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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 Conventional pesticides, including chlorinated hydrocarbons, organophosphates and carbamates, are being withdrawn because of their undesirable effects on humans and non-target species, and overall environmental impact.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