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# Sublethal effects of spinosad and emamectin benzoate on larval development and reproductive activities of the cabbage moth, *Mamestra brassicae* L. (Lepidoptera: Noctuidae)

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ABSTRACT

Lepidopteran insect pest management has relied heavily on synthetic chemical pesticides, but their efficiency is declining as a result of emerging insecticide resistance. Recently biopesticides have become the most promising products employed in pest management strategies. We investigated the sublethal effects of two bioinsecticides, spinosad and emamectin benzoate, on larval and pupal development, and reproductive activity including calling behaviour, pheromone production, fecundity and fertility of the cabbage moth, *Mamestra brassicae*. To assess sublethal effects, second instar larvae were fed with 0.005, 0.05, or 0.5 μg a.i. spinosad/g diet or 0.00005, 0.0005, or 0.005 μg a.i. emamectin benzoate/g diet. Both bioinsecticides significantly increased larval and pupal development time and negatively affected reproductive activity of *M. brassicae*. The calling activity of females decreased very significantly in the highest sublethal concentration of spinosad and in all treatments by emamectin benzoate. The results suggest that, both spinosad and emamectin benzoate are promising alternatives to conventional insecticides for the control of *M. brassicae* if successfully introduced into Integrated Pest Management (IPM) programs.



## 1. Introduction

Pesticide applications have increased in frequency as a result of development of pesticides resistance, as documented in more than 954 insect pest species (Tabashnik et al., 2014). Conventional pesticides, including chlorinated hydrocarbons, organophosphates and carbamates, are being withdrawn because of their undesirable effects on humans and non-target species, and overall environmental impact. Consequently, environmentally-friendly methods have become fundamental for pest management (Metspalu et al., 2013). Biopesticides, produced by living microorganisms, or other natural products, provide an alternative approach used in crop protection for the last 50 years (Chandler et al., 2011).

Spinosad is an important bioinsecticide is spinosad, composed of spinosynes A and D, which are unsaturated tetracyclic esters and produced during the fermentation process of the soil actinomycete *Saccharopolyspora spinosa* (Bret et al., 1997; Sparks et al., 1998; Moulton et al., 2000). Spinosad has an Insecticide Resistance Action Committee (IRAC) class 5 mode of action, affecting the post synaptic nicotinic acethylcholine receptors and γ-aminobutyric acid (GABA) receptors (Salgado et al., 1997). Spinosad causes excitation and persistent activation of the nicotinic acetylcholine receptors, acts as a contact gastric poison (Salgado, 1998) and is effective against a wide range of insect pests including Lepidoptera, Coleoptera, Diptera, Hymenoptera, Thysanoptera and Isoptera (Bret et al., 1997; Sparks et al., 1998; Thompson et al., 2000). Experimentally, spinosad significantly increased larval mortality, while decreasing the pupation percentage and emergence of *Spodoptera littoralis* after 3rd instar larvae were exposed to sublethal concentrations (El-Sheikh, 2015). It has already been used successfully in Integrated Pest Management (IPM) as a reduced-risk product based on its low acute mammalian toxicity to rodents; $LD_{50}$ ranges from 3738 to 5000 mg/kg (Bret et al., 1997; Cleveland et al., 2001).

Emamectin benzoate is a semi-synthetic derivative of abamectin

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(Jansson et al., 1997), which is composed of a mixture of ~90% avermectin $B_{1a}$ and ~10% of avermectin $B_{1b}$ (Mushtaq et al., 1997). These two active ingredients are macrocyclic lactones produced through the fermentation process of the soil microorganism, *Streptomyces avermilitis* (Crouch et al., 1997). Emamectin benzoate has a class 6 IRAC mode of action, affecting the glutamate- or GABA-gated chloride channels causing a flow of chloride ions into neuronal cells. This action disrupts the nerve impulses leading to rapid paralysis, cessation of feeding and death within 3–4 days (Jansson et al., 1997; Grafton-Cardwell et al., 2005). Emamectin benzoate is active against Lepidoptera including *Spodoptera exigua*, *Helicoverpa zea*, and *S. littoralis* (Trumble et al., 1987; López et al., 2010; El-Sheikh, 2015). In contrast to spinosad, emamectin benzoate has low activity against non-lepidoptera and most beneficial arthropods (Jansson et al., 1997).

*Mamestra brassicae* L. (Lepidoptera: Noctuidae) is an important polyphagous pest on over 70 host plant species including cabbage and other vegetable crops (Hill, 1987; Rojas et al., 2000). For *M. brassicae* management, treating 2nd instar larvae with 0.5% active ingredient of neem extract (NeemAzal-T) decreased larval development and subsequent oviposition (Seljåsen and Meadow, 2006); neem also has growth disrupting and antifeedant effects on the larva (Karelina et al., 1992; Meadow and Seljåsen, 2000). However, there is no published information on sublethal effects of bioinsecticides on reproductive behaviour including calling behaviour (Noldus and Potting, 1990) and sex pheromone production of *M. brassicae* (Bestmann et al., 1987, 1988; Jacquin et al., 1994). Such behavioural elements should be equally important factors in the context of pest control.

Sex pheromones in most Lepidoptera are aliphatic compounds (Ando et al., 2004), synthesized in the pheromone gland (PG) (Percy and Weatherston, 1974) of the females that is generally located between the 8th and 9th abdominal segments. Sex pheromone production in the majority of moths, including *M. brassicae* (Bestmann et al., 1987, 1988; Jacquin et al., 1994), is regulated by pheromone biosynthesis activating neuropeptide (PBAN) (Raina et al., 1987). It is synthesized in the subesophageal gangalion transmitted to the *corpora cardiaca*, and then released into the haemolymph according to a circadian rhythm at the onset of scotophase to activate pheromone production (Raina, 1993; Bloch et al., 2013). The stimulating effect of PBAN on pheromone biosynthetic pathway has been demonstrated in a number of lepidopteran species (Matsumoto et al., 2007; Rafaeli, 2011) including *M. brassicae* (Jacquin et al., 1994; Köblös et al., 2015).

Sublethal doses of insecticides such, as spinosad, elicited decreased population growth of *Plutella xylostella* by affecting its survival, development and reproduction (Yin et al., 2008). In respect of synthetic pesticides, sublethal permethrin treatment decreased calling behaviour activity in pink bollworm, *Pectinophora gossypiella* (Haynes and Baker, 1985) and *Trichoplusia ni* females (Clark and Haynes, 1992a). A sublethal concentration of chlordimerform stimulated in early scotophase pheromone emission and increased calling activity in *T. ni* (Clark and Haynes, 1992b). The effect of *Bacillus thuringiensis* mixed with abamectin (BtA) on sex pheromone communication in *Helicoverpa armigera* moths may contribute to assortative mating (Shen et al., 2013). Indoxacarb and chlorantraniliprole significantly influenced in a negative way the chemical communication system in *P. xylostella* (Wang et al., 2011; Guo et al., 2013). Sex pheromone titers decreased after 1st, 2nd, and 4th day post-treatment of female Asian corn borer, *Ostrinia furnacalis* moths with deltamethrin (Yang and Du, 2003).

The current work presents the first information on the effects of sublethal treatments of two bioinsecticides, spinosad and emamectin benzoate, on *M. brassicae*, a suitable model for estimating effects on pheromone production and calling behaviour. This comprehensive study includes assessment of mortality, duration of larval and pupal stages, rate of adult emergence, reproductive activity and finally fecundity and fertility in *M. brassicae*.

## 2. Materials and methods

### 2.1. Insect culture

*Mamestra brassicae* larvae were obtained from a laboratory colony established from individuals collected from three different regions of Hungary in August 2012. The stock colony was maintained in a rearing room at 25 ± 1 °C, 60% relative humidity under a 16L:8D (light:dark) regime. Reverse photoperiod conditions were used to harmonize with working hours (i.e. lights were off at 8:00 a.m. and on at 4:00 p.m./16:00 h). Larvae were kept on a semi-artificial diet (Nagy, 1970). Pupae were collected from soil 8–10 days prior to eclosion, sexed and kept separately in 25 × 15 × 8 cm plastic containers with a layer of tissue paper, and held under the same conditions as larvae in the experimental room. After eclosion, adults were kept separately in cylindrical glass jars (12 × 10 cm) covered with fine mesh, fed with 15% sterilized honey solution applied on a piece of cotton, and furnished with folded brown paper for shelter. The newly emerged adults were designated as day 0 (D0), collected daily at 4:00 p.m., and accordingly the following days are declared as D1, D2 up till D7. For all observations and experiments, adults were retained in the experimental room equipped with a dim red light suitable for scotophase monitoring (i.e. calling behaviour).

### 2.2. Bioinsecticides

The tested bioinsecticides were spinosad (Tracer® 24% SC, Wadi El-Nile Co., Egypt) Dow AgroSciences, and emamectin benzoate (Elactor, 2% EC, Egypt Agricultural Development Co., Egypt) Syngenta.

### 2.3. Bioassay and determination of lethal median concentration ($LC_{50}$), $LC_{90}$ and sublethal concentrations

Bioinsecticidal activities of the spinosad and emamectin benzoate were tested on early 2nd instar larvae of *M. brassicae*. Larvae chosen for the bioassay were transferred onto diets containing 50, 5, 0.5, 0.05, and 0.005 μg/g of spinosad or 0.5, 0.05, 0.005, 0.0005, and 0.00005 μg/g of emamectin benzoate. Each stock concentration of spinosad or emamectin benzoate was prepared in distilled water and mixed with diet with a ratio of 1:25 (v:w; insecticide solution:diet) to give the appropriate concentrations. Freshly prepared diet was smeared in cylindrical glass jars (12 × 10 cm). Early 2nd instar larvae were exposed to treated and untreated diets (controls) and their mortality was recorded after 72 h. Each concentration was tested on 150 larvae in 3 equal groups as replicates and mortality was analyzed by a probit analysis (see: 2.5.). As the three lower concentrations of both tested bioinsecticides elicited less than 20% mortality (= sublethal concentrations) (Wei et al., 2004; Wang et al., 2011), treated larvae were transferred to clean jars containing untreated diet for the following experiments.

### 2.4. Sublethal effects

#### 2.4.1. Effects of spinosad and emamectin benzoate on development

Sublethal concentrations of spinosad (0.005, 0.05, and 0.5 μg/g) and emamectin benzoate (0.00005, 0.0005, and 0.005 μg/g) were used to determine effects on development time of larval and pupal stages, accumulative mortalities and adult emergence. Larval duration and mortality were recorded daily until the last instar of

the larval stadium. Non-feeding last instar larvae were transferred individually to a clean cup containing autoclaved soil for pupation, and covered. Each pupa was gently removed from the soil after 6 days, sexed and weighted, and kept separately in the same cup with moist cotton to record the total pupal time duration and percentage of emergence.

*2.4.2. Recording of calling behaviour*

Calling behaviour was recorded from D1 to D7 virgin female moths developed from treated larvae with sublethal concentrations and controls (9 individuals per treatment group) in glass jars (12 × 10 cm). The observation was carried out in the experimental room with a dim incandescent red backlight at 60 min intervals for 8 h under two different photoregimes (6 h in scotophase, from 10:00 h to 15:00 h and 2 h in photophase, from 16:00 h to 18:00 h). Calling behaviour, visually conspicuous at the tip of the abdomen under the red light, was characterized as calling (PG protruded) or not calling (PG not visible).

*2.4.3. Studies on pheromone blend production*

*2.4.3.1. Preparation of pheromone gland extracts*

For pheromone blend determination and characterization, PGs were excised from D2 virgin females at the last hour of scotophase (at 15:00 h) and extracted individually in 150 μl *n*-hexane for 8 min. The extract was transferred into a glass insert placed into a 1.5 ml vial (suitable for Gas Chromatography Mass Spectroscopy analysis) and after addition of the internal standard Z13-18Ald (300 ng/3 μl) (Pherobank BV, The Netherlands) it was concentrated using a thermo-block (at 65 °C) to a final volume of 20 μl. Finally the vials were sealed with a Teflon cap and stored under −80 °C until analysis.

*2.4.3.2. Gas chromatography-mass spectrometry analysis*

An aliquot of 1 μl from each sample was measured (automatic injection method, splitless mode) under the following conditions using a RESTEC (Rxi-5SI) column (0.25 mm internal diameter × 30 m and 0.25 μm film thickness) with helium as a carrier gas at a flow rate of 1 ml/min on a Gas Chromatograph-Mass Selective Detector (Hewlett Packard GC 6890, HP MSD 5973). The following running conditions were applied: 50 °C for the first min, then increased to 180 °C until 7.5 min (20 °C/min) and 190 °C until 8.17 min (15 °C/min), followed by an increase of 5 °C/min up to 220 °C at 14.17 min ending by 30 °C/min to 300 °C at 17.83 min. The SIM mode of the program allows a rapid, but sensitive fractionation of the two main components: Z11-16Ac (222 *m/z*; Ret. time 13.64 min) and 16Ac (224 *m/z*; Ret. time 13.77 min). Quantification was performed using the internal standard method and calculation was performed with the MSD Chemstation ver. D.01.02.16 software.

*2.4.4. Studies on fecundity and fertility*

After eclosion, groups of 5 (D0) females and 7–8 (D1-2) males of each concentration were transferred to glass jars (12 × 10 cm), layered with a brown paper and covered with fine mesh on the top and fed as described above (2.1.). The paper was removed on D2, on D4 and D6 and was replaced by a new paper. The eggs were counted each time and finally summarized. Egg batches were cut from the paper and each time eggs were transferred to a clean Petri dish with a piece of wet cotton and kept for 5–7 days to record fertility (hatchability). Consequently three sets of fertility data were generated for each concentration. It should be noted that only one replicate was performed of this mating study due to mortality and developmental drag during the experimental period especially relevant for emamectin benzoate.

*2.5. Statistical analyses*

Probit analysis Version 1.5 (EPA Probit Analysis Program) was used to estimate the $LC_{50}$ and $LC_{90}$ of each compound. Data analysis of all biological parameters, and statistical comparisons of calling behaviour of virgin females, sex pheromone production and reproductive activity were performed using ANOVA (Graph Pad Prism version 6) followed by Duncan's multiple range test.

## 3. Results

*3.1. Toxicity of spinosad and emamectin benzoate of treated 2nd instar larvae*

The toxicity results indicate that emamectin benzoate exhibited significantly higher toxicity than spinosad with $LC_{50}$ values of 0.18 and 4.37 μg/g respectively, while $LC_{90}$ values were 53.1 and 228.6 μg/g, respectively with 95% confidence limits (Table 1). It should be mentioned, although not similar concentrations were used, that larval mortality elicited by the sublethal concentrations of spinosad was 25.3, 34.7, and 40%, respectively, as compared with 25.3, 30, and 48% for emamectin benzoate; all values were significantly higher than the control (16.67%; $P > 0.05$) (Table 2).

*3.2. Sublethal effect of spinosad and emamectin benzoate on development to eclosion*

Both spinosad and emamectin benzoate significantly increased larval and pupal developmental time. Time to pupation was significantly longer for emamectin benzoate, while percentage of emergence was significantly lower with spinosad and emamectin benzoate treatments compared to controls (Table 2; $P > 0.05$ in all analyses). Female and male pupal weight (343 mg female and 297 mg male) decreased significantly after larval treatment with 0.05 μg/and 0.5 μg/g spinosad (316 mg female and 291 mg male) and with 0.005 μg/g (320 mg for females and 281 mg for males) emamectin benzoate, respectively, compared with control values (350 mg for females and 324 mg for males). Although female pupal weight was always heavier than that of corresponding males, the difference was not significant. The sex ratio was also not influenced significantly by the exposure to sublethal concentrations in comparison to controls (Table 2). Adult emergence rates ranged

**Table 1**
The calculated $LC_{50}$ and $LC_{90}$ values of spinosad and emamectin benzoate in *Mamestra brassicae* larvae. The bioinsecticides were mixed into the semi-artificial diet in different concentrations: spinosad was applied at 50, 5, 0.5, 0.05, and 0.005 μg/g, while emamectin benzoate at 0.5, 0.05, 0.005, 0.0005, and 0.00005 μg/g. Early 2nd instar larvae of *M. brassicae* were fed on the diet for 3 days. Treatments were performed in triplicate, each group contained 50 larvae at the beginning.

| Insecticides | [a]$LC_{50}$ (μg/g), 95% [c]CL | [b]$LC_{90}$ (μg/g), 95% [c]CL | Slope ± SE |
|---|---|---|---|
| spinosad | 4.37 (1.04–36.28) | 228.57 (29.80–831.96) | 0.746 ± 0.125 |
| emamectin benzoate | 0.184 (0.103–0.368) | 53.11 (15.65–283.29) | 0.520 ± 0.044 |

[a] $LC_{50}$: concentration causing 50% mortality.
[b] $LC_{90}$: concentration causing 90% mortality.
[c] CL: confidence limits.

**Table 2**
Effects of spinosad and emamectin benzoate on development of *Mamestra brassicae* from 2nd larval instar till emergence. Spinosad and emamectin benzoate were applied in three different sublethal concentrations. The early 2nd instar larvae were exposed to the insecticide-containing diet for 3 days, and then transferred to control diet to assess developmental parameters. Treatments were performed in triplicate, each group contained 50 larvae at the beginning.

| Treatments | Concentrations (μg/g) | Larval mortality% | Larval duration[A] | Pupation% | Pupal duration[B] | Pupal weight (mg) | | Sex ratio | | Emergence % |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Female | Male | Female | Male | |
| control | 0.0 | $16.7^{c} \pm 3.5$ | $15.1^{c} \pm 0.3$ | $87.3^{a} \pm 4.0$ | $22.9^{b} \pm 0.8$ | $350^{a} \pm 8$ | $324^{a} \pm 6$ | $48.5^{a} \pm 6.4$ | $51.5^{a} \pm 6.4$ | $79.5^{a} \pm 3.9$ |
| spinosad | 0.005 | $25.3^{ab} \pm 3.5$ | $16.1^{c} \pm 0.3$ | $81.9^{a} \pm 2.7$ | $22.5^{b} \pm 0.8$ | $351^{a} \pm 9$ | $313^{a} \pm 9$ | $48.4^{a} \pm 5.9$ | $51.6 \pm 5.9$ | $71.2^{ab} \pm 3.2$ |
| | 0.05 | $34.7^{a} \pm 4.7$ | $17.4^{b} \pm 0.4$ | $81.0 \pm 4.4$ | $25.5^{ab} \pm 1.2$ | $343^{ab} \pm 11$ | $297^{cb} \pm 9$ | $53.2^{a} \pm 5.6$ | $46.8^{a} \pm 5.6$ | $64.8^{b} \pm 2.9$ |
| | 0.5 | $40.0^{a} \pm 6.1$ | $17.4^{b} \pm 0.4$ | $81.0^{a} \pm 9.7$ | $27.7^{a} \pm 1.8$ | $316^{b} \pm 9$ | $291^{c} \pm 6$ | $45.3^{a} \pm 14.6$ | $54.7^{a} \pm 14.6$ | $65.4^{b} \pm 4.8$ |
| emamectin | 0.00005 | $25.3^{bc} \pm 2.9$ | $17.4^{b} \pm 0.4$ | $72.1^{b} \pm 3.7$ | $25.0^{ab} \pm 0.9$ | $361^{a} \pm 9$ | $344^{a} \pm 12$ | $63.7^{a} \pm 6.6$ | $36.3^{a} \pm 6.6$ | $65.7^{b} \pm 13.6$ |
| benzoate | 0.0005 | $30.0^{b} \pm 2.0$ | $17.0^{a} \pm 0.5$ | $69.7^{b} \pm 5.1$ | $25.8^{ab} \pm 1.2$ | $358^{a} \pm 12$ | $336^{a} \pm 10$ | $47.8^{a} \pm 9.7$ | $52.2^{a} \pm 9.7$ | $67.2^{b} \pm 5.6$ |
| | 0.005 | $48.0^{a} \pm 3.0$ | $19.8^{a} \pm 0.6$ | $67.5^{b} \pm 3.8$ | $26.2^{a} \pm 1.4$ | $320^{b} \pm 7$ | $281^{b} \pm 12$ | $52.2^{a} \pm 8.5$ | $47.8^{a} \pm 8.5$ | $67.5^{b} \pm 1.7$ |

Values marked with the same letters are not significantly different ($P > 0.05$: Duncan's multiple range test).
A = number of days from 2nd instar larvae till pupation.
B = number of days from the pupation till the eclosion.

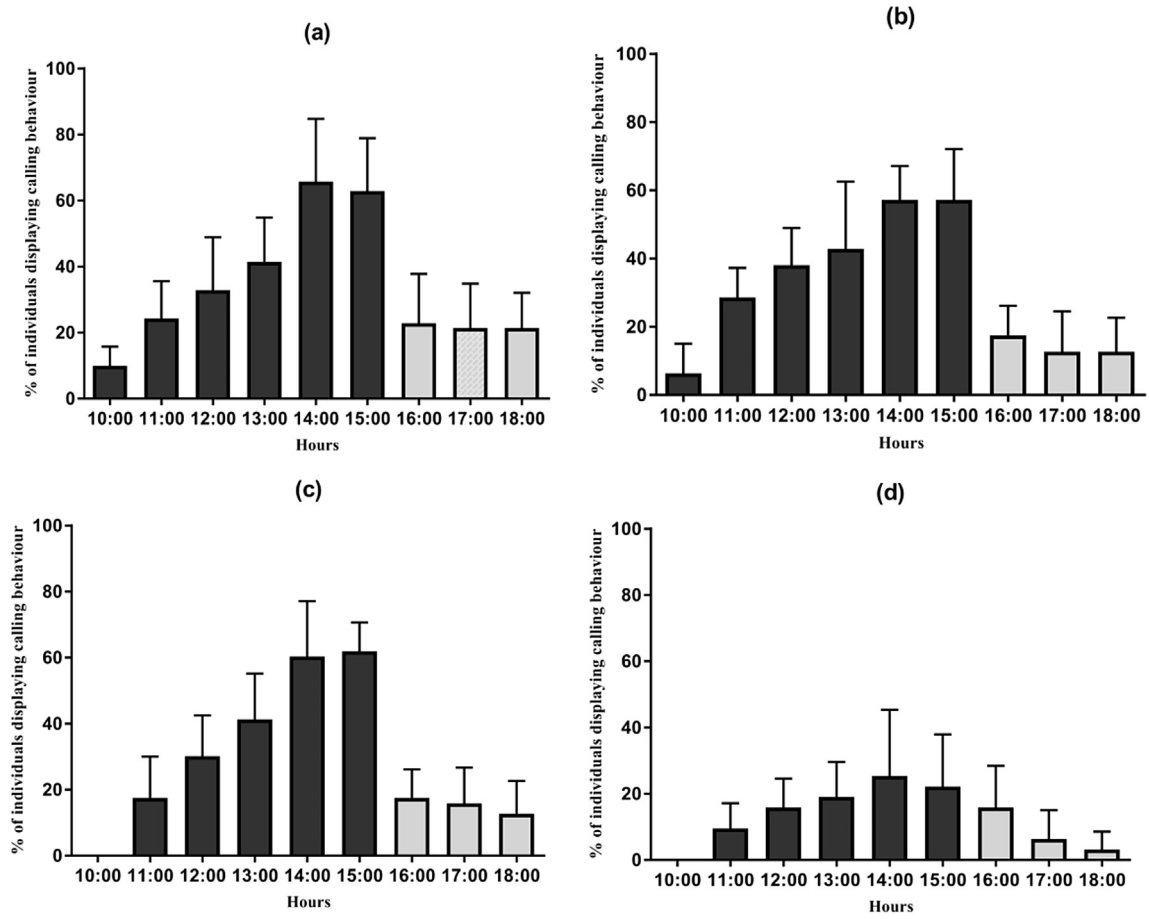


**Fig. 1.** Calling behaviour of *Mamestra brassicae* females (larvae fed with spinosad). Percentages (bars represent means ± SD; n = 9) of individuals displaying calling behaviour of *M. brassicae* females in scotophase (dark grey columns) and in photophase (light grey columns). The 2nd instar larvae of the respective females were untreated control (a) or fed with 0.005 μg/g (b), 0.05 μg/g (c), and 0.5 μg/g (d) subletheal concentrations of spinosad for three days.

between 71.2 or 65.7%, 64.8 or 67.2%, and 65.4 or 67.5% for larvae treated with the increasing concentrations of spinosad or emamectin benzoate, respectively. The value for untreated controls was 79.5% (Table 2) which was significantly higher to all treatments except for the lowest concentration of spinosad.

### 3.3. Calling behaviour of females

Calling activity increased gradually during the scotophase and reached its climax at the end of the scotophase (Fig. 1a). For spinosad, the peak calling percentages of the control females occurred at the end of scotophase at 14:00 h (65.7% ± 7.2) and 15:00 h (62.9% ± 6.1). Spinosad, at the highest concentration, decreased calling to 25.4% ± 7.6, and to 22.2% ± 5.9 at 14:00 h, and 15:00 h, respectively (Fig. 1d). Highly significant decreases were elicited by emamectin benzoate in the following concentrations: 0.00005 μg/g (26.2% ± 6.2 at 14:00 h and 23.8% ± 8 at 15:00 h), 0.0005 μg/g (16.1% ± 3.6 at 14:00 h and 14.3% ± 3.7 at 15:00 h) and 0.005 μg/g (7.1% ± 4.9 at 14:00 h and 7.14% ± 3.36 at 15:00 h) compared to control values (58.7% ± 5.26 at 14:00 h and

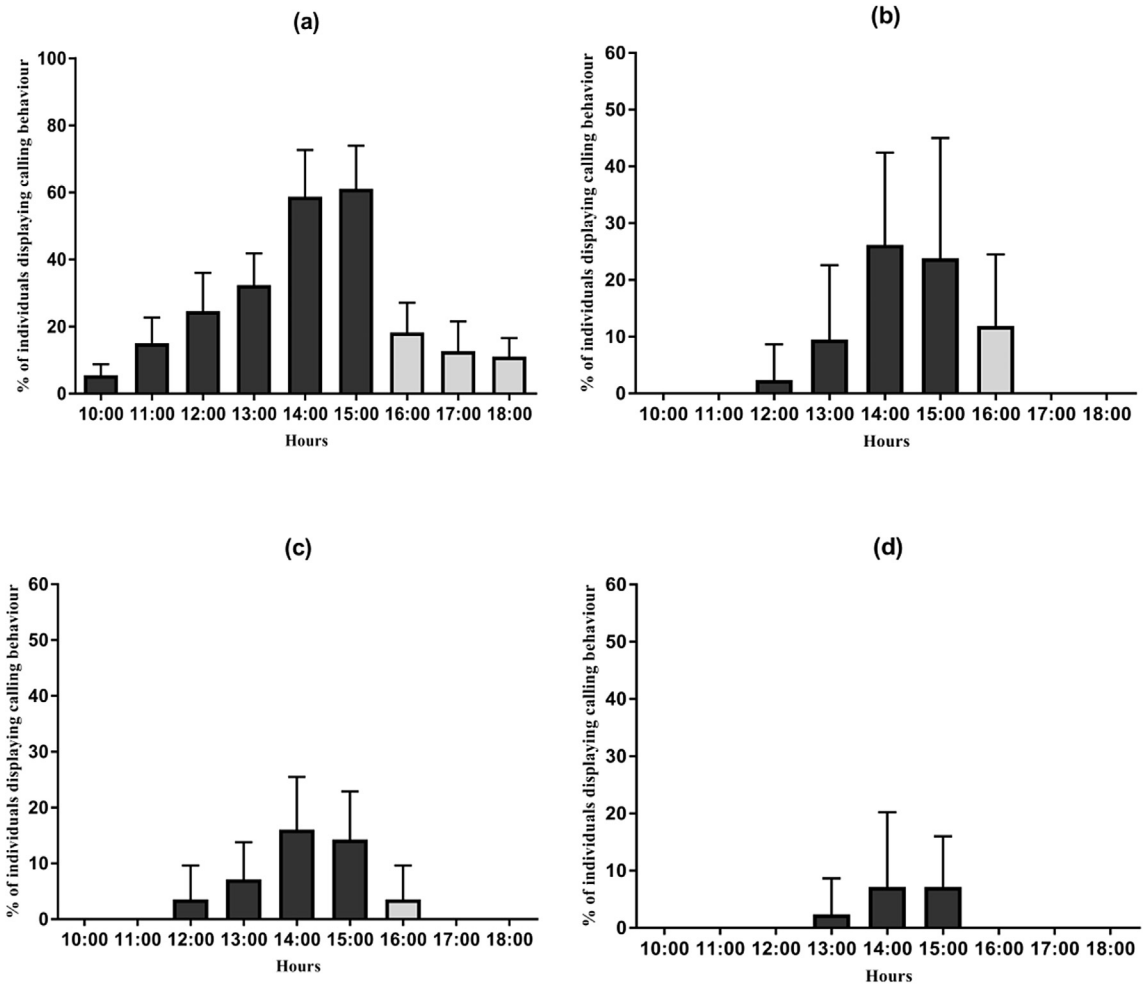


**Fig. 2.** Calling behaviour of *Mamestra brassicae* females (larvae fed with emamectin benzoate). Percentages (bars represent means ± SD; n = 9) of individuals displaying calling behaviour of *M. brassicae* females in scotophase (black columns) and in photophase (light grey columns). The 2nd instar larvae of the respective females were untreated control (a) or fed with 0.00005 μg/g (b), 0.0005 μg/g (c) and 0.005 μg/g (d) sublethal concentrations of emamectin benzoate for three days.

61.1% ± 4.8 at 15:00 h) (Fig. 2).

### 3.4. Sex pheromone production

Sex pheromone titers of D2 virgin females decreased after exposure of 2nd instar larvae to spinosad or emamectin benzoate although the differences were not always statistically significant (Table 3). However, blend ratios of Z11–16Ac and 16Ac were unaffected in comparison to controls (Table 3).

### 3.5. Fecundity and fertility

Spinosad significantly decreased fecundity at all concentrations tested and fertility at the highest concentration. In contrast, emamectin benzoate significantly decreased fecundity only at the highest sublethal concentration (0.005 μg/g), with no effect on

**Table 3**
Pheromone production of *Mamestra brassicae* females. Mean pheromone titer (ng)/female ± SE (CV%, SD/Mean, n = 9) and blend ratio of 2-day-old *M. brassicae* females (at the last hour of scotophase) treated with different sublethal concentrations of spinosad or emamectin benzoate at 2nd larval instar.

| Treatment | Concentrations (μg/g) | Mean titer (ng)/female ± SE (CV%)[A] | | Blend ratio[B] |
|---|---|---|---|---|
| | | Z11-16Ac | 16Ac | |
| control | 0.0 | $218.7^{a}$ ± 26.26 (36.0) | $15.86^{a}$ ± 3.00 (56.8) | 93.2:6.8 |
| spinosad | 0.005 | $217.7^{a}$ ± 22.92 (29.7) | $11.12^{a}$ ± 2.54 (64.8) | 95.1:4.9 |
| | 0.05 | $121.6^{ab}$ ± 35.16 (76.5) | $9.07^{a}$ ± 2.43 (70.7) | 93:7 |
| | 0.5 | $142.7^{ab}$ ± 48.25 (89.4) | $7.53^{a}$ ± 2.72 (95.6) | 94.9:5.1 |
| emamectin benzoate | 0.00005 | $106.2^{b}$ ± 29.14 (72.5) | $8.54^{a}$ ± 2.55 (79.7) | 92.5:7.5 |
| | 0.0005 | $110.3^{b}$ ± 24.17 (57.9) | $12.06^{a}$ ± 4.38 (96.1) | 90.1:8.9 |
| | 0.005 | $157.4^{ab}$ ± 31.06 (52.2) | $11.79^{a}$ ± 2.70 (60.5) | 93:7 |

Values marked with the same letters are not significantly different (P > 0.05: Duncan's multiple range test).
[A]CV = SD/Mean.
[B]The blend ratio is represented as: Z11–16Ac:16Ac.

**Table 4**
Fecundity and fertility of *Mamestra brassicae*. The emerged adults were placed into mating jars (5 females + 7–8 males) following exposure of 2nd instar larvae to different sublethal concentrations of spinosad and emamectin benzoate. Fecundity was estimated by counting the eggs from the second day till the sixth day (total number of eggs laid by the 5 females). Fertility is calculated by counting of the emerged larvae from eggs batches collected every second day (day 2, day 4 and day 6 after the establishment of mating jars).

| Treatments | Concentrations (μg/g) | Fecundity | Fertility % ±SE |
|---|---|---|---|
| control | 0 | $3231^{a}$ | $87.5^{a} \pm 1.1$ |
| spinosad | 0.005 | $1951^{b}$ | $84.2^{ab} \pm 8.3$ |
| | 0.05 | $1718^{b}$ | $69.2^{ab} \pm 3.7$ |
| | 0.5 | $1055^{c}$ | $67.7^{b} \pm 6.3$ |
| emamectin benzoate | 0.00005 | $4571^{a}$ | $91.8^{a} \pm 4.5$ |
| | 0.0005 | $3678^{a}$ | $97.3^{a} \pm 0.2$ |
| | 0.005 | $2674^{b}$ | $87.8^{a} \pm 8.8$ |

Values marked with the same letters are not significantly different (P > 0.05: Duncan's multiple range test).

fertility (Table 4). Mating of females was confirmed visually. This experiment was performed in one replicate only as explained under section 2.4.4.

## 4. Discussion

Control of *M. brassicae* is mainly achieved by using synthetic insecticides, but the emerging side effects, including decrease of biodiversity in the cropping system or recorded pesticide residues in agricultural products, are strongly undesirable (Metspalu et al., 2013). Most of the bioinsecticides are potentially safer compounds for ecosystems, but our knowledge is still limited regarding their mode of action, especially in relation to various pest species. From this perspective, the main aim of the study was to investigate the effect of sublethal concentrations of bioinsecticides spinosad and emamectin benzoate on mortality, duration of larval and pupal stages, sex ratio, adult emergence rate, and reproductive activity with special emphasis on calling behaviour and pheromone production in *M. brassicae*.

The surviving *M. brassicae* larvae, fed on semi-artificial diets amended with the three sublethal concentrations of spinosad or emamectin benzoate, showed an overall significant reduction in development from the 2nd larval instar till emergence. Emamectin benzoate was found to be more effective on 2nd instar larvae of *M. brassicae* after three days of exposure than spinosad (Table 2). This is in agreement with results when emamectin benzoate significantly decreased the development of progeny larvae to the pupal stage, when females of *H. zea* were fed with a mixture of different sublethal concentrations (0.0125–0.1 ppm) of 2.5 M sucrose as a feeding stimulant (López et al., 2010). In the present study only the highest sublethal concentrations of spinosad or emamectin benzoate decreased female and male pupal weight significantly. However, in *P. xylostella*, larval weight, pupation rate, pupal weight decreased significantly and prolonged developmental time after exposing 3rd instar larvae to sublethal concentrations of spinosad (Yin et al., 2008). The sublethal concentrations of emamectin benzoate had a deleterious effect on the development of *H. armigera* after neonate larvae were treated (Kandil et al., 2014). Recent experiments (Shen et al., 2013) have demonstrated that the mortality of 3rd instar larvae of *H. armigera* treated with BtA was significantly higher than that of untreated controls, and that treatment negatively affected the development of *H. armigera* when the larvae were treated with different sublethal concentrations of that mixture, from 0.5 mg/g to 8 mg/g. In some other studies it was found that sublethal concentrations of indoxacarb (Wang et al., 2011) and chlorantraniliprole (Guo et al., 2013) inhibited development of *P. xylostella*. However, the treatments of the yellow sugarcane borer, *Diatraea flavipennella*, larvae with sublethal concentrations of lufenuron showed similar larval and pupal development times and adult longevity, as was recorded with untreated larvae (Fonseca et al., 2015).

We observed the highest calling activity at the end of scotophase (Figs. 1a and 2a), which was significantly reduced in bioinsecticide-treated moths, except in the two lower spinosad concentrations (Fig. 1b and c). On the other hand, a shift in the time point for starting of calling behaviour and in the peak of calling time of BtA-treated females was recorded in *H. armigera* (Shen et al., 2013). A similar trend was described for *P. xylostella* resistant to tebufenozide and abemectin (Xu et al., 2010), as well as for *S. litura* (Wei et al., 2004) and *O. furnacalis* females that survived larval deltamethrin treatment (Wei and Du, 2004). The calling behaviour of *P. xylostella* females following treatment of 3rd instar larvae with a sublethal dose of indoxacarb was high at the first scotophase, but had decreased calling percentages and less calling hours during the subsequent scotophases (Wang et al., 2011).

Sex pheromone biosynthesis in insects involves a complex coordination of physiological activities which are under hormonal -primarily PBAN- and neuronal controls (Matsumoto et al., 2007; Rafaeli, 2011; Bloch et al., 2013). In Noctuids, pheromone production occurs mainly towards the end of scotophase and at the beginning of photophase under long day conditions (Raina, 1993). This is supported by earlier studies in *Pseudaletia separata* and in our test species *M. brassicae* as well (Fónagy et al., 2011; Köblös et al., 2015). Sampling was performed in the last hour of scotophase of 2-day-old virgin females when the highest amount of pheromone production is expected in *M. brassicae* (Köblös et al., 2015). Our results demonstrated a significant reduction in the amounts of the major component, Z11–16Ac for spinosad or emamectin benzoate-treated females in most sublethal concentrations tested compared to control individuals.

The ratio of Z11–16Ac and 16Ac was found to be 93.2:6.8 in extracts from PGs of *M. brassicae* which were in line with results observed to be 93:7 in the same species (Köblös et al., 2015). In contrast, the ratio of Z11–16:Ald and Z9–16:Ald produced by BtA-treated *H. armigera* females decreased from 9.76 to 5.80, which also resulted in a lower mating rate (Shen et al., 2013). In another study, the survived *O. furnacalis* females following the treatment of 1st and 3rd instar larvae with deltamethrin showed a significantly higher amount of sex pheromone production and wide variation of the ratio of E12-14Ac to its Z-isomer (Wei and Du, 2004). Although the amount of 16Ac did not show a significant drop compared to the control in the present study, it should be noted that the mean coefficient of variations of Z11–16Ac and 16Ac of spinosad and emamectin benzoate-treated *M. brassicae* were significantly higher compared to the control (Table 3). This indicates that production of sex pheromone from spinosad and emamectin benzoate-treated females were not as fit as controls. This is consistent with results in *O. furnacalis* and *H. armigera* survivors reared from larvae treated with deltamethrin or BtA (Yang and Du, 2003; Wei and Du, 2004; Shen et al., 2013). In *O. furnacalis* it was also shown that PBAN-like activity decreased following deltamethrin treatment, which likely explains the reduction of pheromone production (Yang and Du, 2003). Our results support the fact that sublethal concentrations of bioinsecticides, especially emamectin benzoate as demonstrated, can strongly affect sex pheromone production as well as calling behaviour critical for attracting conspecific males. The significant drop of calling behaviour following treatments with sublethal concentrations is an important finding, because the perturbation of pheromone emission -even though the amount is less- may negatively affect mating under natural condition. These effects may be explained by the fact that spinosad is a neurotoxin (Salgado et al., 1997; Salgado, 1998), while emamectine benzoate

disrupts nerve impulses due to chloride ion flux modification (Jansson et al., 1997). Consequently, both bioinsecticides influence the series of events of pheromone production and emission, though in a different way. Alternative explanation may be that the lower sex pheromone titer, in female moths reared from surviving larvae treated with spinosad or emamectin benzoate, may be a direct negative feedback of a reduction in the protrusion of the PG from the abdomen as shown in Figs. 1 and 2.

A decrease in fecundity and fertility was found in *M. brassicae* following the treatment of 2nd instar larvae with spinosad at all concentrations. However, the reduction of the egg number was observed only at the highest concentration of emamectin benzoate. These findings could be related to some physiological effects (Yin et al., 2008) as a result of treated larvae with the pesticides including life span (Stark and Rangus, 1994), development rate, fecundity and fertility (Liu and Trumble, 2005). Spinosad is known to have an ovicidal activity in lepidopteran pests including *H. virescens*, *H. zea*, and *S. littoralis* (Peterson et al., 1988; Bret et al., 1997; Pineda et al., 2004). The fecundity and egg size of *P. xylostella* were also reduced after treatment of 3rd instar larvae with spinosad at $LC_{25}$ or $LC_{50}$ (Yin et al., 2008). In an opposite way, spinosad at $LC_{10}$, $LC_{25}$, and $LC_{50}$ was more effective compound on fecundity, hatchability than emamectin benzoate when 2nd instar larvae of *S. littoralis* were treated (Korrat et al., 2012). Moreover, spinosad and chlorpyrifos (0.444 μg/g and 4.01 μg/g, respectively) had a significant effect on longevity and fecundity of the *S. littoralis* adults (Kandil et al., 2014).

In conclusion, the use of insecticides may result in multiple sublethal effects on insect pests with detrimental impacts on some physiological or behavioural process in the surviving insects. Our results are the first to demonstrate sublethal effects of spinosad and emamectin benzoate on *M. brassicae* affecting larval development time, pupation, pupal duration and weight, emergence percentages, and reproductive activity. Accordingly, spinosad and emamectin benzoate have good potential to become a surrogate chemical in *M. brassicae* control. Further studies are required to investigate the sublethal effect of these bioinsecticides on field populations of this pest.

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