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Short note

Effect of Some Environmental Factors on the Toxicity of Azadirachtin to the Egyptian Cotton Leafworm Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae)

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Abstract. Although the bio-degradation of azadirachtin was well studied under storage or environmental conditions, its toxicity was not characterized so far under the impact of environmental conditions. Therefore, the present study aims to elucidate the toxicity of azadirachtin, at the level of LC_{50}, to the fourth larval instars of *Spodoptera littoralis* (Boisduval) under the stress of temperature, ultraviolet radiation (365 nm) (UV-A) and of artificial sunlight. The increase in post-treatment temperatures insignificantly affected azadirachtin toxicity. Likewise, insignificant change was also attained for the increase in the exposure periods of both UV and sunlight, except for exposure to sunlight without UV filter starting from 10 h, where the toxicity of azadirachtin was significantly declined. It can be concluded that azadirachtin toxicity will last efficiently against *S. littoralis* larvae under the stress of the environmental factors prevailing during the season of cotton cultivation in Egypt.

Key words: Azadirachtin, *Spodoptera littoralis*, sunlight, temperature, toxicity, ultraviolet.

Introduction

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisduval) is the major cotton pest in Egypt. The use of synthetic pesticides during the last half century has often been careless and indiscriminate which resulted in malicious effects on the environment and leads to "ecological backlash" (SUNDARARAJ, 1997). Concern about this has led to a surge of research into alternative pest control approaches. One of the efforts is the development of botanical insecticides as a novel and safer strategy. Azadirachtin is one of the most promising natural compounds of plant origin. It is a tetranortriterpenoid isolated from the seeds of the neem tree, *Azadirachta indica* A. Juss (Meliaceae) acting as an insect growth regulator (REMBOLD *et al*., 1982), and displayed insecticidal activity to nearly 550 insect species including lepidopteran ones (DEBASHRI & TAMAL, 2012; HUMMEL *et al*., 2012). Moreover, azadirachtin seems to be selective, non-mutagenic, readily degradable, with low toxicity to non-target and beneficial organisms and causes minimal disruption to ecosystem (SUNDARAM, 1996). Recently, RAIZADA *et al*. (2001) proved that azadirachtin did not produce any signs of toxicity, mortality, and change in tissue weight, pathogenicity and serum blood parameters to mammals.

Most studies dealt with the bio-degradation of azadirachtin under storage or environmental conditions (SCHMUTTERER, 1988; SUNDARAM, 1996; RAM *et al*., 2001; RADWAN & EL-SHIEKH, 2012). However, there are no studies dealt directly with its toxicity under the influence of environmental factors. Therefore, the

present study aims to explore the toxicity of azadirachtin to *S. littoralis* fourth larval instars under the stress of temperature, ultraviolet radiation (UV-A) and artificial sunlight.

Material and Methods

Insects. A susceptible strain of *S. littoralis* was established from egg masses obtained from Faculty of Agriculture, Cairo University, Egypt. Newly hatched larvae were transferred into plastic boxes (18×12×10 cm) containing about 0.5 cm thick semi-synthetic diet on which larvae was fed (SHOREY & HALE, 1965). The pupae were sexed and placed in sterilized containers (18× 15×30 cm) for adult emergence. The containers were lined with tissue paper as an ovipositing stratum. Adult moths were fed 10% sugar solution. Insects were reared under laboratory conditions of 25-27°C, 65-70% RH and 12h: 12 h (L: D) photoperiod.

Azadirachtin. Azadirachtin (96% purity) was obtained as a powder from Carl Roth GmbH + Co. Kg. A stock solution (200 ppm) was prepared in sterile distilled water and stored at 4°C till use.

Bioassay. A preliminary experiment was carried out to determine the toxicity of azadirachtin against the fourth larval instars of *S. littoralis*. Six serial concentrations (50, 25, 12.5, 6.25, 3.125 and 1.5625 ppm) of azadirachtin-acetone solution were prepared. One ml of each concentration was poured onto the surface of the same semi-synthetic diet used for rearing, thoroughly swirled and air-dried. The treated diet was offered for 24 h to newly-molted fourth larval instars, pre-starved for 4 h. Then, the larvae were fed on fresh non-treated diet until pupation or death. The control experiment consisted of larvae fed on semi-synthetic diet treated with distilled water only. Each concentration was repeated three times of 50 larvae each (Total n = 150 larvae). All the experiments were incubated at 25°C. The percentage of mortality was recorded.

Temperature. Five groups (50 larvae each) of newly molted fourth larval instars of *S. littoralis* were fed on semi-synthetic diet of a surface area of 500 mm^2 treated with one ml of 23.15 ppm azadirachtin-acetone solution (LC_{50}). These groups were kept respectively at five controlled temperature regimes (15, 20, 25, 30 and 35°C). The control experiments consisted of larvae fed on a diet treated with acetone only and kept also at the same tested temperatures. Larval mortality was observed until pupation or death for determining the toxicity of azadirachtin. Each experiment was replicated three times.

UV radiation. Sunlight-UV was simulated using a series of Vilber-Lourmat T-15 LN fluorescent lamps, which primarily radiate energy in the UV-A (315-400 nm) and UV-B range (280-315 nm), with a peak at 365 nm (IGNOFFO *et al.*, 1997). These lamps were held into a heat-controlled chamber (20°C). Eight glass Petri dishes containing one ml of the LC_{50} of azadirachtin-acetone solution each were respectively irradiated for 2, 5 and 10 min, and for 1, 3, 5, 10 and 24 h. Azadirachtin solutions were set at a vertical distance of 30 cm from T-15 LN UV lamps. Another eight dishes, each containing one ml of the LC_{50} of azadirachtin-acetone solution covered with aluminum foil were used as the shielded samples. These dishes were then exposed to the same previous exposure periods. Azadirachtin-acetone solution, which was not exposed to UV and the non-azadirachtin sample (non-treated control) were also run. After exposure to UV radiation, all azadirachtin samples (non-shielded exposed, shielded exposed, non-exposed and non-treated control samples) were then dispersed onto the surface of the semi-synthetic diet for determining their toxicity against newly molted fourth larval instars of *S. littoralis*. Each experiment was repeated three times of 50 larvae each.

Sunlight radiation. The sunlight radiation experiments were carried out using a sunlight simulator (ELDONET), which measures solar or artificial radiation in three biological important spectral bands: UV-B (280-315 nm), UV-A (315-400 nm) and PAR (photosynthetic active radiation, 400-700 nm). Azadirachtin-acetone solutions, at the level of LC_{50}, were exposed to sunlight for 1, 3, 5, 7, 10 and 12 h. One ml was used for each exposure period. The samples were

surrounded by ice to avoid the increase of the temperature absorbed by the sample solution, and set at a vertical distance of 30 cm from the light source. Non-exposed and non-treated samples were prepared. After exposure to sunlight, all azadirachtin-acetone samples were dispersed onto the semi-synthetic diet surface for determining their toxicity against newly molted fourth larval instars of *S. littoralis*. Each experiment was repeated three times of 50 larvae each. The same previously described procedures were also carried out, except for the use of UV filter to eliminate UV radiation.

Statistical analysis. The percentage of mortality of treated larvae was corrected against that of non - treated ones (control) using Abbott's formula (ABBOTT, 1925) as follows: Corrected mortality (%) = (% Observed mortality - % Control mortality/100 - % Control mortality) 100. The corrected mortality was then subjected to propit analysis (FINNEY, 1971), from which the LC_{50} was estimated. The toxicity bioassay was determined with POLO–PC software (LEORA SOFTWARE, 1987).

Data were analyzed by one–way analysis of variance (ANOVA) using Costal Statistical Software (Cohort Software, Berkeley). When the ANOVA statistics were significant (at $p<0.05$), the means were compared by Duncan's multiple range test (SAS INSTITUTE INC., 2008).

Results and Discussion

The tested temperatures insignificantly affected the toxicity of azadirachtin against *S. littoralis* larvae, where the percentage of mortality remained approximately around the 50% (> 44 to ≤ 50%) (Table 1). Whereas, it was found that heat storage at 72 ± 2°C for 3 days decreased the insecticidal activity of commercial neem oil against *S. littoralis* larvae (RADWAN & EL-SHIEKH, 2012). They speculated that as the commercial neem formulations contain not only azadirachtin, but also other minor potential bioactive liminoids, the insecticidal effect of the preparation is more complex than that of the pure azadirachtin. Moreover, heat storage reduced significantly azadirachtin bioactivity against *Spodoptera litura* larvae compared to the refrigerated storage (RAM *et al.*, 2001).

Table 1. Toxicity of azadirachtin to *Spodoptera littoralis* fourth larval instars treated with the LC_{50} (23.15 ppm) under different temperature regimes.

Temperature °C	% Mortality ± SE
15	48.01 ± 4.2[a]
20	49.44 ± 3.9[a]
25	44.50 ± 4.2[a]
30	47.21 ± 5.7[a]
35	49.92 ± 2.8[a]

Each experiment was repeated three times of 50 larvae each. Figures within columns followed by different letters were significantly different from each other ($P < 0.05$), using one-way analysis of variance (ANOVA).

The present results indicate that the toxicity of azadirachtin against *S. littoralis* larvae was insignificantly affected by exposure to UV radiation (UV-A) up to 24 h in both shielded and non-shielded samples, where the percentage of larval mortality was around 50% (Table 2). In comparison, at least 200 h of exposure to UV radiation was necessary to significantly reduce the biological activity of azadirachtin (BARNBY *et al.*, 1989).

Table 3 shows that exposure of azadirachtin to sunlight up to 12 h using a UV filter insignificantly affected its toxicity to *S. littoralis* fourth larval instars. The same pattern was also attained up to 7 h of exposure without using a UV filter, whereas toxicity of the same samples exposed to 10 and 12 h was significantly decreased by about 11.2 and 17.8 %, respectively. These results are more or less similar to the findings of JOHNSON *et al.* (2003) which indicated that the biological activity of azadirachtin had been retained even after 30 days of exposure to sunlight when some stabilizers were added. Also, SCHMUTTERER (1988) reported that the effect of azadirachtin-containing extracts normally lasts about 4-8 days under field conditions.

Table 2. Toxicity of azadirachtin to *Spodoptera littoralis* fourth larval instars treated with the LC_{50} (23.15 ppm) under different exposure periods of ultraviolet radiation (UV-A) (365 nm)

Exposure time	% Mortality ± SE	
	Non-shielded samples	Shielded samples
0 min	48.8 ± 8.6[a]	50.0 ± 3.7[a]
2 min	49.4 ± 6.7[a]	50.0 ± 3.4[a]
5 min	48.8 ± 8.6[a]	50.0 ± 2.9[a]
10 min	48.3 ± 7.1[a]	49.4 ± 3.0[a]
1 h	50.0 ± 9.3[a]	50.0 ± 4.1[a]
3 h	48.9 ± 6.1[a]	47.0 ± 3.9[a]
5 h	50.0 ± 5.3[a]	48.9 ± 2.4[a]
10 h	47.7 ± 4.0[a]	50.0 ± 2.9[a]
24 h	48.3 ± 2.5[a]	50.0 ± 3.7[a]

Each experiment was repeated 3 times of 50 larvae each. Figures within columns followed by different letters were significantly different from each other ($P < 0.05$), using one-way analysis of variance (ANOVA).

Table 3. Toxicity of azadirachtin to *Spodoptera littoralis* fourth larval instars treated with the LC_{50} (23.15 ppm) under different exposure periods of artificial sunlight

Exposure time (h)	% Mortality ± SE	
	Without UV filter	With UV filter
0	50.0 ± 4.7[a]	50.0 ± 3.7[a]
1	50.0 ± 5.1[a]	50.0 ± 2.6[a]
3	48.9 ± 4.9[a]	50.0 ± 1.1[a]
5	48.8 ± 3.6[a]	50.0 ± 2.1[a]
7	47.7 ± 2.3[a]	50.0 ± 3.6[a]
10	44.4 ± 1.7[b]	50.0 ± 1.7[a]
12	41.1 ± 3.7[b]	49.4 ± 1.3[a]

Each experiment was repeated 3 times of 50 larvae each. Figures within columns followed by different letters were significantly different from each other ($P < 0.05$), using one-way analysis of variance (ANOVA).

Conclusions

The present study evaluated the toxicity of azadirachtin under the stress of some environmental factors which are comparable to those prevailing during the season of cotton cultivation in Egypt. The findings the toxicity of azadirachtin was not affected up to 35˚C and up to 24 h of exposure to UV in both shielded and non-shielded samples indicate that its toxicity against *S. littoralis* larvae will last effective under the temperature prevailing during the season of cotton cultivation in Egypt (27-35˚C), and at any time of the photophase.

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