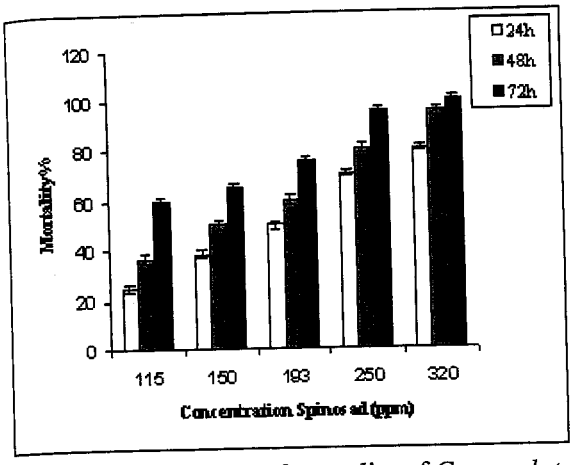


**Figure 2.** Percentage of mortality of *C. maculatus* to Spinosad for 24, 48 and 72 hours post-treatment at 24 °C.

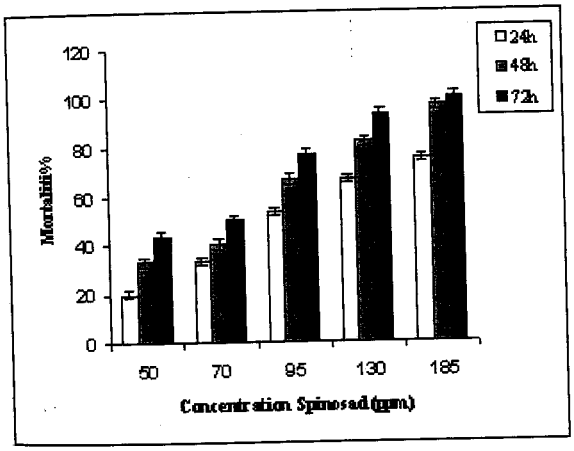


**Figure 3.** Percentage of mortality of *C. maculatus* to Spinosad for 24, 48 and 72 hours post-treatment at 28 °C.

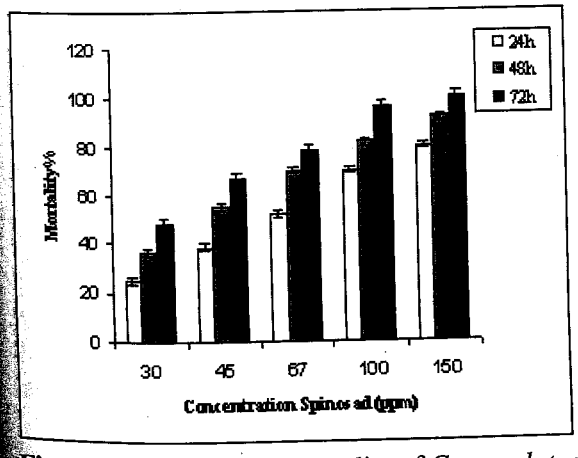


**Figure 4.** Percentage of mortality of *C. maculatus* to Spinosad for 24, 48 and 72 hours post-treatment at 32 °C.

## Discussion

Results of laboratory studies conducted under controlled conditions indicated that temperature and exposure time affect the toxicity of Spinosad. There was a direct relationship between temperature and toxicity of Spinosad. Musser and Shelton reported negative interaction between temperature and efficacy of Spinosad for *O. nubilalis* from 24 to 35 °C. But other study indicated that Spinosad provided differing levels of mortality at each temperature and mortality of Grasshoppers was lowest at 15°C but afterward increased as temperature decreased (Amarasekare and Edelson, 2004).

Mortality of adults *C. maculatus* 72 hours post-treatment was more than 24 and 48 hours after treatment. Other research showed that toxic effects of Spinosad on predatory insects increased with increasing of exposure time (Cisneros et al., 2002). Mortality of *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum* and *Cryptolestes ferragineus* to Spinosad increased as exposure periode increased (Nayak et al., 2005).

Results revealed that Spinosad has considerable contact toxicity against adults of Cowpea Weevil and it may be an alternative biopesticide for control of this pest in storage period. When insecticides from different classes are available to control a pest, knowledge of a product's temperature coefficient will enable pest managers to select a product that is efficacious under the given environmental conditions; therefore, a field test using a similar design is essential to confirm the laboratory findings.

## Refrences

Amarasekare, K.G., Edelson, J.V., 2004. Effect of temperature on efficacy of insecticides to differential Grasshopper (Orthoptera: Acrididae). J. Econ. Entomol. 97, 1595-1602.

Cisneros, J., Goulson, D., Derwent, L.C., Penagos, D.I., Hernandez, O., Williams, T., 2002. Toxic effect of spinosad on predatory insects. Biol. Cont. 23, 156-163.

Copping, L.G., Menn, J.J., 2000. Biopesticides a review of their action, application and efficacy. Pest. Manag. Sci. 56, 651-676.

Crouse, G.D., Sparkes, T.C., Schoonover, J., Gifford, J., Dripps, J., Bruce, T., Larson, L.L., Garlich, J., Hatton, Ch., Hill, R.L., Worden, T.V., Martynow, J.G., 2001. Recent advances in the chemistry of Spinosyns. Pest. Manag. Sci. 57, 177-185.

DeVrise, D.H., Georghiou, G.P., 1979. Influence of temperature on toxicity of insecticides to susceptible and resistant houseflies. J. Econ. Entomol. 72, 48-50.

Huignard, J., Leori, B., Alzoma, I., Germain, J.F., 1985. Oviposition and development of *Bruchidius atrolineatus* (Pic) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in *Vigna ungiculata* (Walp) cultures in niger. Insect. Sci. Appl. 5, 41-49.

Johnson, D.L., 1990. Influence of temperature on toxicity of two pyrethroids to Gasshoppers (Orthoptera: Acrididae). J. Econ. Entomol. 83, 366-373.

Kèita, S.M., Vincent, C., Schmit, J.P., Ramaswamy, S. Bélanger, A., 2000. Effect of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J. Stored Prod. Res. 36, 355-364.

Musser, F.R., Shelton, A.M., 2005. The influence of post-exposure temperature on the toxicity of insecticides to *Ostrinia nubilalius* (Lepidoptera: Crambidae). Pest. Manag. Sci. 61, 508-510.

Nayak, M.K., Daglish, G.J., Bryne, V.S., 2005. Effectiveness of Spinosad as a grain protectant against resistant Beetle and Psocid pests of stored grain in Australia. J. Stored Prod. Res. 41, 455-467.

Ouedraogo, A.P., Sou, S., Sanon, A., 1996. Influence of temperature and humidity on population of *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its parasitoid *Dinarmus basalis* (Peteromalidae) in two climatic zones of Burkina Faso. Bull. Entomol. Res. 86, 695-702.

SPSS, 1993. SPSS for windows user's guide release 6. SPSS. Inc. Chicago.

Tapondjou, L.A., Adler, C., Bouda, H., Fontem, D.A., 2002. Efficacy of powder and essential oil from *Chenopodium ambresioides* leaves as post-harvest grain protectants against six-stored product beetles. J. Stored Prod. Res. 38, 395-402.

Thompson, G.D., Dutton, R., Sparkes, T.C., 2000. Spinosad-a case study: an example from a natural products discovery programme. Pest. Manag. Sci. 56, 698-702.

Toews, M.D., Subramanyam, B., 2003. Contribution of contact toxicity and wheat condition to mortality of stored-product insects exposed to Spinosad. Pest. Manag. Sci. 59, 538-544.

Watson, G.B., 2001. Action of insecticidal Spinosyns on ã-aminobutyric acid responses from small-diameter Cockroachneurons. Pest. Bio. Physio. 71, 20-28.



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**Measuring**

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