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Impact of spinosad and buprofezin alone and in combination against the cotton leafworm, *Spodoptera littoralis* under laboratory conditions

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ABSTRACT

Toxicity of the two biorational insecticides, spinosad and buprofezin and a mixture of the two was tested against the fourth instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisduval). The results showed that spinosad was more effective on the fourth instar larvae than buprofezin. The LC_{50} values for spinosad and buprofezin were 70.7 and 278.2 ppm, respectively. When spinosad was mixed with buprofezin, the percent of mortality increased; it was 85 %, compared with 63.3% in spinosad and 43.3% in buprofezin treatment. This means that the biorational insecticides spinosad and buprofezin can be used in a combination and cause good results with the cotton leafworm. This result suggested that the mixture of spinosad and buprofezin was more active than spinosad or buprofezin alone in all concentrations used. The larval duration, pupal period and adult longevity were not affected by all tested treatments. The number of eggs laid per female and percent of hatchability were affected in buprofezin and spinosad buprofezin in combination treatments compared with that in control. These results suggest that the combination of lethal effects of spinosad and buprofezin might affect pest population dynamics significantly by decreasing its survival and reproduction and by delaying its development.

Key words: *Spodoptera littoralis*, buprofezin, spinosad, combination, biological aspects.

INTRODUCTION

Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) is one of the most destructive pests of several crops such as cotton, *Gossypium hirsutum* L., peanut, *Arachis hypogaea* L., soybean, *Glycine max* L. and vegetables in Africa, Asia and Europe (El-Aswad *et al*., 2003). In addition to its direct damage reducing photosynthetic area, its larval presence, feeding marks and excrement residues reduce marketability of vegetables and ornamentals (Pluschkell *et al*., 1998). Over the past 25 years, the intensive use of broad-spectrum insecticides against *S. littoralis* has led the development of resistance to many registered pesticides for its control (Aydin and Gurkan, 2006).

Spinosad is a bioinsecticide based on the fermentation product of the soil bacterium *Saccharopolyspora spinosa* (Sparks *et al.*, 1998). This compound has two unique modes of action, acting primarily on the insect nervous system at the nicotinic acetylcholine receptor, and exhibiting activity at the GABA receptor (Watson, 2001). It has a low toxicity for mammals with an LD_{50} of 3783 - 5000 mg/kg for rats (Tomlin, 2000). Spinosad has been registered in over 30 countries for the control of Lepidoptera, Coleoptera, Diptera, and Thysanoptera (Thompson *et al.*, 2000; Williams *et al.*, 2004; Aarthi and Murugan, 2010). Pineda *et al*. (2007) tested spinosad against the fourth instar larvae of the cotton leafworm by using five concentrations. The author concluded that the combination of lethal and sublethal effects of methoxyfenozide (IGR) and spinosad might exhibit significant effects on the population dynamics of *S. littolaris* (Aydin and Gurkan, 2006). Osman and Mahmud (2008) found that spinosad was potentially a viable compound for the management of *S. littoralis.* Later, Daglish (2008) examined binary combinations of several insecticides, including spinosad and methoprene either alone or in combination against five stored-grain beetles. Parental mortality was less in the combination treatments compared to spinosad alone, suggesting a possible detrimental effect of methoprene on the toxicity of spinosad to this species. The author reported parental mortality for *Sitophilus oryzae*.

Buprofezin prevents the adult emergence from the pseudopupa of *Bemisia tabaci*. Valle *et al*. (2002) considered a chitin synthesis inhibitor against larvae of Lepidoptera because it interferes with chitin formation by blocking the polymerizations process of N- acetyl glucose amine units (Ishaaya and Horowitz, 1998). Nasr *et al*. (2010) found that buprofezin caused reasonable mortality in *Spodoptera littoralis* larvae. Christos *et al.* (2011) tested that combination of spinosad and methoprene against six stored-product insect species, *Rhyzopertha dominica*, *Sitophilus oryzae*, *S.*

granarius, *Cryptolestes ferrugineus*, *Oryzaephilus surinamensis*, and *Liposcelis bostrychophila* and observed that the speciûc combinations of spinosad and methoprene evaluated in this study would have no beneût over spinosad used alone for control of any of the six species tested. However, no information is available on the impact of buprofezin and spinosad against *Spodoptera littoralis.* In this work, it has been found that trying new types of insecticides that kill or disrupt the physiological processes of the target pest could be useful as an alternative for the integrated management approach. Based on their low ecotoxicological profile and short persistence in the environment, spinosad and buprofezin represent an important pest control option to integrated pest management (IPM). The aims of this work is to evaluate toxicity of spinosad and buprofezin as a biorational insecticide and its mixture on the fourth instar larvae of *S. littoralis* and also the effect of these treatments on some biological aspect.

MATERIALS AND METHODS

Larvae of the cotton leafworm, *Spodoptera littoralis*, were reared on clean and fresh castor leaves, *Ricinus communis* L., in the Laboratory at a temperature of 25 ± 2°C and 65 ± 5% R.H. with a photoperiod of 16:8 (L:D).

Chemical tested

Two pesticides were used with the recommended rate and four lower concentrations. Spinosad, Tracer 25% SC, 50 ml/ 400 l water / feddan (Feddan = 4200 m2), obtained from Dow Agro Sciences (Indianapolis, IN, USA). Buprofezin, Applaud 24% SC, 200 ml/ 400 l water /feddan, obtained from Nihon Nohyaku Co., Japan.

Bioassay

Five concentrations were prepared from the two compounds, buprofezin (120, 60, 30, 15 and 7.5 ppm) and spinosad (31.3, 15.6, 7.8, 3.9 and 1.9 ppm). Each concentration had three replicates. Each replicate included 30 healthy starved larvae. Other three replicates were dipped in water as a control. Castor leaves were dipped into the tested concentrations for 5 s and left to surface-dry and then placed into glass cages containing moistened filter papers to avoid desiccation of leaves. Ten larvae were transferred into the leaves in each replicate. Mortality of fourth instar larvae was recorded after 24 h with spinosad, while 4 days with buprofezin. These cages were incubated in 25 ± 2°C and 65 ± 5% R.H. with a photoperiod of 16:8 (L:D). The LC_{50} (lethal concentration for 50% from population) was calculated by Proban probit analysis program.

Five concentrations also, were used from buprofezin and spinosad combinations (120 + 31.3, 60 + 15.6, 30 + 7.8, 15 + 3.9 and 7.5 + 1.9 ppm). Each concentration had three replicates. Each replicate included 30 healthy starved larvae. The percent of mortality was recorded after four days from treatment.

Biological parameters

The larvae which survive from all treatments were taken and some biological aspects were counted compared with untreated larvae (control). These biological aspects include; larval duration, pupal period, adult longevity, number of laid egg per female and percent of hatchability.

Statistical analysis

Data of the percents mortality in all treatments whether in second instar larvae or the adults were analyzed by one way ANOVA analysis (SAS Institute Inc 2003).

RESULTS AND DISCUSSION

As mentioned in Table 1 the first and second concentrations in spinosad treatment (31 and 15.6 ppm) caused higher mortality than that with buprofezin. However, insignificant difference was recorded between spinosad and buprofezin with other concentrations. It indicates that spinosad was highly toxic against the fourth instar larvae of *S. littoralis* at the field rate only and the lower concentrations were not toxic. When spinosad concentrations were mixed with buprofezin (Table1), the percent of mortality sharply increased (85 and 71.7% for 31 and 15.6 ppm, respectively). Statistical analysis showed that there is a significant difference between spinosad and buprofezin with the first and second concentrations ($F = 13.4$ and 118.5; $df = 3$ and 3; and $P = 0.006$ and 0.0 for 31 and 15.6 ppm, respectively). There is a significant difference among spinosad, buprofezin and spinosad buprofezin combination. This result means that buprofezin has a synergistic action to spinosad and vise versa .The use of spinosad and buprofezin in combination increases the toxic action of both insecticides.

The LC_{50} for spinosad and buprofezin were 70.7 and 278.2 ppm, respectively. The slope values for spinosad and buprofezin were 1.2 and 0.9, respectively. This result means that buprofezin was a low toxic against the forth instar larvae of *S. littoralis* compared with spinosad as observed by Nasr *et al*. (2010). They recorded that buprofezin caused 46.7% mortality and also observed that the lower concentrations were non-toxic. Aydin and Gurkan (2006) recorded the LC_{50} values for field and susceptible strains of *S. littoralis*. Darriet *et al.* (2010) stated that the mixture of pyriproxyfen (insect growth regulator) + spinosad remained active for at least 8 months, compared with 3 months for spinosad alone, and 5

Table 1. Effect of spinosad and buprofezin and its mixture on the fourth instar larvae of *Spodoptera littoralis* under laboratory conditions.

Concentrations	Mortality percents												df	f	P
	Spinosad				Buprofezin				Spinosad +Buprofezin						
	[1]R1	R2	R3	Mean ± SE	R1	R2	R3	Mean ± SE	R1	R2	R3	Mean ± SE			
1.	60	60	70	[2b]63.3±5.8	60	30	40	[a]43.3±15.8	90	80	85	[c]85±5	2	13.4	0.006
2.	40	30	40	[b]36.7±5.8	20	20	25	[a]21.7±2.9	75	70	70	[c]71.7 ± 2.9	2	118.5	0.00
3.	20	40	20	[a]26.7±11.5	15	15	20	[a]16.7±2.9	55	60	50	[b]55±5	2	21.4	0.002
4.	15	25	10	[a]16.7±7.6	10	15	10	[a]11.7±2.9	35	35	30	[b]33.3±2.9	2	15.4	0.004
5.	10	15	15	[a]13.3±2.9	10	10	10	[a]10±0.0	20	15	20	[b]18.3±2.9	2	9.5	0.014
6.	10	10	15	11.7 ± 2.9	0	10	10	6.7±5.8	10	15	10	11.7±2.9	--	--	--

[1]R1. the first replicate

[2]Means under each variety sharing the same letter in a column are not significantly different at $P<0.05$

months for pyriproxyfen alone against the larvae of *Aedes aegypti*. Xie *et al.* (2010) found that when buprofezin mixed with nitenpyram (neonicotinoid acts on the same target of spinosad) the efficiency was enhanced significantly against the third instar nymphs. On the other hand, Christos *et al*. (2011) stated that the specific combinations of spinosad and methoprene (insect growth regulators) have no benefit over spinosad used alone for control of six stored-product insect species.

Biology

The larval duration, pupal period and adult longevity were not affected in all treatment compared with control (Table 2). The statistical analysis also shows that there is no difference among all treatments (Table 2) on larval duration, pupal period and adult longevity (F = 0.27, 1.03 and 3.23; df =3, 3 and 3; and P = 0.56, 0.40 and 0.06 for larval and pupal duration respectively). The number of eggs laid per female were affected in all treatments compared with control especially when spinosad and buprofezin was used in combination (F= = 3.05; df = 3, P = 0,059 Table 2). The percentage of hatchability was reduced in all treatments especially in buprofezin spinosad mixture (45%) compared with control (61.2%). Wang *et al.* (2009) found that spinosad at sub-lethal concentrations significantly extended the developmental time of *Helicoverpa armigera* and decreased the emergence ratio, fecundity and longevity of adults. Antonio *et al*. (2009) found that no significant differences were detected in the adult longevity of *Aedes aegypt*i when the larvae were treated with spinosad and control. Deng *et al*. (2008) found that buprofezin significantly reduced the percentage hatching of the wolf spider *Pirata piratoides* eggs but had only a slight effect on egg production. No negative effects on the development and growth were observed.

Insecticides that work in synergy when mixed together are an avenue to explore in *S. littoralis* control for the needs of public health. Negative aspects of such combinations are those shared with conventional insecticides, in that resistance is ultimately expected to evolve in response to prolonged use and that it is not possible to clearly predict how efficient mixtures will remain if resistance to one of the compounds already exists or develops. Nonetheless, combinations of insecticides with different modes of action could make an efficient contribution in the *S. littoralis* control, notably in regions where *S. littoralis* already shows high levels of resistance to conventional insecticides. The availability of new families of insecticides has been scarce in the last 10 years and relying on the appearance of new products is not a realistic option for the control of resistant populations in the short- to medium-term future. In contrast, the option of associating insecticides with different modes of action is available now.

Table 2. Effect of buprofezin, spinosad and buprofezin spinosad in combination on some biological aspects of the cotton leafworm, *S. littoralis*

Aspects / pesticides	Larval Duration/day	Pupal stages/day	Adult Longevity/day	No. laid eggs/ female	Hatchability Percentage
Buprofezin	[a]15.2 ± 1.3	[a]9.2 ± 0.45	[a]12.4 ± 1.14	[ab]353 ± 23.6	[b]49.2 ± 3.9
Spinosad	[a]15 ± 1	[a]9 ± 0.7	[a]11.2 ± 0.84	[ab]349 ± 25.8	[a]59.2 ± 5.2
[2]Bp and Sp	[a]15.2 ± 1.3	[a]9.6 ± 0.55	[a]10.8 ± 0.84	[b]337 ± 20.5	[b]45 ± 4.1
Control	[a]15.6 ± 0.55	[a]9.4 ± 0.54	[a]11 ± 0.70	[a]375.8 ± 8.7	[a]61.2 ± 2.4
Df	3	3	3	3	3
F	0.27	1.03	3.23	3.05	18.8
P> 0.05	0.56	0.40	0.06	0.059	0.00

[1]Means in a column followed by the same letter do not significantly differ at the 5% level according to Fisher's LSD test.

[2] Buprofezin spinosad in combination

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