CONTROL OF PULSE BEETLE, *CALLOSUBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE) IN DIFFERENT CEREALS USING SPINOSAD DUST IN STORAGE CONDITIONS

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Abstract: Effectiveness of spinosad dust formulation, that contains 0.125% spinosad, was evaluated against adults *Callosubruchus maculatus* (F.) on four commodities: chickpea, split pea, cowpea and lentil. Spinosad was applied at three dose rates: 0.1, 0.2 and 0.3 g/kg, corresponding to 0.125, 0.25 and 0.375 mg/kg of the active ingredient, respectively. The experiment was carried out at 27°C and 55±5% relative humidity. Adults mortality was measured after 1, 2, 5 and 10-days of exposure. After the 10-day mortality count, all surviving insects were removed and samples retained under the same conditions for a further 35 days to assess progeny reduction. Mortality of exposed individuals in all treated commodities was low at 1-day exposure even at 0.3 g/kg and did not exceed 20%. As expected, mortality increased with the increase of exposure interval and dose rates. A significant difference was observed among the four commodities. After 10 days of exposure, mortality reached 100% in all commodities except for split pea. The application of spinosad significantly reduced progeny production in four commodities tested in comparison with the untreated ones. High reduction in progeny production was recorded when spinosad was applied at the rate of 0.3 g/kg on split pea and cowpea (94.33 and 94.21%, respectively). The results of our study clearly revealed that spinosad dust could be successfully used as a grain protectant against *C. maculatus*. Further experimentations still need to be done to examine higher dose rates and long-term use in different commodities.

Key words: spinosad, *Callosubruchus maculatus*, chickpea, split pea, cowpea, lentil

INTRODUCTION

Pulses (members of the Leguminosae family) are one of the important groups of worldwide crops and play a major role in the daily diet of low-income groups of people (Rohitha Prasantha *et al*. 2003). The pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is a serious cosmopolitan and polyphage pest of stored pulses such as bean, cowpea, lentil, chickpea or other legume grain. The pulse beetle can cause losses of up to 30% in a short period of time (Raja and John 2008).

Due to the development of pesticide resistance, hazards effects to non-target species and beneficial organisms, and environmental contamination, the tendency to use safe and low toxicity insecticides has increased (Odeyemi *et al*. 2006; Sadeghi *et al*. 2006). Spinosad is a reduced-risk commercial insecticide based on the fermentation products of an actinomycete bacterium. This reduced-risk commercial insecticide, has been labeled for use on over 250 crops in more than 50 countries (Thompson *et al*. 2000; Huang and Subramanyam 2007). Spinosad is toxic to insects by ingestion or contact, and it acts on an insect's nervous system at the nicotinic acetylcholine and gamma-aminobutyric acid (GABA) receptor sites. The US Environmental Protection Agency (EPA) has classified it as a reduced risk insecticide product because of its: (1) low mammalian toxicity, (2) low toxicity to wildlife and (3) because of its safe and beneficial compatibility with integrated pest management (Saunders and Bret 1997; Salgado 1998). Furthermore, in 2005, the EPA registered spinosad at 1 mg/kg active substance (a.s.) as a grain protectant on commodities including wheat, corn, rice, millets, oats, sorghum, and barley (Huang and Subramanyam 2007).

Previous studies with different formulations of spinosad (liquid or dust) showed that spinosad could be effective against several stored-product pest especially on resistant strains of the pests (Fang *et al*. 2002; Mutambuki *et al*. 2003; Nayak *et al*. 2005; Huang and Subramanyam 2007; Huang *et al*. 2007; Chintzoglou *et al*. 2008; Khashaveh *et al*. 2009; Vayias *et al*. 2009)

To our best knowledge, insecticidal efficacy of spinosad dust formulation has not been studied on various legume commodities, against *C. maculatus*. The aim of the present study was to assess the efficacy of a dust formulation of spinosad on four different pulse commodities

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against the pulse beetle. The effect of this protectant on the amount of progeny reduction was also evaluated.

MATERIALS AND METHODS

Insects

Insects were obtained from laboratory cultures. The cultures had been maintained for at least 4 years, with no history of exposure to insecticides. Adults of *C. maculatus* were reared on cleaned and disinfested cowpea at 27°C and 55±5% relative humidity (RH) in continuous darkness. All adults used in the experiments were 0-24 hours old, and males and females were used.

Commodities

Cowpea (*Vigna unguiculata* L.), chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medikus) and split pea (*Pisum sativum* L.) (split peas from smooth yellow dry pea varieties) were used for the experiments. The commodities were in a good condition, clean and infestation-free. The moisture content of the four commodities as determined by a Dickey–John moisture meter (Dickey-John Multigrain CAC II, Dickey-John Co, USA) ranged between 9.34 and 10.17%. In order to equilibrate moisture content to RH level, seeds were kept at ambient conditions, and after a seven day interval, the moisture content reached to about 12±0.3% (Hall 1980) .

Bioassay

The spinosad dust formulation (Dow AgroScience) contained 0.125% a.s. of spinosad.

Spinosad was applied at dose rates of 0.1 g/kg (corresponding to 0.125 mg/kg a.s.), 0.2 g/kg (corresponding to 0.25 mg/kg a.s.) and 0.3 g/kg (corresponding to 0.375 mg/kg a.s.) in three replicates. For each commodity, three lots of 1 kg of cereal seed were prepared and placed in separate cylindrical jars (2-liter capacity with screwed lids) and treated with appropriate dose rates. Untreated sample served as a control. The vials were shaken manually for approximately 5 min to obtain an even distribution of the dust on the entire seed sample. After a day, four samples, of 50 g each, were taken from each jar (lot) as replication. Then the samples were placed in glass vials which were 8 cm in height and 5 cm in diameter. Next, thirty adults were introduced into each glass vial that was covered with muslin cloth to provide sufficient aeration. The vials were then placed in incubators set at 27°C and 55±5% RH in darkness. The desired RH was maintained by using a saturated salt solution of sodium bromide, as recommended by Greenspan (Greenspan 1977). The number of dead adults was counted after 1, 2, 5 and 10-days of exposure. When there was no observed leg or antennal response to a hot needle, insects were considered dead. After the 10-day mortality count, for each commodity, all adults (dead and alive) were removed from the vials, and vials were left under the same conditions for a further 35 days to assess progeny production.

Data analysis

Mortality counts were corrected by using Abbott's formula (Abbott 1925). The percentage of reduction in progeny production was determined by using the following formula (Aldryhim 1990):

$$\left[\frac{\text{No. progeny in control} - \text{No. progeny in treatment}}{\text{No. progeny in control}} \times 100\right]$$

To equalize variances, mortality percentage of adults and percentage of reduction in progeny production were transformed using the square root of the arcsin. The morality data were analyzed by the General Linear Model (GLM) of the Statistical Analysis System (SAS Institute 2000), with insect mortality as the response variable and commodity, and dose rate and exposure interval used as main effects. The same procedure was also followed for progeny production counts. Means were separated by using the Tukey-Kramer (HSD) test at p = 0.05 (Snedecor and Cochran 1989).

RESULTS

Adult mortality

The mortality within the control group 10 days after treatment was 4.16±1.61, 6.65±1.36, 3.49±0.83 and 1.16±0.59% in chickpea, splitSe pea, cowpea and lentil, respectively. All of the main effects as well as all associated interactions were significant at the $p < 0.0001$ level for dose rate (df = 2, 575; F = 1587.22), commodity (df = 3, 575; F = 741.74) and exposure interval (df = 3, 575; F = 5057.94), dose rate x commodity (df = 6, 575; F = 35.07), dose rate x exposure interval (df = 6, 239; F = 134.17), exposure interval x commodity (df = 9, 575; F = 23.93) and dose rate x exposure interval x commodity (df = 18.575; F = 11.29). In all experiments, as expected, mortality increased with an increase of exposure interval and dose rates. The mortality of *C. maculatus* adults exposed to spinosad in all the treated commodities was generally low the first time of exposure (1 day) even at a 0.3 g/kg rate and did not exceed 20%. Mortality ranged between 0.83% (split pea, lowest dose) and 19.9% (cowpea, highest dose) (Fig. 1). Significant differences were noted among the three doses for different commodities except split pea. A noticeable increase in adult mortality was recorded 24 h later. Hence, at 2-day exposure interval, mortality ranged between 13.32% (split pea, lowest dose) and 61.66% (cowpea, highest dose) (Fig. 2). In addition, a significant difference was observed within all commodities. Five days of exposure resulted in a significant increase in *C. maculatus* mortality in all treated commodities and complete adults mortality (100%) was recorded for cowpea and lentil at the highest dose. However, for chickpea mortality ranged between 36.13% at the lowest dose to 87.39% at the highest dose and for split pea 28.81% at the lowest dose to 62.71% at the highest dose (Fig. 3). Finally, at the 10-day exposure interval and at the highest dose, all adults were dead in chickpea, cowpea and lentil, but mortality was only 79.46% in the case of split pea. In addition, 100% mortality was noted at the rate of 0.2 g/kg only in cowpea (Fig. 4). Generally, of the four commodities, significantly lower mortalities were recorded on split pea.

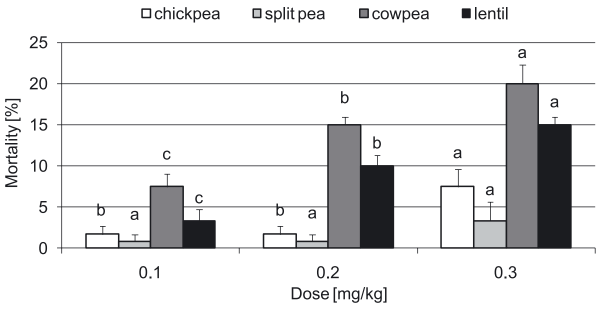

Fig. 1. Mean mortality (% ±SE) of *C. maculatus* adults, in different commodities, treated with spinosad dust after 1 day of exposure (with commodity, means fallowed by same letter are not significantly different; Tukey-Kramer (HSD) test at p = 0.05)

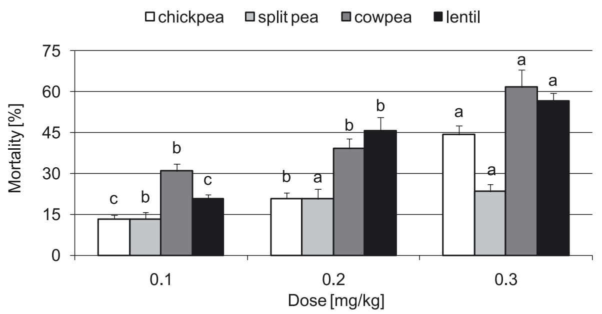

Fig. 2. Mean mortality (% ±SE) of *C. maculatus* adults, in different commodities, treated with spinosad dust after 2 days of exposure (with commodity, means fallowed by same letter are not significantly different; Tukey-Kramer (HSD) test at p = 0.05)

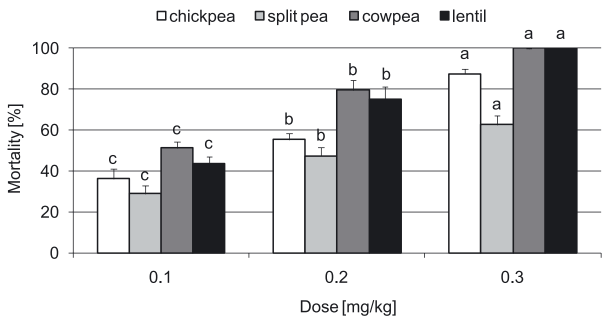

Fig. 3. Mean mortality (% ±SE) of *C. maculatus* adults, in different commodities, treated with spinosad dust after 5 days of exposure (with commodity, means fallowed by same letter are not significantly different; Tukey-Kramer (HSD) test at p = 0.05)

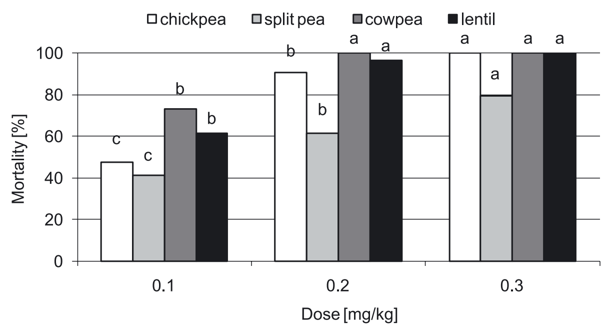

Fig. 4. Mean mortality (% ±SE) of *C. maculatus* adults, in different commodities, treated with spinosad dust after 10 days of exposure (with commodity, means fallowed by same letter are not significantly different; Tukey-Kramer (HSD) test at p = 0.05)

Progeny suppression

The mean number of adult offspring produced (±SE)/vial in the control group was 119±14, 181.75±12.18, 60±6.64 and 15.5±2.25 for chickpea, cowpea, lentil and split pea, respectively. Preliminary analysis of variance (ANOVA) for the amount of progeny production in the control vials revealed that significantly fewer adults emerged in split pea (p = 0.01) in contrast with the other commodities.

Table 1. Mean percentage of reduction (±SE) in progeny production (F1) of *C. maculatus* in different commodities treated with 0.1, 0.2 and 0.3 g/kg of spinosad dust

Dose [g/kg]	Chickpea	Split pea	Cowpea	Lentil
0.1	62.18±5.58 cd	83.49±3.85 b	56.39±6.19 cd	40.83±3.71 d
0.2	73.31±3.45 bc	88.67±4.87 ab	82.02±1.95 b	64.58±6.95 c
0.3	89.28±6.13 ab	94.33±2.88 a	94.21±1.42 a	79.16±2.23 bc

Means followed by the same letter are not significantly different; Tukey-Kramer (HSD) test at p = 0.05

For progeny reduction in the treated vials, all the main effects and associated interaction were significant for dose rate (df = 2, 144; F = 170.95; p = 0.0001), commodity (df = 3, 144; F = 47.32; p = 0.0001) and dose rate × commodity (df = 6, 144; F = 2.49; p = 0.0260). The application of spinosad significantly reduced progeny production in the four commodities tested. The highest percentage of progeny reduction was recorded for split pea and cowpea at 0.3 g/kg, which was 94.33 and 94.21%, respectively (Table 1).

DISCUSSION

This is the first report that characterizes the efficacy of spinosad dust formulation against *C. maculatus* on stored commodities. There is no comparative data on the efficacy of spinosad dust. Previous work that was done by Sadat and Asghar indicated that liquid formulation of spinosad (Tracer® contained 22.8% a.s./l) had considerable contact toxicity against adults of *C. maculatus* and it may be an alternative biopesticide for controlling this pest. In that study, spinosad that was applied at 4 dose rates, 400, 300, 185 and 150 ppm caused 75–100% mortality at different temperatures. By comparing previously published data with our results, we can conclude that spinosad dust formulation is more effective than a liquid formulation against *C. maculatus* adults (Sadat and Asghar 2006).

For all commodities tested except split pea, spinosad was very effective against *C. maculatus* with an application rate of 0.3 g/kg (corresponding to 0.375 mg/kg a.s.) after 10 days of exposure. Generally, the percentage of *C. maculatus* mortality was higher in the case of cowpea in contrast with the other three commodities tested. While, adult mortality for all exposure intervals and dose rates was significantly lower in split pea. The number of eggs laid (personal observation during experiments) for split pea was significantly lower in both untreated and treated samples. In addition, for the split pea commodity the highest percentage of progeny reduction in all dose rates was recorded. Plausible reasons for the obtained results may be related to the nutritional preferences of this pest. The mean number of emerged adults for split pea in comparison with other commodities in the control group was very low (15.5±2.25 adults per vial). These results may show that this commodity is a non-preference food for pulse beetle and this factor could influence and limit the movement and egg laying of *C. maculatus* in treated and untreated vials. In the treated vials, the reduction of movement could reduce contact of insect pests with dust particles, thus decreasing the mortality percentage.

The type of commodity is a key element determining the insecticidal effect of a given seed protectant and even plays a bigger role than the protectant (Ziaee *et al*. 2007; Athanassiou *et al*. 2008; Khashaveh *et al*. 2009; Vayias *et al*. 2009). The reasons why spinosad is not equally effective in different grain commodities have not been investigated in details and little information is available on the adhesion and distribution of spinosad on stored commodities to explain reasons for the significant differences observed. According to Chintzoglou *et al*. (2008), the difference of insect mortality in spinosad treated commodities may be due to the speed of pesticide degradation. The authors used a high-performance liquid chromatography/mass spectrometry method (LC/MS) for the precise and selective quantitative determinations of spinosyns residues in wheat, barley and maize grains. They recorded that spinosad dissipation on these commodities were significantly different. Their conclusions were lower mortality of *Sitophilus oryzae* L. in maize is due to the high degree of spinosad degradation.

Fang *et al*. (2002) stated that the efficacy of spinosad as a liquid formulation differ on different varieties of wheat. However, the authors could not find any noticeable correlation between efficacy of spinosad with kernel diameter, hardness, fiber, weight or protein. Recently, Khashaveh *et al.* (2009) demonstrated that the efficacy of spinosad dust in different oilseeds against *Tribolium castaneum* Herbst is significantly different and that type of commodity seriously affects this difference.

Suppression of the subsequent generations is one of the basic characteristics of a successful grain protectant (Arthur 1996). The results obtained from the present study obviously demonstrated that progeny production of *C. maculatus* significantly decreased in spinosad treated commodities in comparison with untreated ones and the degree of progeny suppression was directly related to the treatment rate.

In the other research, the effectiveness of spinosad against seven major stored grain insects has been evaluated on corn. Results indicated that spinosad at 1 or 2 mg/kg was extremely effective in suppressing progeny production, and could provide complete suppression of progeny production (Huang and Subramanyam 2007).

The tests further certify our results that spinosad effectiveness on *C. maculatus* mortality and suppressing progeny production depends on treatment rates and intervals exposed to it (Fang *et al*. 2002; Huang and Subramanyam 2007; Huang *et al*. 2007; Vayias *et al*. 2009).

CONCLUSION

The results of our research clearly revealed that spinosad dust formulation could be successfully used as a grain protectant against pulse beetle. But further experimentations are required to assess the efficacy of this low toxic protectant in the long-term use and higher dose rates for other commodities of Leguminosae family.

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POLISH SUMMARY

ZWALCZANIE STRĄKOWCA CZTEROPLAMEGO *CALLOSUBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE) NA NASIONACH MOTYLKOWATYCH W OKRESIE PRZECHOWYWANIA PRZY POMOCY PREPARATU BAKTERYJNEGO SPINOSAD

Badano skuteczność preparatu spinosad w formie proszku, zawierającego 0,125 % spinosadu przeciwko dojrzałym osobnikom *Callobruchus maculatys* (F.) zasiedlających nasiona: ciecierzycy pospolitej, grochu, fasolnika wężowego i soczewicy. Preparat spinosad zastosowano w trzech dawkach 0,1; 0,2 i 0,3 g/kg nasion, co w przeliczeniu na składnik aktywny wynosiło odpowiednio 0,125; 0,25 i 0,375 mg/kg nasion. Doświadczenia wykonywano w temperaturze 27 ^{0}C, przy wilgotności względnej 55 ± 5%. Śmiertelność dorosłych osobników oceniano po upływie 1, 2, 5 i 10 dniach ekspozycji. Osobniki, które przeżyły okres 10 dniowej ekspozycji zostały zebrane i umieszczone w takich samych warunkach w celu oceny redukcji płodności, w ciągu kolejnych 35 dni. Śmiertelność owadów w przypadku wszystkich traktowanych nasion poszczególnych gatunków roślin po 1 dniowej ekspozycji była niska, a nawet przy dawce 0,3 g/kg nasion nie przekraczała 20%. Jak się spodziewano śmiertelność owadów wzrastała wraz z wydłużeniem czasu ekspozycji oraz zwiększeniem zastosowanej dawki preparatu. Wystąpiły różnice pomiędzy badanymi gatunkami roślin. Po 10 dniowej ekspozycji śmiertelność owadów osiągała 100% w przypadku nasion wszystkich badanych gatunków roślin z wyjątkiem grochu. Zastosowanie preparatu spinosad istotnie ograniczyło płodność chrząszczy we wszystkich testowanych obiektach w porównaniu do kontroli. Stwierdzono silne ograniczenie płodności owadów po zastosowaniu preparatu spinosad w dawce 0,3 g/kg nasion grochu i fasolnika wężowego (odpowiednio 94,33 i 94,21%). Wyniki prezentowanych badań dowiodły, że preparat spinosad w formie proszku może być z powodzeniem stosowany w ochronie nasion roślin strączkowych przeciwko *C. maculatus*. Dalsze badania są wskazane celem przetestowania wyższych dawek i wydłużonego czasu ekspozycji w przypadku nasion innych gatunków roślin.