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Effect of *Jatropha gossypiifolia* leaf extracts on three Lepidoptera species

Efecto de extractos de hojas de *Jatropha gossypiifolia* sobre tres especies de Lepidoptera

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Abstract. Leaf extracts of *Jatropha gossypiifolia* L. (Euphorbiaceae) contain compounds that are toxic to insects. In this study, these extracts were tested against larvae of three lepidopteran species, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae) and *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae), which are important pests of maize in Africa, Europe and Mediterranean countries, respectively. Leaf extracts were shown to be highly toxic to neonate larvae of *B. fusca* and *O. nubilalis* quickly after they were ingested. In contrast, no effect was found on fourth instar *O. nubilalis* and a low level of toxicity was observed on neonates of *S. nonagrioides*. Given the toxicity of *J. gossypiifolia* to larval neonates of *B. fusca* and *O. nubilalis*, this extract can be used for the control of these species when they are colonizing the plant.

Key words: Euphorbiaceae. Noctuidae. *Busseola fusca*. Pyralidae. *Ostrinia nubilalis*. *Sesamia nonagrioides*. LC_{50}

Resumen. Los extractos foliares de *Jatropha gossypiifolia* L. (Euphorbiaceae) contienen compuestos que son tóxicos a los insectos. En este estudio, se probaron estos extractos sobre las larvas de tres especies de lepidópteros, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae), *Ostrinia nubilalis* Hubner (Lepidoptera: Pyralidae) y *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae); las cuales son plagas importantes del maíz en África, Europa y países mediterráneos respectivamente. Se encontró que los extractos foliares eran altamente tóxicos contra larvas neonatas de *B. fusca* y *O. nubilalis* rápidamente después de que fueron ingeridos. En contraste, no se encontró toxicidad frente a larvas en cuarto instar de *O. nubilalis* y un bajo nivel de toxicidad fue observado con neonatos de *S. nonagrioides*. Debido a la toxicidad de *J. gossypiifolia* hacia larvas neonatas de *B. fusca* y *O. nubilalis*, se puede usar este extracto para el control de estas especies cuando están colonizando plantas de maíz.

Palabras clave: Euphorbiaceae. Noctuidae. *Busseola fusca*. Pyralidae. *Ostrinia nubilalis*. *Sesamia nonagrioides*. LC_{50}

Introduction

Maize and sorghum are two important food crops in Africa for commercial and resource-poor small-scale farmers (Kfir 1998; Seshu Reddy 1998); these crops are cultivated primarily for human consumption, and surpluses are used for feeding livestock (Sibanda 1985). In Africa, the productivity of these crops is very low partly due to the damage caused by lepidopteran stemborers (Haile and Hofsvang 2002). Among them, the stem borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) is considered one of the major insect pests (Kfir *et al*. 2002).

The use of insecticides to control stem borers has been proven inefficient due to the cryptic habit of the larvae, which protects them against insecticide sprays. In addition, insecticides are generally not favorable in durable pest management systems due to eco-toxicity and are not affordable to African peasant farmers. Botanicals are one of the alternatives considered environmentally friendly.

This method does not only reduce application of synthetic insecticides, but also reduce the cost with pest management, which is an important factor for farmers in developing countries. The efficacy of botanicals are largely demonstrated in grain storage insects (Huang *et al.* 1997, 2000; Liu and Ho 1999; Dal Bello *et al.* 2001; Taponjou *et al*. 2002). Furthermore, extracts from the Indian neem tree, *Azadirachta indica* A. Juss. (Meliaceae), are widely used to control various insect species (Saxena 1989; Schmutterer 1990).

The bellyache bush (*Jatropha gossypiifolia* L. [Euphorbiaceae]), native of tropical America is now widespread in the tropics. It is used for medicinal purposes in Africa, Thailand and tropical America and is cultivated as an ornamental plant in Florida. Few insects have been observed to be associated with this plant species apart of a single whitefly species (Sauvion N., pers. observ.) and occasional infestations by thrips and a polyphagous mealybug species (Calatayud P.-A., pers.

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observ.). Leaf extracts of the plant were shown to be toxic to *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and *Phenacoccus herreni* William & Cock (Sternorrhyncha: Pseudococcidae) (Dev and Koul 1997; CIAT 2001). To our knowledge, nothing have been reported showing toxicity of *J. gossypiifolia* leaves to Lepidotera insects.

The purpose of this work was to evaluate the toxicity of *J. gossypiifolia* leaf extracts towards *B. fusca*, to *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), and to *Sesamia nonagrioides* Lef. (Lepidoptera: Noctuidae), which are important pests in many countries in Africa and Europe respectively.

Materials and Methods

Insects and plant material. *Busseola fusca* (Fuller) was provided by the ICIPE mass-rearing unit (Nairobi, Kenya) and was reared on an artificial diet under laboratory conditions (Onyango and Ochieng'-Odero 1994). To regenerate the colony, new insects collected from the field were added three times per year. *Ostrinia nubilalis* and *Sesamia nonagrioides*. larvae were collected from the South part of France and then reared for more than two generations on an artificial diet at the Institut National de Recherche Agronomique (INRA, Versailles, France) (Poitout and Bues 1974). The insects were maintained in a controlled chamber under the following conditions: 25.3 ± 0.9 °C, 68.6 ± 12.8 % r.h. (means ± SE) and L12:D12 reversed photoperiod. Both neonate larvae (0 to 24 h old) and fourth instar larvae (about 20-25 days old) were used in these experiments. All experiments were carried out in France at the INRA, Versailles.

Two-month old *J. gossypiifolia* L. plants were used. Cuttings were planted in individual cylindrical plastic pots (ID = 30 cm; height = 22 cm) containing peat and sand, and kept in a glasshouse at 28-35 °C and L12:D12 photoperiod. Only mature leaves were selected for active material extractions (CIAT 2001).

Leaf sample collection and crude extraction method. Leaves were harvested and frozen in liquid nitrogen. Thirty minutes later, extraction, based on the method described by Valencia-Jiménez et al. (2000) for plant proteins, was performed. One gram of fresh leaves was powdered in a mortar containing liquid nitrogen. Thereafter, 4 ml of 0.1 M sodium chloride solution were added. The mixture was stirred for 6h at 4 °C. The slurry was filtered and centrifuged at 8,000 rpm at 4°C during 20 min. The supernatant was centrifuged one more time until no pellet was obtained. Then, the supernatant was dialyzed against water with a MWCO 3.5 kDa cellulose membrane at 2 - 4°C for 3 days and freeze-dried. The resulting powder was stored at –20°C and used for toxicity experiments.

Toxicity tests. Leaf extract was added to the artificial diet of Onyango and Ochieng'-Odero (1994) at six concentrations ranging from 0.01 to 100 mg/ml. Its toxic effect was tested on *B. fusca* larvae. The highest concentration (100 mg/ml) was also tested on larvae of *O. nubilalis* and *S. nonagrioides* by mixing the extract with the artificial diet of Poitout and Bues (1974). In another experiment, the leaf extract was boiled for 10 min before adding it to the artificial diet, and then the toxicity was tested at 100 mg/ml for each Lepidoptera species.

Five individual insects were introduced into a Petri dish (∅ 35 mm) containing 1g of diet. Mortalities were recorded 24 and 48 hours later. Each test was replicated four times. To verify that the larvae fed normally, a mixture containing diet and 1% (w/w) of bromocresol purple pH indicator as described by Sinha (1959) was prepared and used in the same condition.

All experiments were conducted at 25.3 ± 0.9 °C, 68.6 ± 12.8 % r.h. (means ± SE) and L12:D12 reversed photoperiod. For each experiment, the controls corresponded to the meridic diets without leaf extract.

Data analysis. Statistical analyses were performed with Statview version 5.0 (© 1998, SAS Institute Inc., Abacus Concept, USA). When possible to calculate the variance, homogeneity of variance and data normality were examined by *F*-test and Kolmogorov-Smirnov methods respectively, before running the ANOVAs. All proportions were transformed to arcsin before being subjected to ANOVA. Fisher's PLSD (Protected Least Significant Difference) test was used for mean separation. For *B. fusca* neonates, the four replicates used for each leaf extract concentration yielded four mortality percentages, allowing toxicity indices to be calculated. The Log (C) and probit (%) transformations were used to calculate LC_{50} and LC_{90}, and their confidence intervals at 5% level (Bliss 1935). A program allowing the easy calculation and statistical analyses of these indices is freely available from the authors (Febvay and Rahbé 1991).

Results and discussion

The effect on the mortality of *B. fusca* neonate larvaes was analyzed using six leaf extract concentrations. The results are presented in Table 1. With the increase in the concentration, a significantly level of larval mortality were found at both, after 24 and 48 hours of feeding, indicating that *J. gossypiifolia* leaf extracts are highly toxic to the insect. The LC_{50} and LC_{90} after 24 hours were 0.9 and 79 mg/ml, respectively. After 48 hours feeding, only the LC_{90} could be calculated for the lower concentration (9 mg/ml). A high mortality at 70% was still obtained with the lowest concentration tested.

The highest mortality of *B. fusca* after 24 hours was found at 100 mg/ml. Based on this data, the toxicity of *J. gossypiifolia* leaf extracts to both *O. nubilalis* and *S. nonagrioides* was determined using this concentration.

The table 2 shows that 100 mg/ml of extract in the artificial diet induced 75% and 100% of mortality of neonates of *O.*

Table 1. Percent mortality, LC_{50} and LC_{90} of *B. fusca* neonate larvae due to exposure to leaf extract of *J. gossypiifolia* at different concentrations in the diet.

Extract concentration in the diet (mg/ml)	%[1] Mortality (means ± SE[2]) 24h after	48h after
0	0 a	0 a
0.01	30.0 ± 10.0 b	70.0 ± 7.0 b
0.1	45.0 ± 17.1 c	80.0 ± 6.2 bc
1	50.0 ± 5.8 bc	85.0 ± 5.8 bc
10	65.0 ± 5.0 cd	90.0 ± 10.0 c
50	70.0 ± 5.8 d	100 d
100	100 e	100 d
LC_{50} [confidence interval, *P*=0.05] (mg/ml)	0.9 [0.3-1.5]	not possible to calculate
LC_{90} [confidence interval, *P*=0.05] (mg/ml)	79 [67-91]	9 [6-12]

[1]% without correction; [2]Means followed by the same letter are not significantly different at 5% level (Fisher's PLSD test following ANOVA).

Table 2. Percent mortality of *B. fusca*, *O. nubilalis* and *S. nonagrioides* neonate larvae due to exposure to leaf extract of *J. gossypiifolia* at 100 mg/ml in the diet before or after boiling.

Insect	% Mortality (means ± SE[1], n=20)	
B. fusca	**24h after**	**48h after**
Control diet	0	0
With extract	100	100
With extract (after boiling)	100	100
O. nubilalis		
Control diet	5.0 ± 5.0 a	10.0 ± 5.8 a
With extract	75.0 ± 9.6 b	100 b
With extract (after boiling)	65.0 ± 9.6 b	100 b
S. nonagrioides		
Control diet	5.0 ± 5.0	10.0 ± 10.0
With extract	25.0 ± 12.6	45.0 ± 9.6
With extract (after boiling)	20.0 ± 8.2	20.0 ± 8.2

[1]Means followed by the same letter are not significantly different at 5% level (Fisher's PLSD test following ANOVA). For *B. fusca*, no letter was given because the data do not fit ANOVA requirements. For *S. nonagrioides*, no letter was given because p>0.05 for ANOVA.

nubilalis after 24 hour and 48 hour of feeding period, respectively. In contrast, no effects were found when the same extract was evaluated with *S. nonagrioides* larvae.

For each insect species, the bromocresol-containing diet induced a color change of the larvae intestinal duct, after 60 min of feeding. This confirms that larvae fed normally on the modified diet and indicate that the mortality was linked to diet toxicity. Moreover, its toxicity to *B. fusca* and *O. nubilalis* was not affected after boiling the leaf extracts (Table 2), indicating that the toxicity could be due to thermo-stable compound(s). For the fourth instar larvae of *B. fusca*, no mortality was recorded after 24 hours at 100 mg/ml; however 70% of mortality was obtained after 48 hours. Thus, the toxicity of *J. gossypiifolia* leaf extract for *B. fusca* decreases with the age of the larvae. In the case of *O. nubilalis* and *S. nonagrioides*, no mortality was recorded at 100 mg/ml in the diet after 48 hours or after five days. All species larvae showed a colored intestinal tract when these had been fed on the diet containing pH indicator, thus, the low toxicity on old larvae was not related with starvation but more probably to an increased tolerance of the larvae to the toxin.

In conclusion, *J. gossypiifolia* leaf extracts demonstrated to be highly toxic to both *B. fusca* and *O. nubilalis*. Neonate larvae revealed to be more sensitive than older larval stage. Other reports demonstrated the presence of several secondary compounds from *J. gossypiifolia* leaves and its implication in the toxicity, including flavonoids (e.g. apigenin, isovitexin, vitexin) and diterpenoids (e.g. jatrophone) (Kupchan *et al*. 1970; Subramanian *et al*. 1971). However, these compounds are generally not water soluble, and thus could not have been extracted from the leaves in our study. In addition, these molecules possess a molecular weight lower than 3.5 kDa and would have been removed during the dialysis. Only molecules with molecular weight greater than 3.5 kDa could therefore be involved in the toxicity. Also, Euphobiaceae plants are known to possess polyisoprenes with high molecular weights, in the form of latex (Archer 1980). Such compounds are mostly soluble in organic solvents such as benzene and chloroform. Their presence in the leaf extract described here can be ruled out. Therefore, compounds having molecular weights of over 3.5 kDa and a thermostable characteristic appeared as the most plausible chemical involved in the toxicity for moth neonates.

Plant extracts have been proven successful for the control of grain storage insects in the form of essential oil from plant leaves (Taponjou *et al*. 2002), specially using neem seed oil (Schmutterer 1990). To control maize stemborers, the treatments should be aimed at the first instar, when these migrate from the oviposition site to the whorl, where the larval feeding causes conspicuous leaf damage. However, such extract will probably not control all Lepidoptera species to the same extent, as a quasi-absence of toxicity was found in *S. nonagrioides*. Additionally, a formulation and an easier process to extract the leaves should be developed.

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