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Field Testing of Efficacy of Three Environmentally Friendly Insecticides Against Colorado Potato Beetle (*Leptinotarsa Decemlineata* [Say], Coleoptera, Chrysomelidae) on Potato-Evaluation of the Effect on Yield

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Abstract

In 2007 and 2008 the field experiment was conducted to test the efficacy of three environmentally friendly insecticides against the Colorado potato beetle (*Leptinotarsa decemlineata*), with the aim of evaluating their effect on the yield of potato. 0.25 % water emulsion of Neem-Azal (active ingredient azadirachtin) was applied twice, while 3 % water emulsion of Aktiv (a.i. potassium salt of fatty acids) and 1 % water emulsion of Prima (a.i. refined rape oil) were applied eight times. In 2007, the potato yield was higher (25.3±3.2 t ha^{-1}) than in 2008 (8.2 ± 0.8 t ha^{-1}). In 2007 there were no significant differences in potato yield at different control measures and the yield ranged from 7.5 ± 1.3 t ha^{-1} (Aktiv) to 9.4 ± 1.8 t ha^{-1} (Prima). In 2008, the highest potato yield was recorded in Neem-Azal treatment (3.5 ± 0.7 t ha^{-1}), while in two other insecticide treatments the potato yield did not differ significantly with control treatment neither with the Neem-Azal treatment. Potato tubers were classified into three fractions: fraction 1 (tubers <4 cm), fraction 2 (tubers between 4 and 5 cm), and fraction 3 (tubers > 5 cm). On average we produce 2.11 ± 0.06 t ha^{-1}, 9.93 ± 0.53 t ha^{-1}, and 13.17 ± 0.70 t ha^{-1} of potato in 2007, and 2.11 ± 0.20 t ha^{-1}, 4.68 ± 0.37 t ha^{-1}, 0.84 ± 0.29 t ha^{-1} of potato in 2008, respectivelly.

Keywords: insecticides, azadirachtin, refined rape oil, potassium soap, IPM, colorado potato beetle, yield.

1. Introduction

The Colorado potato beetle [CLB] (*Leptinotarsa decemlineata* [Say]) (Coleoptera: Chrysomelidae) is one of the most economically significant pests in Europe, launching year after year increasingly stronger attacks on potato fields (Ozturk & Yildrim, 2012). The harmful stages consist of larvae and adults. Both are foliar pests and can eat more than 100 cm^2 of leaf surface during their lifetime (Ozturk & Yildrim, 2013). This pest has two to three generations per year (Berry et al., 1997). The first and second generations can cause 100% defoliation of potato foliage if the potato is not protected. By full defoliation the yield of potato can be reduced by more than 50 % (Ozturk & Yildrim, 2013). Laznik et al. (2010) recentlly reported that total defoliation of potato plants influenced the tuber developments in such a way that potato plants produced more small tubers which were unable to grow in size.

Although it was brought to Europe nearly 100 years ago, an effective and suitable 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. Our current overall aim is to reduce, or to stop using, highly toxic pesticides. Already, some environmentally friendly methods of pest control are being studied, including the use of entomopathogenic nematodes for the control of CPB (Laznik et al., 2010).

The Indian Neem tree, *Azadirachta indica* A. Juss, has long been recognized as a multipurpose plant species, with uses varying from medicinal to plant protection (Schmutterer, 1990). Hence, the insecticidal properties of azadirachtin, the most active molecule extracted from its kernels, have been exhaustively studied over the past three decades. These properties include antifeedancy, repellency, ovipositional deterrence, inhibition of fertility, reduction of fitness, and disruption of insect growth. The mechanisms involved and the potential of neem-based insecticides to control a wide range of insects, including CPB, have been reviewed by Schmutterer (1990) and Mordue and Blackwell (1993). In addition, to contact toxicity, systemic activity has been documented (Gill & Lewis, 1971; Osman & Port, 1990). The efficacy of azadirachtin to CPB has been demonstrated under laboratory conditions by Trdan et al. (2007). Several neem extracts have been commercialized in the United States and Europe (Immaraju, 1998).

Fatty acids and their derived soap products were first regarded as contact insecticides over 80 years ago (Fulton, 1930). However, research on pesticidal soaps was discontinued because of the popularity of synthetic insecticides, until the resurgence of interest in the 1970s. Since the early 1980s, commercial formulations of soap have been available for the control of soft-bodied pests, such as mites, aphids, and psyllids, on ornamentals as well as on selected fruit and vegetable crops (Koehler et al., 1983). Trdan et al. (2006) also confirmed moderate efficacy of potassium soap against the cabbage stink bugs in white cabbage. Soaps induce rapid mortality by disrupting the permeability of insect cuticles and by causing asphyxiation through obstruction of the spiracles (Koehler et al., 1983).

Insecticidal oils, including those of botanical or mineral origin, are favorable biorational pesticides for management of numerous pest insects, especially soft-bodied insects (Yang et al., 2010). Oils have different effects on pest insects. The most important is that they block the air holes (spiracles) through which insects breath, causing them to die from asphyxiation (Butler et al., 1989). In some cases, oils also may act as poisons, interacting with the fatty acids of the insect and interfering with normal metabolism (Liang & Liu, 2002). Oils also may disrupt how an insect feed, a feature that is particularly important in the transmission of some plant viruses by aphids (Fournier & Brodeur, 2000).

The aim of our research was to investigate the field efficacy of three natural substances (azadirachtin, refined rape oil and potassium salt of fatty acids known as potassium soap [produced by adding potassium hydroxide to the fatty acids present in animal fats and plant oils]) to control CPB and to evaluate the effect of treatment on potato yield. Trdan et al. (2007) confirmed in a preliminary laboratory investigation a high susceptibility of CPB adults and larvae to refined rape oil (high mortality rate of adults five days after treatment), while azadirachtin was less effective in their research.

2. Material and Methods

2.1 Experimental Field

We planted the Kondor potato variety in a plot measuring 40 x 5.9 m on the experimental field of the Biotechnical Faculty in Ljubljana, Slovenia (46^{o}04'N, 14^{o}31'E, 299 m alt.) on 11 April 2007 and on 28 April 2008. The preparation of the field began in autumn 2006, when stable manure (30 t ha^{-1}) was spread and then the field was ploughed. In spring time the field received the mineral fertiliser, NPK (15:15:15). In both years after the potato harvest we seeded the oil radish variety Raula (20 kg ha^{-1}), which later served as a green manure. The potato was planted with a two-row automatic planting machine with shedding discs. The planting speed was 3 km h^{-1}, planting depth was around 5 cm with row distance of 75 cm. The planting density was 45,000 tubers ha^{-1}, and distance between tubers in a row was 29.7 cm. We divided the field into four blocks, and in each there were four treatments: control (unsprayed), Neem-Azal (0.25 % water emulsion; a.i. azadirachtin; manufacturer and supplier Metrob d.o.o., Ljubečna, Slovenia), Prima (1 % water emulsion; a.i. refined rape oil; manufacturer and supplier Unichem d.o.o., Sinja Gorica, Slovenia) and Aktiv (3 % water emulsion; a.i. potassium salt of fatty acids; manufacturer and supplier Unichem d.o.o., Sinja Gorica, Slovenia). The size of each treatment parcel was 14.75 m^2 (2.5 x 5.9 m).

2.2 Agri-technical Measures

The herbicide Sencor WG 70 (0.75 kg ha^{-1}, a.i. metribuzin; manufacturer: Bayer CropScience, Leverkusen, Germany; supplier: Bayer d.o.o., Ljubljana, Slovenia) was used (29 May 2007) after the potato plants grew to 5 cm. On 14 May 2008 we used the herbicide Plateen (2.5 kg ha^{-1}) (a.i. flufenacet and metribuzin; manufacturer: Bayer CropScience; supplier: Bayer d.o.o.). When the economic treshhold (25 adults counted on 50 potato plants) [4] was exceeded, application of insecticides was conducted. The economic treshold was in 2007 exceeded on 18 May and in 2008 on 15 May. In 2007 the insecticide Neem-Azal was applied on 25 May and on 13 July, the application with Aktiv and Prima was conducted on 18 May, 25 May, 5 June, 15 June, 26 June, 5 July, 13 July and 25 July. In 2008 the insecticide Neem-Azal was applied on 21 May and on 9 July, the application with Aktiv and Prima was conducted on 17 May, 23 May, 1 June, 9 June, 17 June, 1 July, 9 July and 17 July. We applied insecticides using the backpack sprayer Solo 425. The jet stream nozzle number 04F110 with a pressure of 2 bars was used.

Due to the large possibility in appearance of late blight (*Phytophthora infestans* [Mont.] de Bary) we sprayed the potatoes with the fungicide Acrobat MZ (2.5 kg ha^{-1}) (a.i. dimetomorf-9 % and mancozeb-60 %; manufacturer: BASF SE, Ludwigshafen, Germany; supplier: BASF Slovenija, d.o.o., Ljubljana) on 29 May and 13 June 2007. In 2008 the fungicide Melody duo (2.5 kg ha^{-1}) (a.i. iprovalicarb-5.5 % and propineb-61.3 %; manufacturer: Bayer CropScience; supplier: Bayer d.o.o.) was applied for the same purpose on 26 June, and on 24 July. The potatoes were harvested with a back output machine with two rolling plates on 8 August 2007 and on 12 August 2008. On the day of harvest the tubers were classified with a special shaking device into three fractions: fraction 1 (tubers <4 cm), fraction 2 (tubers between 4 and 5 cm), and fraction 3 (tubers > 5 cm) and weighed them separately as well as together. Later we calculated this to the t ha^{-1}.

2.3 Statistical Analysis

Differences in yield were analysed with the use of ANOVA. Prior to analysis, each variable was tested for homogeneity of variance, and the data found to be non-homogenous was transformed to log(Y) before ANOVA. Significant differences ($P \leq 0.05$) between mean values were identified using Student-Newman-Keuls's multiple range test. All statistical analyses were done using Statgraphics Plus for Windows 4.0 (Statistical Graphics Corp., Manugistics, Inc., Rockville, MD, USA). The data is presented as untransformed means ± SE (Laznik et al., 2012).

3. Results

A group analysis demonstrated that in 2007 (F = 4.50; df = 3, 47; P = 0.0559) the potato yield was not affected by measures of pest control, while in 2008 (F = 7.95; df = 3, 47; P = 0.0114) the mentioned measures had significant differences in potato yield. In 2007 the highest yield was obtained in the Prima treatment (9.35 ±1.81 t ha^{-1}) and lowest in the Aktiv treatment (7.51 ±1.26 t ha^{-1}) (Figure 1). In 2008 the highest yield was obtained in the Neem-Azal treatment (3.47 ±0.68 t ha^{-1}) and lowest in the control treatment (2.03 ±0.38 t ha^{-1}). A group analysis demonstrated that in 2007 (F = 216.63; df = 2, 47; P < 0.0001) and 2008 (F = 87.07; df = 2, 47; P < 0.0001) the potato yield was affected by different size fractions. In 2007 the highest yield was obtained with fraction 3 (tubers > 5 cm) [13.17 ±0.71 t ha^{-1}] and the lowest with fraction 1 (tubers <4 cm) [2.11 ±0.06 t ha^{-1}]. In 2008 the highest yield was obtained with fraction 2 (tubers between 4 and 5 cm) [4.40 ±0.43 t ha^{-1}] and the lowest with fraction 3 (tubers > 5 cm) [0.84 ±0.29 t ha^{-1}].

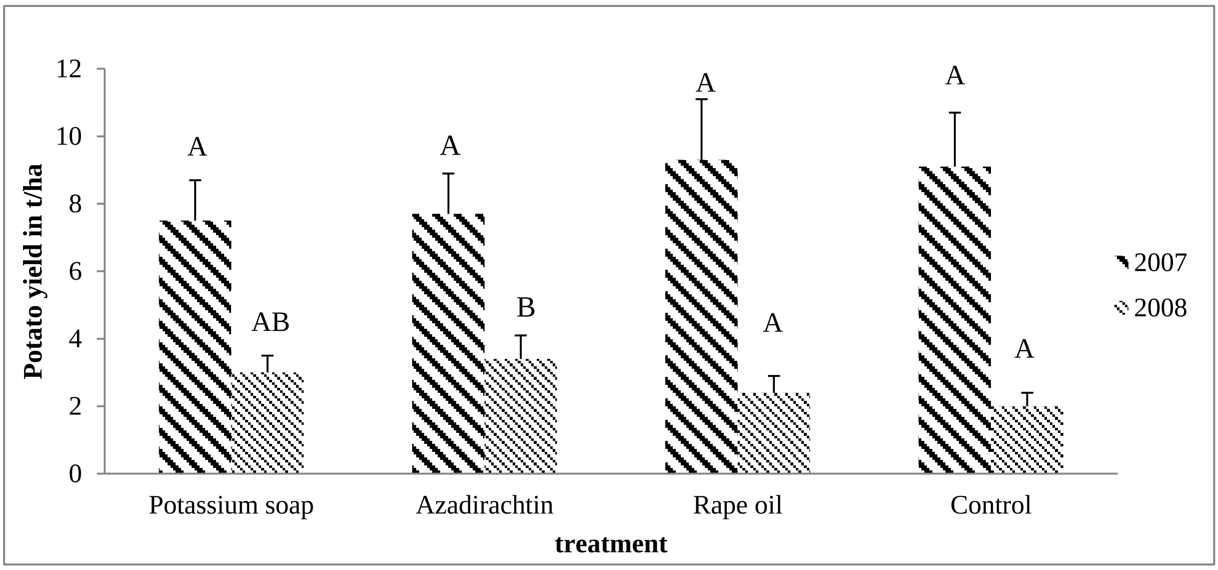

Figure 1. Potato yield [t/ha] at different control treatments in 2007 and 2008. Mean values followed by the same letter do not differ (P≤0.05) according to Student-Newman-Keuls's multiple range test. Bars represent SE of mean potato yield.

No statistically significant differences between the treatments were determined in 2007 (F = 0.75; df = 3, 15; P = 0.5493, while in 2008 (F = 1.03; df = 3, 15; P = 0.0252) such differences were established in fraction 1. On average we produced per treatment 2.11 ±0.06 t ha^{-1} of potato in 2007 and 2.11 ±0.20 t ha^{-1} of potato in 2008

(Figures 2-3). In 2007 the yield ranged from 1.94 ±0.12 t ha^{-1} (Aktiv) to 2.20 ±0.16 t ha^{-1} (control treatment). In 2008 the yield ranged from 2.11 ±0.29 t ha^{-1} (Prima) to 2.88 ±0.48 t ha^{-1} (Aktiv).

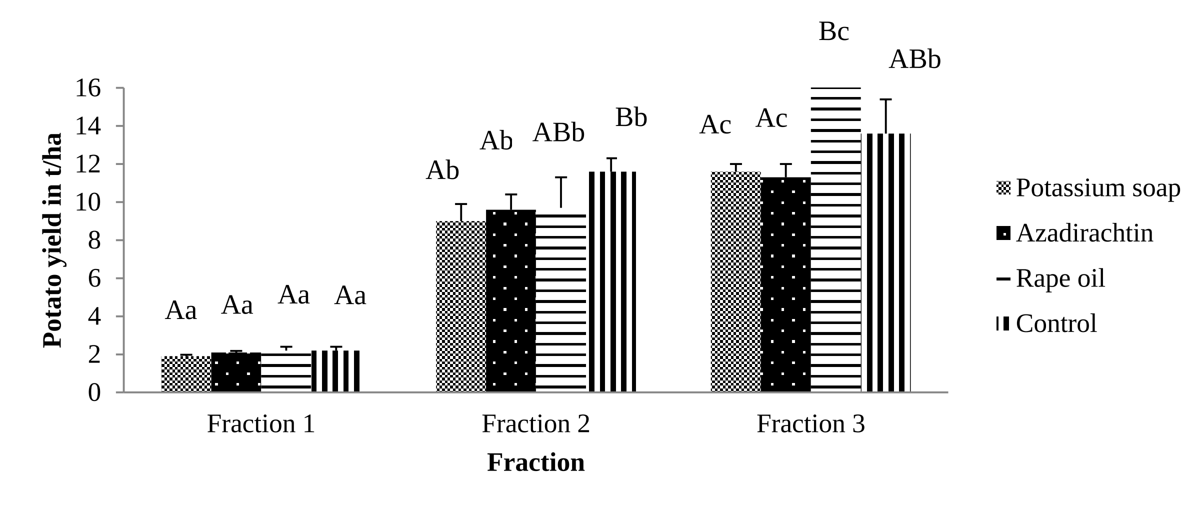

Figure 2. Potato yield [t/ha] at different fractions and treatments in 2007. Mean values followed by the same letter do not differ (P≤0.05) according to Student-Newman-Keuls's multiple range test. Small letters indicates statistical significant differences between different fractions, big letters indicates statistical significant differences between different control treatments. Bars represent SE of mean potato yield. Fraction 1 (tubers < 4 cm); fraction 2 (tubers between 4 and 5 cm); fraction 3 (tubers > 5 cm)

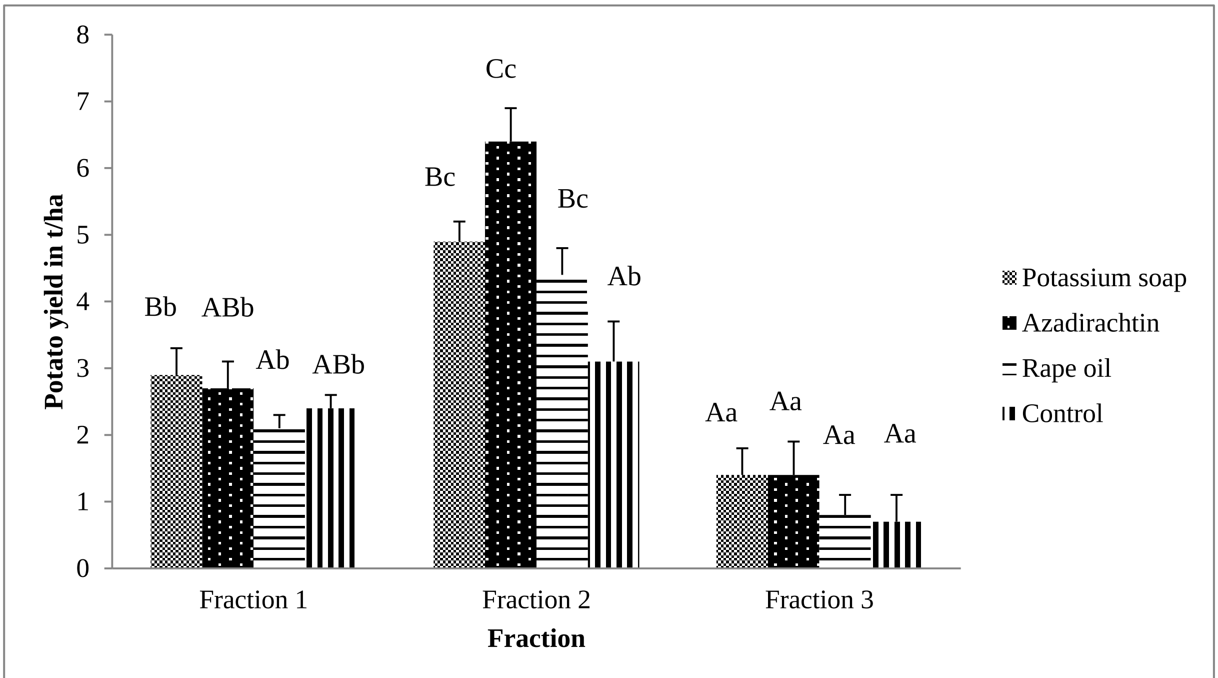

Figure 3. Potato yield [t/ha] at different fractions and treatments in 2008. Mean values followed by the same letter do not differ (P≤0.05) according to Student-Newman-Keuls's multiple range test. Small letters indicates statistical significant differences between different fractions, big letters indicates statistical significant differences between different control treatments. Bars represent SE of mean potato yield. Fraction 1 (tubers < 4 cm); fraction 2 (tubers between 4 and 5 cm); fraction 3 (tubers > 5 cm)

In 2007 at fraction 2 we determined statistically significant differences between different treatments ($F = 0.97$; df = 3, 15; $P = 0.0477$) and the values ranged from 8.98 ± 0.89 t ha^{-1} (Aktiv) to 11.52 ± 0.92 t ha^{-1} (control treatment). The harvested potato as classified in fraction 2, yielded statistically significant differences between the treatments in 2008 (F = 6.45; df = 3, 15; *P* = 0.0127) (Figures 2-3). The highest yield was obtained at treatment Neem Azal (6.35 ± 0.52 t ha^{-1}) and the lowest at the control treatment (3.05 ± 0.63 t ha^{-1}). On average we produced per treatment 9.93 ± 0.53 t ha^{-1} of potato in 2007 and 4.68 ± 0.37 t ha^{-1} of potato in 2008 (Figure 4).

The harvested potato as classified in fraction 3, yielded statistically significant differences between the treatments in 2007 (F = 5.31; df = 3, 15; *P* = 0.0221). The highest yield was obtained at treatment Prima (16.18 ± 1.00 t ha^{-1}) and the lowest at the Neem-Azal (11.35 ± 0.73 t ha^{-1}). In 2008 at fraction 3 we did not determined statistically significant differences between different treatments (F = 0.96; df = 3, 15; *P* = 0.4516) and the values ranged from 0.67 ± 0.36 t ha^{-1} (control treatment) to 1.35 ± 0.42 t ha^{-1} (Aktiv) (Figures 2-3). On average we produced per treatment 13.17 ± 0.70 t ha^{-1} of potato in 2007 and 0.84 ± 0.29 t ha^{-1} of potato in 2008 (Figure 4).

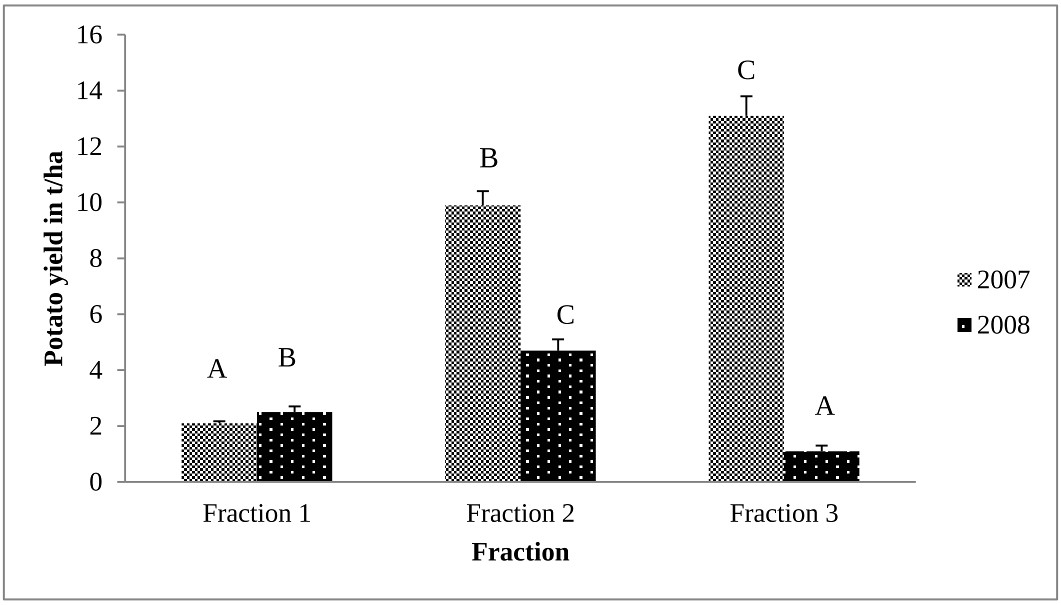

Figure 4. Potato yield [t/ha] at different fractions in 2007 and 2008. Mean values followed by the same letter do not differ (P≤0.05) according to Student-Newman-Keuls's multiple range test. Letters indicates statistical significant differences between different fractions in the same year. Bars represent SE of mean potato yield. Fraction 1 (tubers < 4 cm); fraction 2 (tubers between 4 and 5 cm); fraction 3 (tubers > 5 cm)

4. Discussion

Results of our research showed that in 2007 the potato yield was higher than in 2008. The reason for this is that in 2008 we used as planting material the potato from the previous year (Milošević et al., 2008). The degeneration of potato is appeared to be either due to physiological causes or due to infection of tuber-borne viruses (Chandla et al., 2001). Physiological degeneration can be recovered through proper crop management, but degeneration due to virus can hardly be overcome. Thus the degeneration problem of potato seed tubers due to PVY and PLRV is considered to be the most severe constraint to potato cultivation. It is the most tenacious problem of potato seed tubers resulting spontaneous yield deterioration of the crop (Chandla et al., 2001). When compared to some related research, where the yield of the Kondor potato variety was studied (Ábrahám et al., 2006; Musa et al., 2009) we attained poorer results, also due to the use of small amounts of fertilizers and to high population pressure of CPB in our experiment. We gained similar conclusions also in our related previous research (Laznik et al., 2010).

Although reports of earlier studies indicate that several environmentally friendly insecticides could offer an alterantive to chemical control of CPB (Trdan et al., 2007; Hiiesaar et al., 2009; Amiri-Besheli, 2010) our current research confirm such results only partly. Namely in 2008, the potato sprayed twice with azadirachtin, gave the highest yield. Multiple potato treatments with potassium soap and refined rape oil were less efficient and did not gave a satisfactory results. However, it is also important to note that results from laboratory tests are not always comparable to field testing (Cantelo & Nickle, 1992) as the functioning of bioinsecticides in the open is influenced by an extensive list of factors. Hossain et al. (2008) exposed in their research several factors that influence on the efficacy of azadirachtin in the open. Most often, neem products are applied as foliar sprays to control different foliar pests including CPB. Despite the efficacy of foliar application of neem, major drawbacks are addressed: the fast biodegradability of neem is simultaneously an attractive advantage in domestic areas but a hindering drawback where the short persistence lowers efficacy in field applications. In particular UV light, rainfall and perhaps high acidity on treated surfaces of plants cause a fast degradation or the loss of active material sprayed on foliage (Schmutterer, 1990; Johnson et al., 2003). Moreover, it has been addressed in a number of studies that topical applications of azadirachtin solutions with direct contamination of plant dwelling organism can pose a risk to non-taget beneficials such as predators and parasitoids (Krishnaya & Grewal, 2001). Soil applications such as seed dressing or plant substrate treatments could reduce this risk providing that systemic translocation of the active ingredient is possible (Otto, 1996; Thoeming et al., 2003; Kumar et al., 2005).

Our results demonstrate a poor insecticidal activity of potassium soap and refined rape oil, comparable to some previous research (Liu & Stansly, 2000; Amiri-Besheli, 2010). Nevertheless, previous results were based on excellent to perfect coverage obtained with the spray tower or by leaf dips. The contact activity of these materials requires thorough and complete coverage on the leaf surface (Liu & Stansly, 2000). In addition, insecticidal oils and soaps are only active while wet and become ineffective under drying conditions (Butler et al., 1989).

The population density of CPB in our experiment increased with time and majority of damages appeared in the middle of cultivation period of the potato (end of May, in June, and the beginning of July) (Laznik et al., 2010), which resulted in an expected loss of potato. In our research we found that more damaged plants produced a larger number of small tubers and a small number of large tubers.

Total defoliation of potato plants influenced the tuber developments in such a way that potato plants produced more small tubers which were unable to grow in size. We confirmed previously known facts that the size of tubers at the end of the cultivation period depends on the efficiency of controlling larvae and adults of CPB, because of the influence of defoliation on the poorer development of tubers in the soil (Mannan et al., 1992; Kakaty et al., 1992).

Foliar application of environmentally safe insecticides studied in our research did not show satisfying efficacy of controling CPB and their influence on potato yield although the results from a laboratory test (Trdan et al., 2007) were promissing. The low efficacy of tested insecticides could be explained with the rapid degradation under high temperature and UV light (Johnson et al., 2003; Barrek et al., 2004). Therefore, successful use of these materials requires appropriate application methods and environmental conditions. Further research in the field is required to assess more precisely the most effective rate, timing of application and concentration of tested environmentally safe insecticides used under these conditions against CPB.

5. Conclusions

Results of our research showed that in 2007 the potato yield was higher than in 2008. The reason for this is that in 2008 we used as planting material the potato from the previous year. Our results demonstrate a poor insecticidal activity of potassium soap and refined rape oil, comparable to some previous research. Foliar application of environmentally safe insecticides studied in our research did not show satisfying efficacy of controling CPB and their influence on potato yield although the results from a laboratory test (Trdan et al., 2007) were promissing.

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