Effect of temperature on efficacy of three natural substances to Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae)

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Abstract
Azadirachtin, refined rape oil and slaked lime – three natural substances with insecticidal potential – were tested under laboratory conditions for their efficacy against larvae and adults of Colorado potato beetle, *Leptinotarsa decemlineata*, at 15°C, 20°C, and 25°C. General statistical analysis (all developmental stages and all insecticidal substances taken into account) was performed on the results of the mortality rates of the individuals and no statistically significant differences between the temperatures were observed on the fifth day after application. At all temperatures, the influence of insecticidal substances in question as well as the interaction between the insecticidal substance and the developmental stage of the pest on mortality rates were significant. The individual statistical analysis (a specified developmental stage of the pest at a specified temperature) revealed the maximum efficacy of the refined rape oil at 15°C and 20°C, the adults being the most sensitive developmental stage (mortality rate about 90%). Other substances tested were less effective, and in general the mortality rate of either larvae or adults did not exceed 50%. In the case that the efficacy of the refined rape oil in controlling the population of the adults of the Colorado potato beetle is confirmed also in field trials, this could be the basis for a better strategy in controlling the overwintered Colorado potato beetles on potato.

Keywords: Environment, integrated pest management, mortality rate, natural insecticides, potato.

Introduction
Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is among the economically most harmful insects. Although it was brought to Europe nearly 100 years ago, an effective and sustainable control of this potato pest is still lacking. A too intensive use of synthetic insecticides resulted in resistance of this pest in more than one case (Stanković et al., 2004), while the use of environmentally more acceptable substances – though some of them do show considerable efficacy in controlling this insect (Scott et al., 2003) – has not been generally applied in Europe until now. The reason for this situation can be attributed also to the slow mode of action of these substances, as many growers judge the efficacy of a pesticide according to its immediate effect.

The aim of our research was to investigate the efficacy of three natural substances to control larvae and adults of the pest in question. The contact insecticidal effects of slaked lime and refined rape oil were compared to that of the natural insecticide azadirachtin. The efficacy of the latter on Colorado potato beetle has been well established (Schroeder et al., 1996; Zabel et al., 2002; Martel et al., 2005), but the availability of such data for the first two is scarce (Abdalla et al., 1991; Loockwood et al., 2001; Trdan et al., 2006). We started with two hypotheses: namely, that the temperature of the environment has a significant influence on the efficacy of the tested substances and that there is...
also a significant difference in the susceptibility of different developmental stages to the substances in question. Answers to these questions could improve the strategy of the sustainable control of Colorado potato beetle on potato.

Materials and methods

Natural insecticidal substances

Efficacies of 1% and 5% water solution of slaked lime (IGM Zagorje d.d., Zagorje ob Savi, Slovenia), 1% water solution Prima (a.i. refined rape oil, 750 g/l [±4%] + emulsifier 100 [150 g/l]; Unichem d.o.o., Sinja Gorica, Slovenia), and 0.25% water solution Neem-azal T/S (a.i. azadirachtin, 1%; Metrob d.o.o., Ljubežna, Slovenia) were tested in glass Petri dishes (14 cm diameter). The insecticide Prima is not registered in Slovenia for controlling Colorado potato beetle. For this reason we applied it at a concentration recommended for controlling Homopteran insects and mites during the growing season. We used slaked lime in two concentrations due to the absence of insecticides with this active ingredient on the list of registered pesticides in Slovenia. Therefore we have not had an insight into the level of efficacy of this substance. Insecticide Neem-Azal T/S, which is registered in Slovenia also for control of Colorado potato beetle, was applied at the recommended concentration.

Laboratory bioassay

The individuals for the trial were collected from potatoes grown on the experimental field of the Biotechnical Faculty in Ljubljana, Slovenia (46°04'N, 14°31'E, 299 m a.s.l.). The bottom of each 14 cm-diameter Petri dish was covered with potato leaves and ten individuals of the pest belonging to the same developmental stage (Bounhiol, 1927) were placed in each dish. The trial was performed in a rearing chamber (RK-900 CH, Kambič Laboratory equipment, Semič, Slovenia) at three temperatures (15, 20 and 25°C) in ten repetitions, i.e., each natural insecticide was tested on 100 individuals at each temperature for each of three developmental stages (L1/L2, L3/L4, and adults). The substances tested were applied to the individuals using a house sprayer (volume 0.5 dm³), and immediately after the application the Petri dishes were transferred to the rearing chamber with a controlled relative humidity (75%). They were kept in the dark and the controls were treated with water. The adequacy of the potato leaves was checked daily and they were replaced if necessary. The mortality rate was evaluated the fifth day after the application.

Statistical analysis

A multifactor analysis of variance (ANOVA) was performed to determine the differences in mortality rates (%) between three developmental stages of Colorado potato beetle, reared at three different temperatures. Before the analysis, each variable was tested for homogeneity of treatment variances. The mortality rate data were corrected for control mortality according to Abbott’s formula (Abbott, 1927) and the arcsine square-root was transformed before analysis. Duncan’s multiple range test (p ≤ 0.05) was used to separate mean differences among the parameters in all the treatments. All statistical analyses were performed with Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Maryland, USA). The data are presented as untransformed means ± SE.

Results and discussion

A statistically significant influence of the substance tested (p < 0.014 in all cases) as well as that of the interaction between the substance tested and the developmental stage (p < 0.039 in all cases) on the mortality rate of the tested individuals of the Colorado potato beetle was established for all the temperatures under investigation. The developmental stage of the pest did not exhibit a significant influence (p = 0.8633 at 15°C, p = 0.1189 at 20°C, and p = 0.2118 at 25°C) on the mortality rate of larvae, adults. The refined rape oil was the most effective at all the temperatures studied; at 20°C its efficacy was the same as that of azadirachtin.

Against adults and L1/L2 larvae, refined rape oil, which acts as an insecticide by suffocating the victim, was most effective at 15°C. The mortality rate of adults was higher (90.70 ± 5.70) and comparable to the mortality rate of the beetles treated with the same substance at 20°C (89.13 ± 6.87). Treating L3/L4 larvae at the lowest temperature revealed no significant differences, but the peak of efficacy was at a moderate 23.08 ± 10.53%. At 20°C the mortality rate after five days of no other insecticidal substance exceeded 46%; the efficacy of azadirachtin, an insecticide with anti-feedant activity, was relatively uniform for all the developmental stages under investigation (from 38.72 ± 10.15 for L1/L2 to 45.65 ± 5.95 for adults). At 25°C the only case exhibiting more than a 50% mortality rate was the one where beetles were treated with refined rape oil, while the mortality rate for other insecticidal substances and other developmental stages was never above 36%.

Scarcie investigations on temperature dependence of the insecticide efficacy usually mostly show a
negative correlation between temperature and mortality rate of the insects (Heimbach & Baloch, 1994; Pimentel et al., 2004), although there are some references which prove the opposite to be true (Chandler et al., 1991; Shipp & Zhang, 1999). For obvious reasons the results of similar investigations on other insects, which can considerably differ from Colorado potato beetle, cannot and should not be considered relevant without some serious consideration. This is especially true since slaked lime has not yet been studied in such a context, while in one recent study refined rape oil showed satisfactory field efficacy when reducing harmfulness of cabbage stink bugs on cabbage (Trdan et al., 2006).

The results of related research showed that the activity of azadirachtin on the differential grasshopper, Melanoplus differentialis [Thomas], does not depend on air temperature (Amarasekare & Edelson, 2004). Our results are not in accordance with the findings of the authors, while azadirachtin showed the highest efficacy on all three developmental stages of Colorado potato beetle at 20°C. This and similar examples show specific response of exposed insects to insecticides. This demonstrates the complex relationships between biotic and abiotic factors.

In this connection, special attention should be paid to the fact that results of insecticide efficacy, when comparing laboratory and field applications, are not always comparable. Besides variable air temperature, also other abiotic factors, e.g., light or presence of water, influence the duration of efficacy of insecticidal substances. Also, applied insecticides such as azadirachtin (Barrek et al., 2004) are essentially more exposed to photodegradation and hydrolysis when used outdoors than in the laboratory (Bobe et al., 1998).

Conclusion

The results of the study presented in this contribution are interesting because of the pronounced efficacy of refined rape oil which has not been tested for controlling Colorado potato beetle. Its high efficacy under laboratory conditions (high mortality rate of adults five days after treatment) should be tested under field conditions as soon as possible. Similar efficacy and confirmation of such an insecticidal effect – especially if its highest efficacy at 15°C and 20°C are also taken into account – would provide a sound basis for a better strategy for controlling overwintered Colorado potato beetles.

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References


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