Synergistic interaction between carvacrol and *Bacillus thuringiensis* crystalline proteins against *Cydia pomonella* and *Spodoptera exigua*

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Abstract The aim of our study was to determine the usefulness of mixtures of carvacrol and *Bacillus thuringiensis* crystalline proteins Cry against pests of two different species: *Cydia pomonella* L. (Lepidoptera: Tortricidae) and *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). The novelty of our work lies in showing the interactions between carvacrol and bacterial toxins against insect pests. Moreover, we have demonstrated that carvacrol applied via ingestion exerts toxicity against lepidopteran caterpillars. We have shown that the botanical compound and Cry proteins act in synergy and their mixtures are most effective in reducing the number of L1 and L3 larvae when *B. thuringiensis* toxins constitute up to 0.1% and 0.05% of the mixtures, respectively. Carvacrol and crystalline proteins act in synergy in these combinations and have the potential to be effective in protecting crops against lepidopteran pests. The nature

of the interaction between the components depended on the proportion of their concentrations in the mixture. Mixtures containing Cry toxin concentrations equal or higher than 20% caused lower observed mortality of insects compared to the expected one. Furthermore, we showed that crystalline proteins of *B. thuringiensis* MPU B9, carvacrol and their mixture did not affect the morphology of insect haemocytes, and additionally, had no effect on the immune system.

Keywords Bacterial toxins · Plant substance · Insecticidal activity · Biopesticide · Synergy

Introduction

Interest in the use of commercially available bioinsecticides and future prospects for the development of new biological preparations in plant protection has significantly increased in recent years. This is due to the fact that they are an environmentally safe alternative to chemical pesticides and can be easily degraded with limited persistence in the environment (Chattopadhyay et al. 2017). Additionally, the current strategy of Integrated Pest Management (IPM) programs assumes the application of all available plant protection methods, giving priority to non-chemical approaches. Biopesticides contain natural products derived from animals, plants, fungi, and bacteria. Almost 90% of commercial microbial insecticides are

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based on a spore-crystals mixture of *Bacillus thuringiensis* (Damalas and Koutroubas 2018). Plant products are also recommended as efficient biopreparations against insect pests (Konecka et al. 2018b, 2019; Mossa 2016). Moreover, recent trends in crop protections involve the use of insecticide mixtures (Sharifzadeh et al. 2018). This approach can lead to the prevention or delay of the development of insect resistance (Sudo et al. 2017), especially when individual components of preparations have different modes of action (Zhu et al. 2016). This strategy limits costs (Das 2014) and reduces the dosage of preparations when insecticides enhance each other's activity and act in synergy (Wraight and Ramos 2005).

B. thuringiensis strains synthesize protein crystals during sporulation. Crystals together with bacterial spores are eaten and dissolved into the insect midgut. In the most common model, Cry proteins after activation by midgut proteases, bind to receptors on the surface of epithelial cells and destroy the midgut (Saiyad 2017), allowing bacterial spores to enter the body and circulatory system of insects, where the spores germinate and bacteria multiply (Argôlo-Filho and Loguercio 2014). Insect haemocytes phagocytize bacterial cells and form aggregates that may be encapsulated by other haemocytes or by cells that may be released from the aggregates. During bacterial infection, different changes in haemocyte structures were observed in lepidopteran insects. For example, granulocytes were surrounded by precipitated haemoplasm and various sizes of clear vesicles. Moreover, the membrane of granulocytes was disrupted and the nucleus was distorted. Bodies resembling lysosomes were observed in the cytoplasm of infected haemocytes (El-Aziz and Awad 2010). Some of *B. thuringiensis* strains produce haemolysin with lytic properties that induce death of insect haemocytes and macrophages (Tran et al. 2013). However, spore-free preparation of crystalline proteins, Cry1, Cry2, and Cry9, did not cause changes in the morphology of lepidopteran haemocytes (Konecka et al. 2018a).

Carvacrol or plant extracts with carvacrol as the main component are known to exert negative effects against coleopteran (Szczepanik et al. 2018), homopteran (Zekri et al. 2016), dipteran (Andrade-Ochoa et al. 2018; de Mesquita et al. 2018; Giatropoulos et al. 2018), lepidopteran (Melo et al. 2018), neuropteran

(Castilhos et al. 2018), and hemipteran (Gaire et al. 2019; Rizvi et al. 2018) insects. This phytochemical was shown to exhibit contact (Castilhos et al. 2018; Melo et al. 2018; Rizvi et al. 2018), repellent (Giatropoulos et al. 2018), and fumigant (Gaire et al. 2019) toxicity. The carvacrol-containing essential oil showed neurotoxic effects and was found to inhibit the acetylcholinesterase enzyme in the contact application against insect pests (de Mesquita et al. 2018; Rizvi et al. 2018). Carvacrol has been also reported to have insecticidal activity upon ingestion against Hemiptera (Park et al. 2017) and Lepidoptera (Tang et al. 2011). One study incorporated extracts of *Origanum vulgare* (Lamiales) and *Artemisia dracunculus* (Asterales) with high carvacrol content into the insect diet and the plant compound caused a high loss of body weight of *Alphitobius diaperinus* (Coleoptera) when applied through consumption (Szczepanik et al. 2018). Data about the toxicity of carvacrol to lepidopteran insects determined based on ingestion are still not comprehensive. The mechanism of carvacrol action applied to the insect intestinal tract is unknown.

The aim of this research was to determine the usefulness of mixtures of *B. thuringiensis* crystalline proteins and carvacrol against lepidopteran pests. We combined bacterial and plant products in different ratios and established the nature of interactions between them. The study applied crystals of two *B. thuringiensis* strains: MPU B9 and MPU B54. The MPU B9 strain had a large number and variety of genes coding for insecticidal crystalline proteins Cry: *cry1Aa*, *cry1B*, *cry1C*, *cry1D*, *cry1I*, *cry2Ab*, *cry9B*, and *cry9E* (Konecka et al. 2007b). Moreover, mass spectrometry analysis revealed that MPU B9 crystals contained Cry1Aa, 1Ba, 1Ca, 1D, and 9E toxins (Baranek et al. 2017). Bacterial crystals alone or with spores showed high insecticidal activity against insect pests (Konecka et al. 2018c, 2015, 2012, 2007b). *B. thuringiensis* strain MPU B54 harbored the following genes: *cry1Aa*, *cry1Ab*, *cry1C*, *cry1D*, *cry1I*, *cry2Ab*, *cry9B*, and *cry9E*. Crystals of the strain MPU B54 exhibited significant insecticidal activity against moths (Konecka et al. 2018c).

The insecticidal activity of crystal-carvacrol mixtures was determined against insects of two different species: *Cydia pomonella* (Lepidoptera: Tortricidae) and *Spodoptera exigua* (Lepidoptera: Noctuidae). C.

pomonella is a fruit pest and attacks mainly apples, but also pears, apricots, cherries, plums, peaches (Alston et al. 2010), and even walnuts and chestnuts (Hagstrum et al. 2013). Caterpillars feed on the surface of the fruit for a relatively short time and subsequently enter the fruit and dig tunnels inside it (Alston et al. 2010). Larvae remain there until completing their development (Pajač et al. 2011), and thus are hardly available for the action of insecticides. Recently, a decrease in this insect sensitivity to chemical preparations has been noted (Grigg-McGuffin et al. 2015). *S. exigua* is a phytophagous species. It feeds mainly on vegetables, but also on grains and ornamental plants, both in open fields and in greenhouses. Caterpillars skeletonize foliage and consume fruits (Capinera 1999). Both pest species cause considerable economic agricultural losses (Pajač et al. 2011; Hua et al. 2013). Damages of plants due to eating plant seedlings and cotton bolls caused by *S. exigua* slow the growth of commercial crops such as cotton and reduce crop yield, which has negative impact on the textile industry (Hua et al. 2013). *C. pomonella* infestation can reach 60–80% for pears and apples, respectively, in orchards without pest control treatment (Vreysen et al. 2010). It has become a worldwide pest due to the variety of hosts and the increase in international fruit trade (Thaler et al. 2008).

The insect immune system can be a target for development of novel strategies of suppressing pest viability (Czarniewska et al. 2019a), but there is no information how carvacrol and a mixture of carvacrol and crystalline proteins of *B. thuringiensis* affect insect's immunity. In this work, we examined the in vivo effect of carvacrol and the mixture of carvacrol and bacterial Cry proteins on morphology, viability, and cellular immune response (phagocytosis and nodule formation) of the third instar of *S. exigua* caterpillars to determine whether carvacrol and a mixture of carvacrol with the bacterial toxins are cytotoxic to immunocompetent cells and affect insect immunity. For this purpose, we used a very sensitive haemocyte biotest that we previously developed, through which it is possible to detect changes in the morphology, adhesion, viability, and immunological function of haemocytes that can be induced by various biotic and abiotic factors (Czarniewska et al. 2012, 2018, 2019a, 2019b; Kuczer et al. 2016; Konecka et al. 2018a; Kowalik-Jankowska et al.

2019). Our results should explain the mechanism of interaction between carvacrol and *B. thuringiensis* Cry proteins in the mixture against insects.

Materials and methods

Bacteria

Two *Bacillus thuringiensis* strains were used in the study: MPU B9 and MPU B54. The strains were deposited in the Bacteria Collection of the Department of Microbiology, Adam Mickiewicz University in Poznań, Poland. *B. thuringiensis* MPU B9 was isolated from the intestinal tract of dead caterpillar of codling moth *Cydia pomonella* during epizootics caused by naturally occurred bacterial infection in the laboratory-cultured line of the insect (Konecka et al. 2007a). *B. thuringiensis* MPU B54 was cultured from soil sample (Konecka et al. 2018c).

Plant substance

A plant substance – carvacrol – was used in the study. The preparation of carvacrol (purity $\geq 98\%$) was purchased from SAFC, St. Louis (USA).

Insects

The activity of bacterial crystals and carvacrol were determined against two lepidopteran species: the codling moth *C. pomonella* L. and the beet armyworm *Spodoptera exigua* (Hübner), representing Tortricidae and Noctuidae, respectively. Caterpillars of codling moth and beet armyworm came from standardized laboratory culture lines reared at the Faculty of Biology at Adam Mickiewicz University, Poznań, Poland. Insects were reared on a synthetic medium (McGuire et al. 1997) at 26 °C with a L:D 16:8 photoperiod and 40–60% RH.

Determination of interaction between bacterial crystalline proteins and carvacrol against insects

Isolation of B. thuringiensis crystalline proteins

The isolation procedure was described previously (Konecka et al. 2012, 2015, 2019). The strains were cultured on a medium for bacteria sporulation

(Lecadet and Dedonder 1971) at 30°C for seven days. The collected crystals and spores were suspended in 1 M NaCl, 10 mM KCl, pH 7.5. Subsequently, the crystals were separated from the spore-crystal mixtures by sucrose density gradient centrifugation (Guz et al. 2005). Crystals layer was collected, washed in sterile distilled water and weighed. The purity of crystals was checked by standard light microscopy which was preceded by a staining with amido black and Ziehl's carbol fuchsin (Smirnoff 1962).

Estimation of insecticidal activity of carvacrol, B. thuringiensis crystalline proteins and their mixtures

Carvacrol was homogenized with Tween 80 (1:0.5 ratio). Then, the dilutions of the botanical preparation, *B. thuringiensis* proteins, and their mixtures were prepared in sterile distilled water (Table 1) and applied to *C. pomonella* and *S. exigua* caterpillars of the first instar (L1) and additionally to *S. exigua* larvae of the third instar (L3). Caterpillars of *C. pomonella* and *S. exigua* of the first instar were fed with: (1) carvacrol, (2) *B. thuringiensis* MPU B9 Cry proteins, (3) *B. thuringiensis* MPU B54 Cry proteins, (4) mixtures of carvacrol or MPU B9 proteins, and (5) mixtures of carvacrol and MPU B54 proteins. The activity of each concentration of the preparations was determined for 30 *C. pomonella* caterpillars of first instar (three simultaneous replicates with ten larvae each) or 30 *S. exigua* caterpillars of first instar (three simultaneous replicates with ten larvae each).

S. exigua larvae of the third instar were fed with (1) carvacrol, (2) *B. thuringiensis* MPU B9 Cry proteins, or (3) mixtures of carvacrol and MPU B9 proteins. The activity of each concentration of the preparations was determined for 30 *S. exigua* caterpillars of third instar (three simultaneous replicates with ten larvae each). Bioassays with *C. pomonella* and *S. exigua* were conducted in insect culture boxes, as described previously (Konecka et al. 2012, 2015, 2018a, c, 2019). Each box was divided into 12 separate wells – one well per one larva. Synthetic medium for codling moth and beet armyworm rearing was placed into the wells. Its composition was the same as described by McGuire et al. (1997), but without formaldehyde to avoid its effect on the action of Cry proteins. Considering insect biology, the medium was poured

into wells or formed into cylindrical pieces of 5 mm in diameter and a height of 3 mm for *C. pomonella* or *S. exigua* bioassays, respectively. Each dilution of botanical, bacterial, and mixed preparations was applied on the diet surface in a volume of 10 µl for *S. exigua* or 50 µl for *C. pomonella* bioassays. Different volumes were used to refer to various species due to the use of various surface areas of the medium onto which the preparation was poured. Bioassays with all mixed preparations, as well as their individual components, at concentrations at which they were incorporated into the mixture were performed simultaneously. The Tween 80 in concentration of 10 mg ml⁻¹ and sterile distilled water, instead of the insecticidal substance, was given to 30 caterpillars as a negative control. Insects were reared at 26 °C with a L:D 16:8 photoperiod and 40–60% RH. Seven days after preparation applications, dead and live insects were counted.

Calculation of the synergism, antagonism and addition between carvacrol and crystalline proteins against pests

The expected mortality of insects (M_{EXP}) caused by the mixture of the plant compound and *B. thuringiensis* crystalline proteins was calculated according to the equation: $M_{EXP} = (1 - B \times C) \times 100\%$, where B is the proportion of insects that survived the exposure to crystalline proteins in relation to the total number of insects used, and C is the proportion of insects that survived the exposure to carvacrol in relation to the total number of insects used (Tabashnik et al. 2013).

The character of the interaction between the plant and bacterial substances against insects was determined according to Tabashnik et al. (2013). Synergism between components was recorded when the value of the observed mortality was statistically significantly higher than the value of the expected mortality of insects. Antagonism was observed when the observed mortality was statistically significantly lower than the expected one. If the difference between the values of the expected and observed mortality was not statistically significant, the interaction was recognized as additional. Fisher's exact test (<https://www.graphpad.com/quickcalcs/contingency1/>) was employed to determine statistically significant

differences ($P < 0.05$) between the results (Tabashnik et al. 2013).

Impact of Cry proteins, carvacrol and their mixture on haemocytes and insect cellular immune response

We examined the haemocytes' morphology, viability, adhesion, and phagocytic activity as well as nodule formation in the *S. exigua* L3 larvae fed with (1) 250 ng of bacterial toxins per larva, (2) 500,000 ng of carvacrol per larva, or (3) their mixture. Tween 80 in concentration of 10 mg ml^{-1} instead of the insecticidal substance was used as a negative control. The haemocytes' morphology, viability, and adhesion study was performed according to the method described by Czarniewska et al. (2012) and Konecka et al. (2018a). The phagocytic ability of haemocytes of control and experimental caterpillars was analyzed in vivo by using fluorescent latex beads (Sigma-Aldrich L1030) according to the method described by Czarniewska et al. (2019a). Caterpillars anaesthetized and disinfected with ethanol were injected with $2 \mu\text{l}$ of latex beads suspended in physiological solution (274 mM NaCl , 19 mM KCl , 9 mM CaCl_2) (2: 1000 v/v) through the abdominal foreleg by using a Hamilton syringe (Hamilton Co., Reno, Nevada, USA). The caterpillars were anaesthetized again, disinfected and washed in distilled water two hours after the injection of the latex beads and the samples of haemolymph ($2 \mu\text{l}$) were collected and diluted in $20 \mu\text{l}$ of saline. Haemolymph samples were incubated on the poly-L-lysine-coated glasses (Sigma P4707) for 30 min at room temperature in the dark. The haemocytes were washed with saline to remove unphagocytized beads and stained with DAPI solution for 5 min in the dark to visualize cell nuclei. Phagocytic activity of haemocytes was examined with a Nikon Eclipse TE 2000-U fluorescence microscope. The photos of haemocytes were captured with a Nikon DS-1QM digital camera and analyzed with the ImageJ software (version 2). The results were expressed as a percentage of phagocytic haemocytes in the total number of haemocytes in the images.

Nodule formation in the haemocoel of control and experimental caterpillars was studied according to the

method described by Czarniewska et al. (2018). Briefly, caterpillars anaesthetized and disinfected with ethanol were injected with *Staphylococcus aureus* (Sigma S2014 Saint Louis, Missouri, USA) suspension in physiological saline ($2 \mu\text{l}$, 2:1000 v/v) through the abdominal proleg. Caterpillars were dissected two days after the injection of bacteria to expose the nodules on the dorsal side of the haemocoel. For this purpose, the insect's body was pinned onto a SYLGARD R filled Petri dish, and then the fat body, Malpighian tubules, and the digestive tract were removed. The number of nodules was counted by using an Olympus SZX 12 stereoscopic microscopy (Olympus Co., Tokyo, Japan), and three images of each caterpillar were captured with an Olympus U-LH100HG digital camera (Olympus Co., Tokyo, Japan). The number of nodules on the dorsal side of the haemocoel was counted in all insects. The images were analyzed with the ImageJ (version 2) software.

Results

Different types of interactions were observed between *B. thuringiensis* crystalline proteins and carvacrol depending on the concentration proportion of the components in the mixtures. The combination of *B. thuringiensis* toxins and plant compound in a ratio of 1:25,000 and 1:50,000 resulted in a synergy between these products against *Cydia pomonella* L1 and caused from 1.5- to 1.9-fold higher insect mortality than expected, depending on bacterial strain Cry proteins used. The same type of interaction was observed against *S. exigua* first and third instars when the concentrations of carvacrol in the mixtures were relatively higher. The constituents combined in 1:1000, 1:2000, and 1:10,000 ratios acted in synergy against *S. exigua* first instar and caused from 1.5 to 1.8-fold higher insect mortality than expected. Synergistic interactions were also noted between crystalline toxins and the phytochemical in 1:2000 and 1:5000 ratios against *S. exigua* L3, as the observed insect mortality was from 2 to 2.4-fold in comparison to the expected rate. Bacterial and plant products in a ratio of 1:2000 exhibited synergism against *S. exigua* L1 and the third instar larvae of *S. exigua* (Table 1).

Table 1 Activity of *B. thuringiensis* crystalline proteins, carvacrol, and their mixtures against lepidopteran insects

Insect	Preparation	Concentration (ng per insect)*	Proportion Cry:carvacrol	Mortality (%)		P**	Effect
				Observed ($\pm$ SE)	Expected		
<i>C. pomonella</i> L1	Cry MPU B9	20	–	20 ($\pm$ 5.77)	–	–	–
		10	–	10 ($\pm$ 0)	–	–	–
	Cry MPU B54	20	–	56.7 ($\pm$ 3.33)	–	–	–
		10	–	46.7 ($\pm$ 8.82)	–	–	–
	Carvacrol	500,000	–	23.4 ($\pm$ 3.33)	–	–	–
		5000	–	10 ($\pm$ 5.77)	–	–	–
	Cry MPU B9 + carvacrol	20 + 500,000	1:25,000	70 ($\pm$ 0)	38.4	0.037(s)	Synergistic
		20 + 5000	1:250	30 ($\pm$ 5.77)	28	1.00(ns)	Additive
		10 + 500,000	1:50,000	60 ($\pm$ 5.77)	31	0.037(s)	Synergistic
		10 + 5000	1:500	23.4 ($\pm$ 3.33)	19	1.00(ns)	Additive
	Cry MPU B54 + carvacrol	20 + 500,000	1:25,000	96.7 ($\pm$ 3.33)	66.1	0.006(s)	Synergistic
		20 + 5000	1:250	83.4 ($\pm$ 3.33)	60.4	0.084(ns)	Additive
		10 + 500,000	1:50,000	93.4 ($\pm$ 3.33)	58.4	0.048(s)	Synergistic
		10 + 5000	1:500	56.7 ($\pm$ 8.82)	51.4	0.796(ns)	Additive
	Control***	–	–	0	–	–	–
<i>S. exigua</i> L1	Cry MPU B9	500	–	13.4 ($\pm$ 3.33)	–	–	–
		100	–	13.4 ($\pm$ 6.66)	–	–	–
		50	–	3.4 ($\pm$ 3.33)	–	–	–
	Cry MPU B54	200	–	30 ($\pm$ 11.55)	–	–	–
		100	–	26.7 ($\pm$ 8.82)	–	–	–
	Carvacrol	100,000	–	36.7 ($\pm$ 8.82)	–	–	–
		1,000	–	20 ($\pm$ 10)	–	–	–
		500	–	13.4 ($\pm$ 3.33)	–	–	–
	Cry MPU B9 + carvacrol	500 + 100,000	1:2000	73.4 ($\pm$ 3.33)	44.3	0.035(s)	Synergistic
		500 + 1000	1:2	13.4 ($\pm$ 6.66)	30.4	0.209(ns)	Additive
		500 + 500	1:1	20 ($\pm$ 5.77)	24.3	1.00(ns)	Additive
		100 + 100,000	1:10,000	66.7 ($\pm$ 3.33)	44.3	0.038(s)	Synergistic
		100 + 500	1:5	10 ($\pm$ 0)	24.3	0.299(ns)	Additive
		50 + 100,000	1:2000	66.7 ($\pm$ 3.33)	37.9	0.037(s)	Synergistic
	Cry MPU B54 + carvacrol	50 + 500	1:10	20 ($\pm$ 5.77)	15.6	1.00(ns)	Additive
		200 + 100,000	1:500	60 ($\pm$ 10)	57.1	1.00(ns)	Additive
		200 + 1000	1:5	33.4 ($\pm$ 3.33)	46.4	0.0430(ns)	Additive
		200 + 500	1:2.5	30 ($\pm$ 10)	41.7	0.422(ns)	Additive
		100 + 100,000	1:1,000	83.4 ($\pm$ 3.33)	53.6	0.047(s)	Synergistic
		100 + 500	1:5	30 ($\pm$ 10)	35.6	0.785(ns)	Additive
	Control***	–	–	0	–	–	–
<i>S. exigua</i> L3	Cry MPU B9	500	–	10 ($\pm$ 10)	–	–	–
		250	–	3.4 ($\pm$ 3.33)	–	–	–
	Carvacrol	500,000	–	20 ($\pm$ 5.77)	–	–	–
		250,000	–	13.4 ($\pm$ 3.33)	–	–	–

Table 1 continued

Insect	Preparation	Concentration (ng per insect)*	Proportion Cry:carvacrol	Mortality (%)		P**	Effect
				Observed ($\pm$ SE)	Expected		
	Cry MPU B9 + carvacrol	500 + 500,000	1:5000	56.7 ($\pm$ 3.33)	28	0.035(s)	Synergistic
		500 + 250,000	1:500	26.7 ($\pm$ 8.82)	21.7	1.00(ns)	Additive
		250 + 500,000	1:2000	53.4 ($\pm$ 3.33)	22.4	0.033(s)	Synergistic
		250 + 250,000	1:1000	20 ($\pm$ 11.55)	15.6	1.00(ns)	Additive
	Control***	–	–	0	–	–	–

s significant at $P < 0.05$, ns non-significant

*ng of Cry in 50 μ l or 10 μ l of preparation applied via ingestion against *C. pomonella* or *S. exigua*, respectively (see Methods for details)

**Probability that the difference between observed and expected mortality occurred by chance based on Fisher's exact test (Tabaschnik et al. 2013)

***The Tween 80 in concentration of 10 mg ml⁻¹ and sterile distilled water, instead of the insecticidal substance, was given to insects as a negative control

Reduced concentrations of carvacrol in mixtures to ratios lower than 1:1000 resulted in the additive effect between *B. thuringiensis* proteins and the plant substance against pests. Similar values of the observed and expected insect mortality were recorded in the majority of samples showing an additive effect between the components, in which Cry toxins and the phytochemical were mixed in 1:10, 1:250, and 1:500 ratios. However, a significant decrease in the value of the observed mortality in comparison to the expected one was noted in a bioassay with mixtures containing 71% or lower carvacrol concentration. The values of the observed mortality were from 1.05- to even 2.3-fold lower than the expected mortality when bacterial and botanical products were applied in 1:1, 1:2, 1:2.5, and 1:5 ratios to the insects (Table 1).

B. thuringiensis toxins, carvacrol, and their mixture had no effect on the morphology, viability, adhesion, and immunological function of insect haemocytes. Insecticidal agents did not exert apoptotic and caspase activity in *S. exigua* haemocytes. Cell shrinkage and fragmentation of nuclei were not visible in the haemocytes. The cytoskeleton of the cells was well developed, and thus haemocytes adhered to the cover slip and formed numerous filopodia, similarly as control haemocytes (Fig. 1). Additionally, no differences in cellular immune response were observed in caterpillars treated with bacterial proteins, plant compound, and their mixture in comparison to the control insects. The percentage of phagocytic haemocytes (Fig. 2) and the number of nodules formed in

insect haemocoel during immune response did not differ significantly when compared to the control insects (Fig. 3).

Discussion

The demonstration of interactions between *B. thuringiensis* crystalline proteins and carvacrol against insect pests is the novelty of our research. Additionally, we have shown that carvacrol applied via ingestion is toxic to lepidopteran caterpillars. Although there are studies on the toxicity of mixtures of *B. thuringiensis* and plant products against pests (Abedi et al. 2014; Amizadeh et al. 2015; Nouri-Ganbalani et al. 2016), none of them concern the use of carvacrol as a component of these combinations. Our study is in line with the search for effective biological plant protection products and harmonizes with IPM programs. The use of a mixture of two insecticidal substances that act synergistically is more profitable than employing two different preparations in separate applications. Our approach will not only reduce the costs of plant protection, but also enable applying lower doses of pesticides and reducing the occurrence of insect resistance, which is of great importance due to the emergence of insect resistance to Cry proteins (Peralta and Palma 2017).

Our research showed that Cry toxins and carvacrol acted in synergy and their mixtures were the most effective in reducing the number of L1 and L3 pests

when bacterial toxins constituted up to 0.1% and 0.05% of the mixtures, respectively. The use of these mixtures caused an increase in the observed insect mortality, up to approximately 1.9-fold in comparison with the expected one. However, the type of the interaction between the components depended on the proportion of their concentrations in the mixture. Increasing the concentration of Cry proteins did not result in a synergistic effect between bacterial and plant products. Mixtures containing concentrations of *B. thuringiensis* toxins equal or higher than 20% caused lower observed insect mortality than expected, as if carvacrol prevented the action of bacterial toxins or reduced their effects. Different types of the interactions between substances depending on the

concentration of components were also observed in other studies (Konecka et al. 2018a; Rajguru and Sharma 2012).

We propose the use of a combination of *B. thuringiensis* toxins and carvacrol against lepidopteran pests, because they are considered responsible for the loss of significant amounts of plant crops (Culliney 2014). Bacterial proteins from the Cry1, Cry2, and Cry9 groups, which are produced by *B. thuringiensis* MPU B9 and MPU B54, as well as carvacrol, were found to be insecticidal substances with no negative effect on other animals than plant pests (Gao et al. 2018). In addition, these substances do not persist in the environment for a long time (Mossa 2016). Commercial biopesticides based on

Fig. 1 Fluorescence microscopy images of haemocytes of *Spodoptera exigua* L3 (negative control **a**), fed with 250 ng of *Bacillus thuringiensis* Cry proteins (**b**), 500,000 ng of carvacrol (**c**), and their mixture (**d**). All haemocytes were stained with the

SR-VAD-FMK reagent for caspase activity detection (no red color – no active caspase), with Oregon Green® 488 phalloidin for F-actin cytoskeleton (green color) staining and with DAPI for DNA staining (blue color)

Fig. 2 Fluorescence microscopy images showing the phagocytosis of fluorescent latex beads (green color) by haemocytes of *Spodoptera exigua* L3 (negative control **a**), fed with 250 ng of *Bacillus thuringiensis* Cry proteins (**b**), 500,000 ng of carvacrol (**c**), and their mixture (**d**). All haemocytes were stained with

B. thuringiensis contain bacterial spores. We suggest the usage of an insecticidal preparation with no bacterial spores due to the possibility of synthesis of

DAPI to visualize DNA. The graph shows the percentage of phagocytic haemocytes in haemolymph of the studied caterpillars (**e**) (means $\pm$ SE, $n = 5$). Bars with the same letter do not differ significantly ($P \geq 0.05$)

vertebrate virulence factors by vegetative bacterial cells that germinate from spores in the insect body (Kim et al. 2015). Thus, in this study, we isolated *B.*

Fig. 3 Images showing the nodule formation in haemocoel of *Spodoptera exigua* L3 (negative control **a**), fed with 250 ng of *Bacillus thuringiensis* Cry proteins (**b**), 500,000 ng of carvacrol (**c**), and their mixture (**d**) induced by *Staphylococcus aureus* suspension injection. Arrows indicate nodules. The graph shows

the activity of cellular response in haemocoel of the studied caterpillars (**e**). The number of nodules was the determinant of cellular response (means $\pm$ SE, $n = 5$). Bars with the same letter do not differ significantly ($P \geq 0.05$)

thuringiensis crystals from spore-crystal mixtures by sucrose density gradient centrifugation.

Mixtures can be advantageous compared to individual constituents, because they may have different modes of action and may delay the development of resistance. The general mode of action of *B. thuringiensis* Cry proteins is known (Jouzani et al. 2017; Saiyad 2017). However, many important questions concerning the mechanism by which insect cells are killed by some of the crystalline toxins remain poorly understood (Vachon et al. 2012). Most Cry proteins damage epithelial cell permeability and cellular integrity of the insect midgut (Melo et al. 2016). The mode of action of carvacrol applied to insects via the intestinal track is not explained. It remains unresolved whether the phytochemical is involved in the destruction of insect tissues of the intestinal track or whether it has an impact on other

cells that were exposed to carvacrol after the destruction of midgut cells by Cry proteins. We attempted to explain the latter possibility and determine the impact of carvacrol and its mixtures with MPU B9 Cry proteins on insect haemolymph cells. The effect of *B. thuringiensis* MPU B9 toxins on the morphology and viability of insect haemocytes has been determined previously (Konecka et al. 2018a). However, we performed similar experiments in this study as a comparative assay. To the best of our knowledge, there is no data on the influence of carvacrol and its mixture with bacterial toxins on insect haemocytes. Furthermore, we also considered the consequences for the insect's immune system, resulting from the action of the insecticidal agents. We showed that crystalline toxins of *B. thuringiensis* MPU B9, carvacrol, and their mixture did not affect the morphology, viability of insect haemocytes, and, additionally, they had no

effects on the immunological system. This may indicate that carvacrol acted earlier, before the integrity of the midgut cells was disturbed or affected in other insect tissues than the haemolymph. In topical and fumigant bioassays performed by Gaire et al. (2019), carvacrol demonstrated neuro-inhibitory effects in hemipteran insect. Rizvi et al. (2018) showed that carvacrol inhibited acetylcholinesterase activity in insects of the same order by contact application. Similar activity of carvacrol was found against Diptera (de Mesquita et al. 2018; Park et al. 2016). In our research, the route of carvacrol application was different and the insects used represented a different order in comparison with the other studies mentioned above. The mode of action of ingested carvacrol as well as the explanation of the synergy between Cry proteins and carvacrol against Lepidoptera needs elucidation by additional experimental studies.

In conclusion, the present study has revealed for the first time that crystalline protein-carvacrol mixtures, containing Cry toxins in concentrations up to 0.05% in dietary applications exhibit stronger insecticidal activities as compared to the components assessed separately. The bacterial and plant products act in synergy in these combinations and have the potential to be effective in protecting crops against lepidopteran pests.

Compliance with ethical standards

Conflict of interest The authors have declared that they have no conflict of interest.

Research involving human and/or animal rights This article does not contain any studies with human participants or animals (vertebrates) performed by any of the authors.

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References

- Abedi Z, Saber M, Vojoudi S, Mahdavi V, Parsaeyan E (2014) Acute, sublethal, and combination effects of azadirachtin and *Bacillus thuringiensis* on the cotton bollworm *Helioverpa armigera*. *J Insect Sci* 14:30
- Alston D, Murray M, Reding M (2010) Codling moth (*Cydia pomonella*). Utah pest fact sheets ENT 13-06:1-7. <https://climate.usu.edu/includes/pestFactSheets/Codling-Moth.pdf>. Accessed 04 Mar 2020
- Amizadeh M, Hejazi MJ, Niknam G, Arzanlou M (2015) Compatibility and interaction between *Bacillus thuringiensis* and certain insecticides: perspective in management of *Tuta absoluta* (Lepidoptera: Gelechiidae). *Biocontrol Sci Technol* 25:671–684
- Andrade-Ochoa S, Sánchez-Aldana D, Chacón-Vargas KF, Rivera-Chavira BE, Sánchez-Torres LE, Camacho AD, Noguera-Torres B, Nevárez-Moorillón GV (2018) Oviposition deterrent and larvicidal and pupacidal activity of seven essential oils and their major components against *Culex quinquefasciatus* Say (Diptera: Culicidae): synergism–antagonism effects. *Insects* 9(1):25
- Argôlo-Filho RC, Loguercio LL (2014) *Bacillus thuringiensis* is an environmental pathogen and host-specificity has developed as an adaptation to human-generated ecological niches. *Insects* 5(1):62–91
- Baranek J, Konecka E, Kaznowski A (2017) Interaction between toxin crystals and vegetative insecticidal proteins of *Bacillus thuringiensis* in lepidopteran larvae. *BioControl* 62:649–658
- Capinera JL (1999) *Spodoptera exigua* (Hübner) (Insecta: Lepidoptera: Noctuidae). Featured creatures EENY-105. Entomology and Nematology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL, USA. https://entnemdept.ufl.edu/creatures/veg/leaf/beet_armyworm.htm. Accessed 06 Aug 2019
- Castilhos RV, Grütmacher AD, Coats JR (2018) Acute toxicity and sublethal effects of terpenoids and essential oils on the predator *Chrysoperla externa* (Neuroptera: Chrysopidae). *Neotrop Entomol* 47(2):311–317
- Chattopadhyay P, Banerjee G, Mukherjee S (2017) Recent trends of modern bacterial insecticides for pest control practice in integrated crop management system. *3 Biotech* 7:60
- Culliney TW (2014) Crop losses to arthropods. In: Pimentel D, Peshin R (eds) *Integrated pest management*. Springer, Dordrecht, pp 201–225
- Czarniewska E, Mrówczyńska L, Kuczer M, Rosiński G (2012) The pro-apoptotic action of the peptide hormone *Neb-colloostatin* on insect haemocytes. *J Exp Biol* 215:4308–4313
- Czarniewska E, Urbański A, Sz C, Kuczer M (2018) The long-term immunological effects of alloferon and its analogues in the mealworm *Tenebrio molitor*. *Insect Sci* 25(3):429–438
- Czarniewska E, Nowicki P, Kuczer M, Schroeder G (2019a) Impairment of the immune response after transcuticular introduction of the insect gonadoinhibitory and

- hemocytotoxic peptide *Neb-colloostatin*: a nanotech approach for pest control. *Sci Rep* 9:10330
- Czarniewska E, Mrówczyńska L, Jedrzejczak-Silicka M, Nowicki P, Trukawka M, Mijowska E (2019b) Non-cytotoxic hydroxyl-functionalized exfoliated boron nitride nanoflakes impair the immunological function of insect haemocytes *in vivo*. *Sci Rep* 9:14027
- Damalas ChA, Koutroubas SD (2018) Current status and recent developments in biopesticide use. *Agriculture* 8:13
- Das SK (2014) Scope and relevance of using pesticide mixtures in crop protection: a critical review. *IJESTR* 2(5):119–125
- de Mesquita BM, do Nascimento PGG, Souza LGS, de Farias IF, da Silva RAC, de Lemos TLG, Monte FJQ, Oliveira IR, Trevisan MTS, da Silva HC, Santiago GMP (2018) Synthesis, larvicidal and acetylcholinesterase inhibitory activities of carvacrol/thymol and derivatives. *Quim Nova* 41(4):412–416
- El-Aziz NMA, Awad HH (2010) Changes in the haemocytes of *Agrotis ipsilon* larvae (Lepidoptera: noctuidae) in relation to dimilin and *Bacillus thuringiensis* infections. *Micron* 41(3):203–209
- Gaire S, Scharf ME, Gondhalekar AD (2019) Toxicity and neurophysiological impacts of plant essential oil components on bed bugs (Cimicidae: Hemiptera). *Sci Rep* 9:3961
- Gao YJ, Zhu HJ, Chen Y, Li YH, Peng YF, Chen XP (2018) Safety assessment of *Bacillus thuringiensis* insecticidal proteins Cry1C and Cry2A with a zebrafish embryotoxicity test. *J Agric Food Chem* 66(17):4336–4344
- Giapopoulos A, Kimbaris A, Michaelakis A, Papachristos DP, Polissiou MG, Emmanouel N (2018) Chemical composition and assessment of larvicidal and repellent capacity of 14 Lamiaceae essential oils against *Aedes albopictus*. *Parasitol Res* 117(6):1953–1964
- Grigg-McGuffin K, Scott IM, Belleros S, Chouinard G, Cormier D, Scott-Dupree C (2015) Susceptibility in field populations of codling moth *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) in Ontario and Quebec apple orchards to a selection of insecticides. *Pest Manag Sci* 71(2):234–242
- Guz K, Kucińska J, Lonc E, Doroszkiewicz W (2005) Differentiated pattern of protein composition of crystalline inclusions of newly isolated *Bacillus thuringiensis* strains from Silesia in Poland. *Pol J Microbiol* 54(4):263–269
- Hagstrm DW, Klejdysz T, Subramanyam B, Nawrot J (2013) Atlas of stored-product insects and mites. AACC International Inc., St. Paul
- Hua W, Zemerov S, Wason E (2013) *Spodoptera exigua*. Animal Diversity Web. https://animaldiversity.org/accounts/Spodoptera_exigua/. Accessed 06 Aug 2019
- Jouzani GS, Valijanian E, Sharafi R (2017) *Bacillus thuringiensis*: a successful insecticide with new environmental features and tidings. *Appl Microbiol Biotechnol* 101(7):2691–2711
- Kim MJ, Han JK, Park JS, Lee JS, Lee SH, Cho JI, Kim KS (2015) Various enterotoxin and other virulence factor genes widespread among *Bacillus cereus* and *Bacillus thuringiensis* strains. *J Microbiol Biotechnol* 25(6):872–879
- Konecka E, Kaznowski A, Ziemnicki J, Ziemnicki K (2007a) Molecular and phenotypic characterisation of *Bacillus thuringiensis* isolated during epizootics in *Cydia pomonella* L. *J Invertebr Pathol* 94(1):56–63
- Konecka E, Kaznowski A, Ziemnicka J, Ziemnicki K, Paetz H (2007b) Analysis of *cry* gene profiles in *Bacillus thuringiensis* strains isolated during epizootics in *Cydia pomonella* L. *Curr Microbiol* 55(3):217–222
- Konecka E, Baranek J, Kaznowski A, Ziemnicka J, Ziemnicki K (2012) Interaction between crystalline proteins of two *Bacillus thuringiensis* strains against *Spodoptera exigua*. *Entomol Exp Appl* 143(2):148–154
- Konecka E, Hrycak A, Kaznowski A (2015) Synergistic effect of *Bacillus thuringiensis* crystalline toxins against *Cydia pomonella* (Linnaeus) (Tortricidae: Lepidoptera). *Entomol Gen* 35(3):157–166
- Konecka E, Czarniewska E, Kaznowski A, Grochowska J (2018a) Insecticidal activity of *Bacillus thuringiensis* crystals and thymol mixtures. *Ind Crop Prod* 117:272–277
- Konecka E, Kaznowski A, Marcinkiewicz W, Tomkowiak D, Maciąg M, Stachowiak M (2018b) Insecticidal activity of *Brassica alba* mustard oil against lepidopteran pests *Cydia pomonella* (Lepidoptera: Tortricidae), *Dendrolimus pini* (Lepidoptera: Lasiocampidae), and *Spodoptera exigua* (Lepidoptera: Noctuidae). *J Plant Prot Res* 58(2):206–209
- Konecka E, Kaznowski A, Stachowiak M, Maciąg M (2018c) Activity of spore-crystal mixtures of new *Bacillus thuringiensis* strains against *Dendrolimus pini* (Lepidoptera: Lasiocampidae) and *Spodoptera exigua* (Lepidoptera: Noctuidae). *Folia For Pol Ser A* 60(2):91–98
- Konecka E, Kaznowski A, Tomkowiak D (2019) Insecticidal activity of mixtures of *Bacillus thuringiensis* crystals with plant oils of *Sinapis alba* and *Azadirachta indica*. *Ann Appl Biol* 174(3):364–371
- Kowalik-Jankowska T, Lesio M, Krupa K, Kuczer M, Czarniewska E (2019) Copper(ii) complexes with alloferon analogues containing phenylalanine H6F and H12F stability and biological activity lower stabilization of complexes compared to analogues containing tryptophan. *Metallomics* 11(10):1700–1715
- Kuczer M, Czarniewska E, Majewska A, Rózanowska M, Rosinski G, Lisowski M (2016) Novel analogs of alloferon: synthesis, conformational studies, pro-apoptotic and antiviral activity. *Bioorg Chem* 66:12–20
- Lecadet MM, Dedonder R (1971) Biogenesis of the crystalline inclusion of *Bacillus thuringiensis* during sporulation. *Eur J Biochem* 23(2):282–294
- McGuire MR, Galan-Wong LJ, Tamez-Guerra P (1997) Bioassay of *Bacillus thuringiensis* against lepidopteran larvae. In: Lacey LA (ed) Manual of techniques in insect pathology. Academic Press, San Diego, pp 91–99
- Melo CR, Picanço MC, Santos AA, Santos IB, Pimentel MF, Santos ACC, Blank AF, Araújo APA, Cristaldo PF, Bacci L (2018) Toxicity of essential oils of *Lippia gracilis* chemotypes and their major compounds on *Diaphania hyalinata* and non-target species. *Crop Prot* 104:47–51
- Melo ALA, Soccol VT, Soccol CR (2016) *Bacillus thuringiensis*: mechanism of action, resistance, and new applications: a review. *Crit Rev Biotechnol* 36(2):317–326
- Mossa ATH (2016) Green pesticides: essential oils as biopesticides in insect-pest management. *J Environ Sci Technol* 9(5):354–378

- Nouri-Ganbalani G, Borzoui E, Abdolmaleki A, Abedi Z, Kamita SG (2016) Individual and combined effects of *Bacillus thuringiensis* and azadirachtin on *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae). *J Insect Sci* 16(1):95
- Pajač I, Pejić I, Barić B (2011) Codling moth, *Cydia pomonella* (Lepidoptera: Tortricidae): major pest in apple production: an overview of its biology, resistance, genetic structure and control strategies. *Agric Consp Sci* 76(2):87–92
- Park ChG, Jang M, Yoon KA, Kim J (2016) Insecticidal and acetylcholinesterase inhibitory activities of Lamiaceae plant essential oils and their major components against *Drosophila suzukii* (Diptera: Drosophilidae). *Ind Crop Prod* 89:507–513
- Park JH, Jeon YJ, Lee CH, Chung N, Lee HS (2017) Insecticidal toxicities of carvacrol and thymol derived from *Thymus vulgaris* Lin against *Pochazia shantungensis* Chou & Lu., newly recorded pest. *Sci Rep* 7:40902
- Peralta C, Palma L (2017) Is the insect world overcoming the efficacy of *Bacillus thuringiensis*? *Toxins* 9(1):39
- Rajguru M, Sharma AN (2012) Comparative efficacy of plant extracts alone and in combination with *Bacillus thuringiensis* subsp. *kurstaki* against *Spodoptera litura* Fab. larvae. *J Biopest* 5(1):81–86
- Rizvi SAH, Ling S, Tian F, Xie F, Zeng X (2018) Toxicity and enzyme inhibition activities of the essential oil and dominant constituents derived from *Artemisia absinthium* L. against adult Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae). *Ind Crop Prod* 121:468–475
- Saiyad SA (2017) Application of *Bacillus thuringiensis* as an effective tool for insect pest control. *IOSR-JAVS* 10(7):27–29
- Sharifzadeh MS, Abdollahzadeh G, Damalas ChA, Rezaei R (2018) Farmers' criteria for pesticide selection and use in the pest control process. *Agriculture* 8:24
- Smirnoff WA (1962) A staining method for differentiating spores, crystals, and cells of *Bacillus thuringiensis* (Berliner). *J Insect Pathol* 4(3):384–386
- Sudo M, Takahashi D, Andow DA, Suzuki Y, Yamanaka T (2017) Optimal management strategy of insecticide resistance under various insect life histories: heterogeneous timing of selection and interpatch dispersal. *Evol Appl* 11(2):271–283
- Szczepanik M, Walczak M, Zawitowska B, Michalska-Sionkowska M, Szumny A, Wawrzenczyk CZ, Swiontek Brzezinska M (2018) Chemical composition, antimicrobial activity and insecticidal activity against the lesser mealworm *Alphitobius diaperinus* (Panzer) (Coleoptera: Tenebrionidae) of *Origanum vulgare* L. ssp. *hirtum* (Link) and *Artemisia dracuncululus* L. essential oils. *J Sci Food Agric* 98(2):767–774
- Tabashnik BE, Fabrick JA, Unnithan GC, Yelich AJ, Masson L, Zhang J, Bravo A, Soberón M (2013) Efficacy of genetically modified Bt toxins alone and in combinations against pink bollworm resistant to Cry1Ac and Cry2Ab. *PLoS ONE* 8(11):e80496
- Tang X, Chen S, Wang L (2011) Purification and identification of carvacrol from the root of *Stellera chamaejasme* and research on its insecticidal activity. *Nat Prod Res* 25(3):320–325
- Thaler R, Brandstätter A, Meraner A, Chabicovski M, Parson W, Zelger R, Dallinger R (2008) Molecular phylogeny and population structure of the codling moth (*Cydia pomonella*) in Central Europe: II. AFLP analysis reflects human-aided local adaptation of a global pest species. *Mol Phylogenet Evol* 48(3):838–849
- Tran SL, Guillemet E, Lereclus D, Ramarao N (2013) Iron regulates *Bacillus thuringiensis* haemolysin *hlyII* gene expression during insect infection. *J Invertebr Pathol* 113(3):205–208
- Vachon V, Laprade R, Schwartz JL (2012) Current models of the mode of action of *Bacillus thuringiensis* insecticidal crystal proteins: a critical review. *J Invertebr Pathol* 111(1):1–12
- Vreysen MJB, Carpenter JE, Marec F (2010) Improvement of the sterile insect technique for codling moth *Cydia pomonella* (Linnaeus) (Lepidoptera Tortricidae) to facilitate expansion of field application. *J Appl Entomol* 134(3):165–181
- Wraight SP, Ramos ME (2005) Synergistic interaction between *Beauveria bassiana*- and *Bacillus thuringiensis tenebrionis*-based biopesticides applied against field populations of Colorado potato beetle larvae. *J Invertebr Pathol* 90(3):139–150
- Zekri N, Handaq N, El Caidi A, Zair T, El Belghiti MA (2016) Insecticidal effect of *Mentha pulegium* L. and *Mentha suaveolens* Ehrh. hydrosols against a pest of citrus, *Toxoptera aurantii* (Aphididae). *Res Chem Intermed* 42:1639–1649
- Zhu F, Lavine L, O'Neal S, Lavine M, Foss C, Walsh D (2016) Insecticide resistance and management strategies in urban ecosystems. *Insects* 7(1):2

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