

Deleterious Activity of Natural Products on Postures of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Diatraea saccharalis* (Lepidoptera: Pyralidae)

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The control of Lepidoptera pests should be carried out before hatching of their caterpillars to avoid damage to the crops. The aim of this work was to assess the activity of neem (trade name: Natuneem[®], producer: Base Fértil, Chapadão do Sul, Brazil) and pyroligneous extracts (trade name: Biopiról 7M[®], producer: Biocarbo, Itabirito, Brazil) at 10 mL/L (1%) and 20 mL/L (2%) contents on egg masses of different ages of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) and of *Diatraea saccharalis* F. (Lepidoptera: Pyralidae) at Embrapa Corn and Sorghum in Sete Lagoas, Minas Gerais State, Brazil. The tests took place in an unbiased casualized design with 12 treatments and four replications. The insecticides were diluted in water, and 0.04 mL of the solution was applied to recently laid and one- and two-day-old eggs of *S. frugiperda* and *D. saccharalis*. Caterpillars hatching from recently laid egg masses of *S. frugiperda* was lower with 2% pyroligneous extract [(0.02 ± 0.00)%]. Recently laid eggs and one- or two-day-old eggs of *D. saccharalis* presented lower caterpillar hatching with 1% neem extract [(0.00 ± 0.00)%], (0.00 ± 0.00)%, and (1.00 ± 0.01)%] and 2% neem extract [(0.00 ± 0.00)%], compared to 1% pyroligneous extract [(27.30 ± 3.22)%, (28.40 ± 3.32)%, and (37.80 ± 4.14)%] and 2% pyroligneous extract [(42.20 ± 4.49)%, (48.70 ± 4.97)%, and (56.60 ± 5.52)%], respectively. Neem and pyroligneous extracts had impact on hatching of *S. frugiperda* and *D. saccharalis* caterpillars.

Key words: Lepidoptera, Neem, Pyroligneous Extract

Introduction

Spodoptera frugiperda Smith (Lepidoptera: Noctuidae) and *Diatraea saccharalis* F. (Lepidoptera: Pyralidae) are important pests and damage the aerial parts of plants such as corn (Moré *et al.*, 2002; Matos Neto *et al.*, 2004; Clark *et al.*, 2007). The control of these species is carried out in the first stages of their culture with synthetic insecticides that increase environmental risks, production costs and the dependence upon synthetic insecticides, intoxication in operators and residues in food (Zanuncio *et al.*, 1998; Rodriguez *et al.*,

2001; Silva *et al.*, 2009). For these reasons, the use of less toxic products, such as neem, pyroligneous, and Asteraceae extracts is important in agricultural areas (Charleston *et al.*, 2005; Habluetzel *et al.*, 2007; Tavares *et al.*, 2009). The effect of insecticides on adults, pupae, and caterpillars of Lepidoptera has been studied (Pereira *et al.*, 2002; Pineda *et al.*, 2009; Senthil-Nathan *et al.*, 2009), but few studies have shown the impact of these products on eggs of insects with different ages.

The neem plant, *Azadirachta indica* A. Juss. (Meliaceae) originated from Asia, is cultivated in several countries of America, Africa, and Aus-

tralia and is one of the most important plants with insecticide activity and accentuated action against pests such as *S. frugiperda* (Lima *et al.*, 2008; Santiago *et al.*, 2008) and mites (Mourão *et al.*, 2004a). Extracts from seeds of *A. indica* are used as insecticide in water-based and alcoholic solutions (Charbonneau *et al.*, 2007). Azadirachtin is the main insecticide compound of this plant, with high concentration in its fruit followed by its peel and leaves, but the content of said compound in the leaves varies according to the period of the year (Bruce *et al.*, 2004; Medina *et al.*, 2004; Mourão *et al.*, 2004b).

The pyroligneous extract is obtained by condensation of the smoke during wood carbonization (Silva and Zanetti, 2007), and it can present insecticide properties (Silva *et al.*, 2005; Azevedo *et al.*, 2007). It is mainly produced from *Eucalyptus grandis* W. Hill ex Maiden, *Piptadenia peregrina* (L.) Benth., *Piptadenia gonoacantha* (Mart.) Macbr. or *Calycophyllum sprussiana* Benth. (Alves *et al.*, 2007).

The aim of the present work was to assess the harmful effects of the neem and pyroligneous extracts on egg masses of *S. frugiperda* and *D. saccharalis* of different ages in order to obtain products with less environmental impacts, and to produce a higher rate of insect mortality.

Material and Methods

Insects and insecticides

The experiments were carried out at (25 ± 1) °C, a 12-h photoperiod and (70 ± 10)% relative humidity at the Laboratory of Insect Rearing (LACRI) of the Brazilian Agricultural Research Company (Embrapa Corn and Sorghum) in the Municipality of Sete Lagoas, Minas Gerais State, Brazil.

Spodoptera frugiperda and *Diatraea saccharalis* were reared in the laboratory with an artificial diet (Freitas *et al.*, 2007; Tavares *et al.*, 2009).

Emulsified neem oil (trade name: Natuneem[®], producer: Base Fértil, Chapadão do Sul, Brazil) and pyroligneous extract (trade name: Biopirol 7M[®], producer: Biocarbo, Itabirito, Brazil) (Table I) were diluted in water (10 mL/L or 20 mL/L) and applied to recently laid eggs or to one- and two-day-old eggs of *S. frugiperda* and of *D. saccharalis*. The control had only water, and it was applied with the same volume as in the insecticidal treatments.

Table I. Active ingredient (a.i.), content of active ingredient in the formula, content of the insecticide solution (i.s.), producer (Pr), town (To) and Country (Co) of the neem oil (Natunem[®]) and pyroligneous extract (Biopirol 7M[®]).

Parameter	Natuneem [®]	Biopirol 7M [®]
Common name	Neem oil	Pyroligneous extract
a.i.	Azadirachtin	n.i. ^a
Content a.i. (mL/L)	0.15	n.i. ^a
i.s. (%)	1.0 or 2.0	1.0 or 2.0
Pr	Base Fértil	Biocarbo
To	Chapadão do Sul	Itabirito
Co	Brazil	Brazil

^a n.i., not informed by the manufacturers.

The neem was chosen in the form of oil because it is found more easily for sale. However, in farms with neem plants, the water-based extract of leaves may be used, and with more advantages than the oil extracted from the seeds. The main problem using seeds is the low production in some regions, as well as the oil extracting process, which demands presses and special processes, making its use difficult. On the other hand, the use of leaves when preparing the extract presents the advantage of its abundant supply in tropical conditions, as well as its easy preparation, making its use feasible, mainly in small rural properties (Schmutterer, 1990).

Experimental

Groups of pieces of paper used for oviposition and with recently laid or one- or two-day-old eggs of *S. frugiperda* and *D. saccharalis* were cut, and 20 eggs per group were left on them. Each group was put into tubes individually (2 cm in diameter × 10 cm in height). The neem and pyroligneous extracts were diluted in water [10 mL/L (1%) or 20 mL/L (2%)], and 0.04 mL of each solution of these products were applied to each group of eggs. The probes were left for 30 min at room temperature in order to dry. Hence they were sealed with PVC film.

The hatching of caterpillars from groups of eggs of *S. frugiperda* and *D. saccharalis* was assessed 5 and 8 d after application of the neem and pyroligneous extracts, respectively. This period is enough for hatching of the insects to occur under normal laboratory conditions (Ng *et al.*, 1993; Tavares *et al.*, 2009).

The tests took place in a completely casualized design with 12 treatments and four replications. Data regarding hatching of *S. frugiperda* and *D. saccharalis* were submitted to the variance analysis, and the averages were compared with the Tukey test ($P < 0.05$) using the computer program MSTAT-C, version 2.1 (Russel, 1989).

Results

With the 1% neem extract the hatching of caterpillars from recently laid eggs (7.50%) was lower than with one- (13.70%) or two-day-old eggs (15.00%) of *S. frugiperda* (Table II). The hatching of caterpillars from recently laid eggs and from one- or two-day-old eggs of *S. frugiperda* was 6.20%, 7.50%, and 7.50%, respectively, with 2% neem extract (Table II).

The 1% pyroligneous extract caused a lower rate of hatching of caterpillars from recently laid (23.70%) and one-day-old (35.00%) eggs compared to two-day-old eggs (75.00%) of *S. frugiperda* (Table III). The hatching of caterpillars from recently laid eggs (0.02%) with 2% pyrolig-

neous extract was lower than that from one-day-old (25.00%) or two-day-old (45.00%) eggs of *S. frugiperda* (Table III).

The hatching of caterpillars from recently laid eggs (0.00%), and one- (0.00%) or two-day-old (1.00%) eggs of *D. saccharalis* was similar with 1% neem extract (Table IV). These values were also similar to those with 2% neem extract (recently laid eggs, 0.00%, and those being one day of age, 0.00%, or two days of age, 0.00%, respectively) (Table IV).

The hatching of caterpillars from two-day-old eggs of *D. saccharalis* (37.80%) was higher than that from the recently laid ones (27.30%) or that being one day old (28.40%) with 1% pyroligneous extract (Table V). The 2% pyroligneous extract caused a lower rate of hatching of caterpillars from recently laid eggs of *D. saccharalis* (42.20%) than from those being one day old (48.70%) or two days old (56.60%) (Table V).

Discussion

The lower hatching of caterpillars from recently laid eggs of *S. frugiperda* as compared to those at

Table II. Hatching (%) of caterpillars from groups of recently laid or one- or two-day-old *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs, five days after application of 1% or 2% water-based neem extract in Sete Lagoas, Minas Gerais, Brazil.

Age of eggs	Hatching (%) of caterpillars of <i>Spodoptera frugiperda</i>		
	1% Neem extract	2% Neem extract	Water
Recently laid	7.50 ± 0.73Aa	6.20 ± 0.48Aa	81.20 ± 7.01Ab
1 d	13.70 ± 1.70Ba	7.50 ± 0.73Aa	90.00 ± 7.48Ab
2 d	15.00 ± 1.87Ba	7.50 ± 0.73Aa	92.50 ± 7.61Ab
CV	9.28%		

Averages followed by the same capital letter per column or lower case letter per line did not differ by the Tukey test ($P < 0.05$). CV, coefficient of variation.

Table III. Hatching (%) of caterpillars from groups of recently laid or one- or two-day-old *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs, five days after application of 1% or 2% pyroligneous extract in Sete Lagoas, Minas Gerais, Brazil.

Age of eggs	Hatching (%) of caterpillars of <i>Spodoptera frugiperda</i>		
	1% Pyroligneous extract	2% Pyroligneous extract	Water
Recently laid	23.70 ± 2.86Ab	0.02 ± 0.00Aa	81.20 ± 7.01Ac
1 d	35.00 ± 3.91ABab	25.00 ± 3.00Ba	90.00 ± 7.48Ac
2 d	75.00 ± 6.66Cb	45.00 ± 4.70Ca	92.50 ± 7.61Ac
CV	9.61%		

Averages followed by the same capital letter per column or lower case letter per line did not differ by the Tukey test ($P < 0.05$). CV, coefficient of variation.

Table IV. Hatching (%) of caterpillars from groups of recently laid or one- or two-day-old *Diatraea saccharalis* (Lepidoptera: Pyralidae) eggs, eight days after application of 1% or 2% water-based neem extract in Sete Lagoas, Minas Gerais, Brazil.

Age of eggs	Hatching (%) of caterpillars of <i>Diatraea saccharalis</i>		
	1% Neem extract	2% Neem extract	Water
Recently laid	0.00 ± 0.00Aa	0.00 ± 0.00Aa	82.30 ± 7.07Ab
1 d	0.00 ± 0.00Aa	0.00 ± 0.00Aa	87.60 ± 7.35Ab
2 d	1.00 ± 0.01Aa	0.00 ± 0.00Aa	93.20 ± 7.65Ab
CV	9.65%		

Averages followed by the same capital letter per column or lower case letter per line did not differ by the Tukey test ($P < 0.05$). CV, coefficient of variation.

Table V. Hatching (%) of caterpillars from groups of recently laid or one- or two-day-old *Diatraea saccharalis* (Lepidoptera: Pyralidae) eggs, eight days after application of 1% or 2% pyroligneous extract in Sete Lagoas, Minas Gerais, Brazil.

Age of eggs	Hatching (%) of caterpillars of <i>Diatraea saccharalis</i>		
	1% Pyroligneous extract	2% Pyroligneous extract	Water
Recently laid	27.30 ± 3.22Aa	42.20 ± 4.49Ab	82.30 ± 7.07Ac
1 d	28.40 ± 3.32Aa	48.70 ± 4.97Bb	87.60 ± 7.35Ac
2 d	37.80 ± 4.14Ba	56.60 ± 5.52Cb	93.20 ± 7.65Ac
CV	8.11%		

Averages followed by the same capital letter per column or lower case letter per line did not differ by the Tukey test ($P < 0.05$). CV, coefficient of variation.

the age of one or two days with 1% neem extract seems to suggest a greater resistance of the latter to natural products, which may be due to the higher level of hardness and difficulty of penetration for external products, which can be verified by the mortality of 97.7% of *S. frugiperda* eggs at the age of one day, treated with *Lychnophora ericoides* Mart. (Asteraceae) or *Trichogonia villosa* Sch. Bip. Ex. Baker (Asteraceae), and of 72.0% for two-day-old insect eggs treated with *Lepidaploa lilacina* (Mart. Ex. DC.) H. Rob. (Asteraceae) (Tavares *et al.*, 2009). Neonate and young caterpillars of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) were more susceptible to extracts of *Piper tuberculatum* Jacq. (Piperaceae) than four-day-old ones, while the extract of *Piper nigrum* L. (Piperaceae) reduced the larval survival rate of this insect up to 70% (Scott *et al.*, 2003). These results suggest that younger insects of some species are more susceptible to botanic extracts. However, this was not observed for the eggs of *D. saccharalis* with 1% or 2% neem extracts, which suggests a higher level of sensitivity of egg masses of this species to external agents. The penetration and action of neem compounds caused a higher

mortality rate at the cellular level of insects, as observed for isolated limonoids of this plant on *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Anuradha *et al.*, 2007) with interference in the growth regulation hormones and with the metamorphosis and reproduction of insects (Huang *et al.*, 2007). However, other neem compounds, such as neembin and salannin, were also toxic to the insects (Jarvis *et al.*, 1997).

The similar mortality of *S. frugiperda* eggs of different ages with 2% neem extract confirms that this content was enough to reduce the hatching of caterpillars of this species in the laboratory, which gives evidence of the impact of neem extracts on Lepidoptera pests. Their more active compounds belong to the limonoid class, and azadirachtin is the main compound which acts on insects, its main source being the fruits along with the peel and the leaves (Schmutterer, 1990). Nevertheless, the similar mortality of recently laid eggs and of one- or two-day-old ones of *S. frugiperda* and *D. saccharalis* with the 1% or 2% neem extract shows a satisfactory impact of the extracts on these insects. The harmful effect of neem was also observed for the lower hatching of caterpillars

from egg masses of *Mamestra brassicae* L. (Lepidoptera: Noctuidae) (Seljasen and Meadow, 2006) and in the fifth instar of *Streblote panda* Hübner (Lepidoptera: Lasiocampidae) caterpillars (Calvo and Molina, 2003). Also, other botanic products were harmful to the Lepidoptera, such as *Porteresia coarctata* Takeoka (Poaceae) to *Spodoptera litura* F. (Lepidoptera: Noctuidae) (Ulrichs *et al.*, 2008) and *Gliricidia sepium* Jacquin (Leguminosae) to corn pests (Montes-Molina *et al.*, 2008). In addition to the ovicide effect, neem causes repellence and feeding reduction in the insects, interfering with the growth regulation hormones, metamorphosis and reproduction. The action on the biological cycle is shown through the longevity of the adults of *S. frugiperda* caterpillars, since the water-based extract of neem reduces feeding, development and, later, causes the death of the caterpillars via ingestion of corn leaves treated with the extract (Schmutterer, 1990).

The higher rate of hatching of caterpillars from *D. saccharalis* and *S. frugiperda* eggs at the age of two days, as compared to that of recently laid or one-day-old insect eggs, with the pyroligneous extract suggests that the older insects are more resistant to this extract. This might be due to the more advanced maturation of the eggs, which makes it difficult to let external agents in (Tavares *et al.*, 2009). The lower hatching rate of caterpillars from eggs of *D. saccharalis* and of *S. frugiperda* with 2% pyroligneous extract suggests that egg masses of these pests might have different susceptibilities to the same extract concentrations. Products based on pyroligneous extract present the possibility of being used for *D. saccharalis* and *S. frugiperda*, mainly the 2% pyroligneous extract for recently laid egg masses or for one-day-old ones. However, the efficiency of botanic products on insects might vary with UV radiation, temperature, and solvent used, or with the concentration of the extract (Moreira *et al.*, 2007). The genetics of the plant, the extraction time of the compounds (Sidhu *et al.*, 2004), leaf areas, organic compounds of leaves, density of leaf trichomes (Leite *et al.*, 2007), and the part of the plant used for producing the extracts further influence the activity of some compounds against insects (Siskos *et al.*, 2007). In addition this product is more efficient in the field, since it activates substances of the secondary metabolism of the plants and thus improving resistance (Tsuzuki *et al.*, 2000).

The prohibition of the use of synthetic products in areas having organic agriculture makes the use of botanic products for the control of insects important. On the other hand, the emergence of adults of *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) or *Trichogramma exiguum* Pinto and Platner (Hymenoptera: Trichogrammatidae) from eggs of *Plutella xylostella* L. (Lepidoptera: Plutellidae) after application of neem, pyroligneous extract or a mixture of these products with synthetic insecticides (Thuler *et al.*, 2008) and that of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) parasitizing eggs of *S. frugiperda* treated with extracts of Asteraceae (Tavares *et al.*, 2009) was lower, suggesting that the use of these products might diminish the emergence of natural enemies from parasitized egg masses. Other botanic products, such as neem, astilbin, and Asteraceae extracts caused mortality of pests [*Oligonychus ilicis* McGregor (Acari: Tetranychidae)] (Mourão *et al.*, 2004a), pollinating insects [*Apis mellifera* L. (Hymenoptera: Apidae)] (Cintra *et al.*, 2005a), leaf-cutting ant [*Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae)] (Cintra *et al.*, 2005b), predators [*Iphiseiodes zuluagai* Denmark and Muma (Acari: Phytoseiidae)] (Mourão *et al.*, 2004b), and parasitoids [*Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae) and *Telenomus remus* Nixon (Hymenoptera: Scelionidae)] (Tavares *et al.*, 2009). *Bacillus thuringiensis* also affected *T. pretiosum* parasitizing eggs of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) (Vianna *et al.*, 2009). The two latter examples suggest that plant extracts or biological products applied to parasitized eggs might decrease the hatching of insects and, therefore, harm the biological control of natural enemies' important tactics of integrated pest management (Matos Neto *et al.*, 2004, 2005).

The mortality of eggs of different ages of *S. frugiperda* and *D. saccharalis* treated with different concentrations of neem or pyroligneous extracts was satisfactory in the laboratory, thus suggesting, that they might be used in the control of these pests.

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