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RAPID COMMUNICATION

Insecticidal activity of *Brassica alba* mustard oil against lepidopteran pests *Cydia pomonella* (Lepidoptera: Tortricidae), *Dendrolimus pini* (Lepidoptera: Lasiocampidae), and *Spodoptera exigua* (Lepidoptera: Noctuidae)

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Abstract

Our research provides novel information concerning the insecticidal activity of *Brassica alba* mustard oil applied to the intestinal tract via insects' diet against pests from the order Lepidoptera: *Cydia pomonella*, *Dendrolimus pini*, and *Spodoptera exigua*. The LC_{50} value of the oil against *C. pomonella* was 0.422 mg · ml^{-1}. The LC_{50} of the plant oil against *D. pini* was 11.74 mg · ml^{-1}. The LC_{50} of the botanical product against *S. exigua* was 11.66 mg · ml^{-1}. The plant substance was the most active against *C. pomonella* in comparison with *D. pini* and *S. exigua*. The LC_{50} values of the oil against *D. pini* and *S. exigua* were similar. The plant oil exhibited high insecticidal activity against pests from the order Lepidoptera and may prove to be an effective biopesticide.

Key words: biopesticide, *Cydia pomonella*, *Dendrolimus pini*, ingestive application, *Spodoptera exigua*

Botanical pesticides constitute an alternative way of reducing the use of chemical insecticides. On 21 October 2009, the European Parliament and the Council of the European Union established Directive 2009/128/WE (http://eur-lex.europa.eu) to achieve the sustainable use of pesticides by reducing the risks and impacts of pesticide use on human health and the environment, and by promoting the use of integrated pest management and of alternative approaches or techniques such as non-chemical alternatives of pesticides. Integrated Pest Management does not exclude the use of chemical insecticides but recommends their application when the number of pests cannot be reduced by employing non-chemical methods. There are biopesticides available, mostly based on baculoviruses, enthomopathogenic bacteria and fungi; however, intense research is being carried out to find new biological preparations. The development of novel insecticides based on natural substances could extend the list of environmentally friendly agents used in pest control and the market of plant protection preparations would be more competitive when offering a greater choice of products.

Some plant substances may be toxic for insects and therefore they would be useful as complementary or alternative methods to the heavy use of classical insecticides. Commonly, essential oils can be inhaled, ingested or skin absorbed by insects. Repellent, antifeedant and insecticidal effects of essential oils have been observed (Khater 2012). One natural preparation active against insects is mustard oil. The effect of the oil applied in the diet of insects has been evaluated against *Bradysia impatiens* from the order Diptera (Main *et al*. 2014) and *Bruchidius incarnatus* from the order Coleoptera (Sabbour and E-Abd-El-Aziz 2010). There is no available data on the toxicity of this plant product applied in the diet of lepidopteran insects. Only contact toxicity causing growth inhibition effects has been shown via topical application of the oil to the dorsum of *Trichoplusia ni* (Akhtar *et al.* 2014).

The aim of this study was to estimate the potential usefulness of *Brassica alba* mustard oil with butyl isothiocyanate in plant protection. In our research the insecticidal activity via ingestion of oil was determined against insects from the order Lepidoptera: *Cydia pomonella* L., *Dendrolimus pini* L., and *Spodoptera exigua* Hübner representing various families. *Cydia pomonella* from the family Tortricidae causes damage to fruit such as apples, pears, plums, and apricots. It is hard to protect the fruit against this insect because insecticides may target eggs and the first stage of larva. Then, the larva tunnels into fruit and becomes unavailable for pesticides (Alston *et al.* 2010). *Dendrolimus pini* from the family Lasiocampidae damages conifers, particularly Scots pine *Pinus sylvestris*, which may cause tree death. It is an important pest with outbreaks in Eastern and Central Europe (Björkman *et al.* 2013). *Spodoptera exigua* from the family Noctuidae is a polyphagous insect that can develop in vegetables, grasses, weeds and flowering plants in fields and greenhouses throughout the world (Hua *et al.* 2013) The problem in the management of *S. exigua* is pesticide resistance of the insect (Capinera 1999).

The essential oil – mustard oil from white mustard *B. alba* was purchased from Synthite Industries Ltd. (India). According to the producer's information, the oil was derived from white mustard seeds by using steam distillation and contained min. 90% butyl isothiocyanate.

The activity of the oil was determined against three insect pest species: *C. pomonella* (codling moth) from the family Tortricidae, *D. pini* (pine-tree lappet) from the family Lasiocampidae, and *S. exigua* (beet armyworm) from the Noctuide family. The insect species represent the order Lepidoptera.

The *C. pomonella* and *S. exigua* insects came from a standardized laboratory culture of the Department of Microbiology, Adam Mickiewicz University. The culture originated from insects reared in the Institute of Plant Protection – National Research Institute (Poland). The pests were cultured at 26°C with 40–60% relative air humidity (RH) and L16 : D8 photoperiod.

The *D. pini* larvae were collected from their natural population in a pine forest in Central Poland. The insects were then reared in the laboratory at 23°C (±2°C), 40–60% relative air humidity, natural photoperiod and fed pine needles.

The mustard oil was suspended in Tween 80 (Sigma-Aldrich, USA): 1 ml of mustard oil was added to 0.5 ml of Tween 80. The following dilutions of the oil were prepared in distilled sterile water: 0.1 mg · ml^{-1}, 0.5 mg · ml^{-1}, 1 mg · ml^{-1}, 5 mg · ml^{-1}, 7.5 mg · ml^{-1}, 10 mg · ml^{-1}, 12.5 mg · ml^{-1}, 15 mg · ml^{-1}, 7.5 mg · ml^{-1}, 20 mg · ml^{-1}, and 25 mg · ml^{-1} (Table 1). Each dilution contained Tween 80%: 0.05 mg · ml^{-1}, 0.25 mg · ml^{-1}, 0.5 mg · ml^{-1}, 2.5 mg · ml^{-1}, 3.75 mg · ml^{-1}, 5 mg · ml^{-1}, 6.25 mg · ml^{-1}, 8.75 mg · ml^{-1}, 15 mg · ml^{-1}, 10 mg · ml^{-1}, and 12.5 mg · ml^{-1}, respectively. The concentrations of mustard oil were established based on preliminary bioassay tests conducted before the experiment. In a pilot testing, each of the ten oil concentrations (0.01 mg · ml^{-1} –100 mg · ml^{-1}) was employed against ten caterpillars in the same manner as described below.

The medium for *C. pomonella* rearing (Guennelon *et al.* 1981) was poured into individual boxes, 23 mm in diameter, of transparent polystyrene multi-well plates. After solidification of the medium, 50 µl of mustard oil of a known concentration were poured on the whole surface of the diet and kept until dry. Then, two-day-old insect larvae of the first instar were put into each well (one caterpillar per well) and the box was covered with a transparent lid enabling air flow.

For bioassay with *D. pini*, *P. sylvestris* needles, 6 cm long and 0.1 cm wide, were used. Aliquots of 10 µl of mustard oil of each dilution were distributed on the surface of each needle and left to dry without touching any surface. One pine needle and one *D. pini* larvae of the first instar were put into a separate 60 mm diameter Petri dish. Additional pine needles were supplemented to avoid insects' death from starvation – after having eaten the infected needle, the larvae were provided with uninfected needles.

The medium for *S. exigua* culturing (Poitout and Bues 1970) was formed into pieces of 5 mm diameter and 3 mm thick, and each piece was placed in a separate box of transparent polystyrene multi-well plates. Ten µl of mustard oil of a known concentration were spread on the top surface of the medium and seven-day-old insect larvae of the second instar were placed

Table 1. Concentrations of mustard oil used in the study

Units	Concentration of mustard oil										
mg · ml^{-1}	0.1	0.5	1	5	7.5	10	12.5	15	17.5	20	25
µg · 10 μl^{-1}	1	5	10	50	75	100	125	150	175	200	250
µg · 50 μl^{-1}	5	25	50	250	375	500	625	750	875	1000	1250

on the medium's surface – one caterpillar per the box. After the piece of medium was eaten, another piece was added to avoid insects' death from starvation.

Each dilution of the oil was provided to 30 larvae – three repetitions with 10 caterpillars each; the repetitions of the bioassay were done simultaneously with the same stock solutions of mustard oil. As a control, 30 larvae were given 10 mg · ml^{-1} Tween 80 instead of the oil. Conditions of insect rearing are presented in Table 2.

Dead and live insects were counted after 7 days. Mortality correction was calculated by using Abbot's formula (O'Callaghan *et al*. 2012) and the lethal concentration (LC_{50}) of the mustard oil was determined by probit analysis using BioStat 2009 Professional 5.8.4 (AnalystSoft Inc., Canada).

The LC_{50} value of mustard oil against *C. pomonella* was 0.422 mg · ml^{-1} (21.1 µg of the oil in 50 µl per one larva) (Table 3). At the concentration of 1 mg of the plant substance in 50 µl per one insect there was 100% mortality. Fiducial limits obtained for LC_{50} values were wide: the lower value was 0.06 mg · ml^{-1} and the upper 3.3 mg · ml^{-1}. The upper limit was 55 times greater than the lower. The reason of this wide range of fiducial limits could be similar mortality of insects treated with mustard oil of different concentrations. We noted similar percentages of dead larvae, 77 and 80%, after treating them with 5 mg · ml^{-1} and 10 mg · ml^{-1} of mustard oil, respectively. Applying 0.1 mg · ml^{-1} and 0.5 mg · ml^{-1} of the oil also caused similar mortality of pests: 43 and 47%, respectively.

LC_{50} of the botanical product against *D. pini* was 11.74 mg · ml^{-1} (117.4 µg of the oil in 10 µl per one larva) (Table 3). The usage of 20 mg · ml^{-1} (200 µg of the plant preparation in 10 µl per one larva) resulted in 100% caterpillar mortality.

LC_{50} of the plant preparation against *S. exigua* was 11.66 mg · ml^{-1} (116.6 µg of the oil in 10 µl per one larva) (Table 3). The botanical product at a concentration of 25 mg · ml^{-1} (250 µg of the oil in 10 µl per one larva) caused 100% caterpillar mortality.

The oil was the most active against *C. pomonella* in comparison with *D. pini* and *S. exigua*. The LC_{50} values of the botanical product against *D. pini* and *S. exigua* in 10 µl per one larva were similar. The concentration of the plant product that caused 100% mortality was similar for the three insect species used in our study.

No data are available about the toxicity of mustard oil for lepidopteran insects determined via ingestive application of the oil. Our research provides new results concerning the insecticidal activity of this plant oil applied to the intestinal tract via insects' diet.

Up to now, only one conference paper has reported insecticidal effects of the essential oil of black mustard – *Brassica nigra* against lepidopteran insects, but the way of the oil application was different. The contact toxicity effect was determined via topical application of the essential oil to the dorsum of third instar larvae of *Trichoplusia ni*. The EC_{50} value (effective concentration causing 50% growth inhibition) of the plant substance was 371.9 ppm (Akhtar *et al*. 2014). Our LC_{50} values, presented as ppm instead of oil µg in 10 µl per one larva, were 422 ppm, 11,740 ppm and 11,660 ppm of the preparation against *C. pomonella*, *D. pini* and *S. exigua*, respectively (assuming that 1 µg · ml^{-1} equals 1 ppm). Due to the different way of oil application and the different plant species from which the essential mustard oil was derived, it is difficult to compare our results with those presented by Akhtar *et al*. (2014). Moreover, we evaluated the lethal concentrations of the botanical product against insects, whereas Akhtar *et al*. (2014) determined the concentration of the oil causing growth inhibition of larvae.

The activity of mustard oil extracted from *Brassica juncea* seeds was evaluated against insect pest fungus gnats *Bradysia impatiens* from the Diptera order. The

Table 2. Conditions of insect rearing

Insect	Temperature [°C]	Relative air humidity [%]	Photoperiod
Cydia pomonella	26	40–60	L16 : D8
Dendrolimus pini	23 (±2)	40–60	natural
Spodoptera exigua	26	40–60	L16 : D8

Table 3. Activity of mustard oil against lepidopteran insects

Insect	LC_{50} of mustard oil against insects				95% Fiducidal limits	Slope ±SE	Chi-square
	[mg · ml^{-1}]	[µg · larva^{-1}]	[mg · cm^{-2}]	[ppm]	[mg · ml^{-1}]		
Cydia pomonella	0.422	21.1	0.0051	422	0.06–3.3	0.5 ± 0.30	2.1
Dendrolimus pini	11.740	117.4	0.1957	11,740	22.53–49.4	7.9 ± 0.10	22.0
Spodoptera exigua	11.660	116.6	0.5941	11,660	10.81–12.64	8.6 ± 0.02	0.48

LC_{50} value of the oil against the insects was 0.8 mg · cm^{-2} of the diet (Main *et al.* 2014). In our study, the oil of a different plant species was used – *B. alba*. Moreover, the manner of the oil application in our study was different than that proposed by Main *et al.* (2014). Main *et al.* (2014) mixed the substance with the insects' diet. In our research the oil was spread on the surface of the insects' diet (synthetic medium or pine needle) that imitated the use of potential bioinsecticides in plant or fruit protection. Thus, in our study, the calculation of LC_{50} per 1 cm^2 of insects' diets is indicative. The mustard oil, spread on the surface of the diet, soaked into it. The amount of diet containing the oil is unknown. Approximate values of LC_{50} of the botanical substance against insects were calculated considering the size of the diet surface on which the oil was spread. The LC_{50} value of the plant substance against *C. pomonella* and *S. exigua* was 0.0051 mg and 0.5941 mg, respectively, of the oil per 1 cm^2 of the diet. LC_{50} of the oil against *D. pini* was 0.1957 mg per 1 cm^2 of pine needle. *Spodoptera exigua* was less susceptible to the plant substance than *C. pomonella* and *D. pini* considering the LC_{50} value of the oil per 1 cm^2 of insect diet. It might be related to the larval stage of *S. exigua,* which was L2. The larval stage of *C. pomonella* and *D. pini* was L1. The oil was the most active against *C. pomonella*. The LC_{50} value of the oil was the lowest against *C. pomonella* of the oil in 10 µl per one larva and per 1 cm^2 of the diet in comparison with LC_{50} of the plant product against *D. pini* and *S. exigua*.

The essential oil from black mustard *B. nigra* was also efficient in reducing the number of bean bruchids beetle *Bruchidius incarnatus* from the Coleoptera order. The usage of 2% of the oil led to 76% insect mortality (Sabbour and E-Abd-El-Aziz 2010). In our study, the application of 2% of the oil caused 100% mortality of *C. pomonella* and *D. pini*. Mortality of 100% of *S. exigua* was noted at a 2.5% concentration of the oil.

The oil had a strong insecticidal activity against lepidopteran pests. It seems to be a promising candidate for protecting crops from insect infestation. The relatively short time of action of the oil applied to the intestinal tract via insects' diet had particular significance considering the short time of feeding on the plant surface of some insect species. One example is *C. pomonella*. The caterpillars tunnel into fruit (Alston *et al.* 2010). Therefore, the usage of pesticides is effective for only a short period of time in which larvae feed on the fruit surface. Moreover, the concentration of the mustard oil needed to be consumed for efficient control of insects is low. The application of 2–2.5% of the oil caused 100% mortality of lepidopteran pests. The plant product may be an alternative for chemical products since bioinsecticides are harmless to the environment, however, the next step in developing new preparations with mustard oil should be gaining a better understanding of fitotoxicity, and the influence on non-targets.

In conclusion, *B. alba* mustard oil exhibited high insecticidal activity against pests from the order Lepidoptera and seems to be an effective biopesticide. It is a fact of great importance due to the fast development of insect resistance against synthetic insecticides and the short list of registered chemical and biological insecticides.

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