Galleria mellonella (L.) (Pyralidae) and *Spodoptera exigua* (Hübner) (Noctuidae): Differences in effects of XenTari® (*Bacillus thuringiensis aizawai*), NeemAzal T/S® and their combinations on survival

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Abstract. *Galleria mellonella* (L.) (Pyralidae) und *Spodoptera exigua* (Hübner) (Noctuidae): Wirkungsunterschiede zwischen XenTari® (*Bacillus thuringiensis aizawai*), NeemAzal T/S® und ihren Kombinationen

Im Labor wurden die Larven mit trockenem Futter versorgt, das vorher für 20 Sek. in die Testlösung getaucht war. Die Testlösungen, auch in der Kontrolle, wurden mit dem anionischen Detergens Triton X 100 versetzt (10%, davon 0.1 ml). XenTari wurde mit 0.5, 1 und 2 mg/l getestet, NeemAzal mit 2, 4 und 8 mg/l. Bei *G. mellonella* war die höchste korrigierte Mortalität bei XenTari nach 4 Wochen 77%, bei NeemAzal T/S 100%. So wird letzteres empfohlen. Bei *Spodoptera exigua* im zweiten Larvenstadium bewirkte XenTari nach 3 Wochen eine maximale Wirkung von 95.6%, während NeemAzal T/S bereits nach 7 Tagen 100% erreichte. Im vierten Larvenstadium erreichte NeemAzal TS eine Mortalität von 46%. Wurden aber beide Präparate bei halber Dosis nacheinander appliziert, war die Wirkung nach 5 Tagen 73% (XenTari zuerst) bzw. 98.8% (NeemAzal T/S zuerst). Letztere Kombination wird für den Bauwollanbau in den Tropen empfohlen, um durch deren Zwischenschaltung die Resistenzbildung gegenüber synthetischen Insektiziden zu vermindern.

Key words: *Galleria mellonella*, *Spodoptera exigua*, XenTari®, NeemAzal T/S®, combinations, mortality

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Both, *G. mellonella* and *S. exigua*, are most important pests in tropical countries. *G. mellonella* has five to six generations per year (Abid et al. 1997; Ali 1996), there, and feeding in bee combs they find, besides wax, residues of honey, insect skin and pollen (Hachiro & Knox 2000). Li et al. (1987) have shown the efficacy of *Bacillus thuringiensis aizawai* against *G. mellonella*. It is registered in the EU as 'Mellonex' for its control, but NeemAzal T/S may also be active, and will have some advantages (Leymann et al. 2000, Melathopoulos et al. 2000). Therefore we conducted new studies here, on the results we shall report.

S. exigua is an important polyphagous pest of crops in tropical areas (Brown & Dewhurst 1975). By repeated control with synthetic insecticides, especially by illiterate farmers (Armes et al. 1992; Aggarwal et al. 2006a) resistance to a lot of those insecticides has been built up, making plant protection very difficult. Therefore the need is pronounced for microbial and botanical pesticides (Nagarkatti 1982; Rao et al. 1990), which have different modes of action than synthetic insecticides. Aggarwal et al. (2006b) have started to test such ingredients, but the time of observation was too short (3 days), since the effects of Neem products occur later than those of synthetic insecticides (Basedow et al. 2002). So we conducted new, longer lasting experiments (with 5 to 30 days), on which we give a report here. The experiments were conducted during guest stays of the three co-authors (from Mymensingh, Bangladesh, from Nazreth, Ethiopia, and from Khartoum, Sudan) at the Experimental Station of the Institute of Phytopathology and Applied Zoology at Giessen Univerity.

Material and Methods

G. mellonella larvae were bought from a zoo shop. They were kept on artificial diet ("Hayduck medium") at 32°C at dark. *Spodoptera exigua* eggs/larvae were obtained from the Laboratory for Entomology, Bayer AG,

Monheim, Germany. They were kept at 23°C at 14h light and 10h dark. For experiments, food of larvae was dipped for 20 sec into the suspension of the ingredients, with addition of Triton X 100 as non-ionic detergent (10%, 1 ml/100ml). Untreated food was dipped into water plus Triton X 100. After dipping, the food was allowed to dry for 3 min, and then it was transferred to round glass jars with a metal lid in *G. mellonella*, and to petri dishes (9 cm Ø) in *S. exigua*, with filter paper moistened daily. Then the larvae were added. *G. mellonella* received 2 g bee wax per two larvae/jar as food, *S. exigua* leaflets of *Vicia faba*. When the treated food in the course of the experiments was eaten up, new untreated food was offered (Hayduck-medium or *Vicia faba* leaflets). Per treatment, in *G. mellonella*, 24 larvae were taken, while in *S. exigua* these were 25, with 4 replicates.

XenTari® (commercial preparation of *Bacillus thuringiensis aizawai*) was used at 0.5, 1.0 and 2.0 g/l water, the botanical insecticide NeemAzal T/S® at 2, 4 and 8 ml/l, the medium concentration, each, being registered.

Larvae were weighed daily, and the mortality was recorded, and corrected according to SCHNEIDER-ORELLI (1947).

For statistical analysis the program SPSS 12 was used, with ANOVA and partly a non-parametric test (KRUSKALL-WALLIS).

Results

1. *Galleria mellonella*

Food intake was diminished by XenTari, but not by NeemAzal T/S (two series; Table 1). But with higher concentration of the ingredients, the weight gain of larvae turned out to be negative, also for NeemAzal T/S.

When comparing the mortality (Table 2), it turned out, that NeemAzal T/S was much more efficient than XenTari (100 versus 77 % mortality within 30 days).

2. Spodoptera exigua

2.1 2nd instar-larvae

Young larvae of *S. exigua* showed differences in mortality between XenTari and NeemAzal T/S (Table 3). In XenTari it took 3 weeks to reach 96% mortality in the highest concentration, while in NeemAzal T/S within 7 to 8 days, 100% mortality was reached in all concentrations (Table 3).

2.2 4th-instar larvae

In the older larvae, which are less susceptible (AGGARWAL et al 2006b), the mortality reached by NeemAzal T/S within 5 days was 46 % (Table 4), which is not sufficient for control. But when NeemAzal T/S and XenTari were combined (with 3 days difference), the result proved to be better (Table 4), depending on the sequence. When XenTari was given first with the food, mortality was 72 % after 5 days, but when NeemAzal T/S was fed first, mortality after 5 days was 99%.

Table 1. Food intake and weight increase of *Galleria mellonella* larvae, when differently treated bee wax was offered. d.a.t. = day after treatment

Preparation and concentration	Wax intake (mg), 5 d.a.t.	Weight gain (mg), 9.d.a.t.
Water	278/330	54/33
XenTari, 0.5 g/l	154	22
1.0 g/l	113	1
2.0 g/l	67	-29
NeemAzal T/S, 2 ml/l	240	5
4 ml/l	290	3
8 ml/l	370	-6

Discussion

For the control of *G. mellonella* NeemAzal T/S had a higher efficacy. But it is not registered (yet) for wax moth control. But if preparations of *Bacillus thuringiensis aiawai* are used frequently, the danger of resistance in the target pests arises (SHELTON et al. 1993, GAUGHY & JOHNSON 1994). So alternatives have to be found, and Neem products seem to be one. They are harmless for bees (SCHMUTTERER & HOLST 1987; NAUMANN

& Isman 1996; Leymann et al. 2000), and additionally are able to control Varroa mite *Varroa destructor* Anderson & Trueman (Liu 1995; Melathopoulos et al. 2000). Therefore NeemAzal T/S should also be registered for the control of *G. mellonella*.

Though very efficient in controlling young larvae, NeemAzal T/S alone was not very efficient in older larvae of *Spodoptera exigua*. Though Prabhakar et al. (1986) report for *S. exigua* a sufficient mortality by Neem products in all larval stages, age specific differences in mortality caused by synthetic and biological insecticides are well known from Noctuidae (Ali & Young 1996). Therefore the combination with XenTari seems to be a very good tool for partly replacing the use of synthetic insecticides, in order to reduce the further rise of resistance. Our findings are in line with those of Parthiban & Ananthan (1998) and Liu et al. (1998), which used *B. t. kurstaki*. Ma at al. (2000) and Hamd et al. (2006) have shown that these bio-rational insecticides are harmless to beneficial arthropods. This is a further advantage of their use.

Table 2. Mortality of *G. mellonella* larvae, four weeks after ingestion of differently treated food. The pupal mortality in XenTari was due to an unavoidable fungal infection, and is not included in the corrected mortality

* Significantly different from Untreated at p=0.05 (Kruskall-Wallis-test)

Preparation and concentration	Larval mortality	Pupal mortality	Corrected mortality (%)
XenTari, 0.5 g/l	4	3	0
1.0 g/l	9	3	25.0
2.0 g/l	19*	0	77.1
NeemAzal T/S, 2ml/l	15	3	63.3
4 ml/l	18	1	69.4
8 ml/l	23*	1	100.0

Table 3. Mortality of 2nd-instar larvae of *Spodoptera exigua* after ingestion of differently treated food

* Significantly different from Untreated at p=0.01 (Kruskall-Wallis-test)

Preparation and concentration	Duration (days)	Larval mortality	Corrected mortality (%)
XenTari, 0.5 g/l	21	16*	63.5
1.0 g/l	21	23*	91.3
2.0 g/l	21	24*	95.6
NeemAzal T/S, 2ml/l	8	25*	100.0
4 ml/l	7	25*	100.0
8 ml/l	7	25*	100.0

Table 4. Mortality of 4th-instar larvae of *Spodoptera exigua* 5 days after different treatment of food. Mortality was significantly different from Untreated at p=0.01 in all treatments

Treatment of food with	dosage per l	Corrected mortality (%)
NeemAzal T/S (NA)	4 ml	46.2
XenTari first and NA after 3 days	0.5 g + 2 ml	71.9
NA first and after 3 days XenTari	2 ml + 0.5 g	98.8

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