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The efficacy of environmentally acceptable products for the control of major potato pests and diseases

Tanja BOHINC, Filip VUČAJNK, Stanislav TRDAN

University of Ljubljana

Jamnikarjeva 101, 1000 Ljubljana, Slovenia

E-mail: tanja.bohinc@bf.uni-lj.si

Abstract

From 2015–2016, different environmentally acceptable products for the control of harmful organisms, including the Colorado potato beetle (*Leptinotarsa decemlineata*), wireworms (*Agriotes* spp.), early blight (*Alternaria solani*) and late blight (*Phytophthora infestans*), were tested on potatoes. To control the Colorado potato beetle, was tested the efficacy of limestone dust at two concentrations, 345 and 690 kg ha⁻¹. Brassica pellets (200 g m⁻²) and calcium cyanamide (1000 kg ha⁻¹) were tested against wireworms. Tincture of propolis and propolis glycolic extract (mentioned as propolis) at 5 and 10 ml l⁻¹ H₂O was tested against early and late blight. All of these products were combined into four treatments. Treatment 1 included treatments with limestone dust (690 kg ha⁻¹), Brassica pellets and propolis (10 ml l⁻¹ H₂O). Treatment 2 included treatments with limestone dust (345 kg ha⁻¹), calcium cyanamide (1000 kg ha⁻¹) and propolis (5 ml l⁻¹ H₂O). Treatment 3 was positive control – use of registered phytopharmaceutical plant protection method, and treatment 4 was negative control (untreated plots). The inspection of all developmental stages: egg clusters, first and second instar larvae after hatching (L1–L2), and third and fourth instar larvae after hatching (L3–L4, adults), of the Colorado potato beetle was performed. After harvest, the tuber yield was evaluated. The evaluation of the yield was conducted on the small, medium and large tubers. The amount of damage caused by wireworms on the potato tubers was also detected in the different tubers. Calcium cyanamide was more effective than Brassica pellets against wireworms, whereas at a dose of 10 ml l⁻¹ H₂O, propolis was proven to be a good alternative for the management of early and late blight under unfavourable weather conditions for an epidemic outbreak. In 2016, the potato tuber yield in all three treatments was significantly higher than that in the untreated plots.

With the combination of the tested products, promising alternative control strategies for future potato production systems might be obtained, which will be suitable for farming under changing climate conditions with a very narrow spectrum of registered phytopharmaceutical plant protection products.

Keywords: Brassica pellets, calcium cyanamide, diseases, limestone dust, pests, potato, propolis, yield.

Introduction

Europe is one of the top regions for food production in the world. Among the cultivated plants in Europe, potato production is fourth, because it is possible in areas with different climates and in different production systems (Pulatov et al., 2016). In Europe, several harmful organisms, including the Colorado potato beetle (*Leptinotarsa decemlineata* Say), wireworms (*Agriotes* spp.), late blight (*Phytophthora infestans* (Mont.) de Bary) and early blight (*Alternaria solani* (Ellis & G. Martin) L. R. Jones & Grout), can cause yield reductions (Kapsa, 2008).

The economically important fungal potato diseases in Europe are late blight and early blight. Frequently excessive and otherwise inappropriate fungicide applications in different potato growing areas are one of the reasons for the fungal resistance of *P. infestans* (Nærstad et al., 2007), whereas increasingly warm and dry summers and new (environmentally more acceptable) production technologies have significantly increased *A. solani* potato infections (Runno-Paurson et al., 2014; Olle et al., 2015). A high level of potato infection caused by *A. solani* can result in a 20–30% lower yield (Christ, Haynes, 2001).

Due to its pronounced resistance to insecticides (Alyokhin et al., 2008; Rinkevich et al., 2012; Scott et al., 2014) and climate change, the Colorado potato beetle (CPB) is still one of the most important potato pests, although it has been almost 100 years since its first occurrence in Europe (Wang et al., 2017). Furthermore, wireworms are also important potato pests in Europe. The suppression of wireworms in arable land is usually influenced by a limited number of insecticides, while knowledge of alternative methods for their suppression is usually insufficient (Ritter, Richter, 2013; Sufyan et al., 2013; Rogge et al., 2017). Several alternative plant protection methods can be used for the suppression of organisms that are harmful to potatoes. Wood ash has been shown to be efficient against CPB (Boiteau et al., 2012), since it serves as a physical barrier. In our case, we used limestone dust as a physical barrier. In the search for natural fungicides to be used in potato production, we tested propolis. The antifungal efficacy of propolis has already been reported by Anjum et al. (2018) but has never been tested in field conditions. The application

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of Brassica pellets and calcium cyanamide has been previously described by Bohinc and Trdan (2014) as a good alternative method against wireworms.

The purpose of our research was to study the effects of different alternative plant protection products for the suppression of four species of organisms that are harmful to potatoes and to introduce the most efficient product into future environmentally friendly potato production systems.

Materials and methods

Plant material. The two-year (2015–2016) field experiment was conducted at the Experimental Field (46°04' N, 14°31' E, 299 m) of the Biotechnical Faculty of the University of Ljubljana, Slovenia. In the first year of the study, the experimental field covered 522 m², whereas in the second year, the area covered 421.2 m². In the first year, the experiment included the cultivar 'Labadia' (producer: KWS, Germany; supplier: Semernarna Ljubljana Ltd., Slovenia), whereas in the second year, the experiment included the cultivar 'Bonnata' (producer: Stet Holland, Holland; supplier: Semernarna Ljubljana Ltd., Slovenia). 'Labadia' is a traditional early-bulking potato cultivar that is suitable for the fresh market. This cultivar is suitable for all soil types but is susceptible to common scab. 'Labadia' is moderately susceptible to late blight in its foliage and tubers, and conventional treatments are recommended. 'Bonnata' is a medium-early cultivar that is not resistant to potato cyst nematodes

but is resistant to the wart disease *fysio 1*. It is rather resistant to common scab and late blight in its foliage. We planted the 'Labadia' on 16 April 2015, and the 'Bonnata' was planted on 4 April 2016.

Crop and soil management practices. The pre-sowing soil preparation in both years involved two passes with a rotary harrow to a depth of 20 cm in the spring. Between the first and second passes, manure with a nitrogen, phosphorus and potassium (NPK) ratio of 7-20-30 at 500 kg ha⁻¹ was added to the soil. The tuber sowing was conducted with a two-row potato planter (Tehnos Ltd., Slovenia) at a speed of 3 km h⁻¹. The weeds were controlled twice (20 April 2015 and 7 April 2016) with the herbicide Plateen WG 41.5 (a.i. metribuzin, 17.5%) at a dose of 2.5 kg ha⁻¹ with 300 l of water per hectare. At the beginning of June 2015, we mechanically hoed the earth, whereas on 18 May 2016, we treated the field with the herbicides Sencor SC 600 (a.i. metribuzin) at a dose of 0.15 l ha⁻¹ and Fusilade Forte (a.i. fluzifop-p-butyl 15%) at a dose of 0.8 l ha⁻¹. In both years, the previous crop was winter wheat. In the experimental years, the potato seeding fields were chosen in different locations at the Experimental Field.

Treatments. The experimental area in both experimental years was divided into three blocks. In each block, we randomly allocated 4 plots (treatments). In each treatment, the occurrence of four harmful organisms was investigated. All the treatments along with the application dates and doses are presented in Tables 1 and 2.

Table 1. The products in the four treatments that were tested against the four organisms that are harmful to potatoes with their application dates in 2015

Pest / disease	Treatment 1	Treatment 2	Treatment 3 (positive control)	Treatment 4 (negative control, untreated plots)
Colorado potato beetle (<i>Leptinotarsa decemlineata</i>)	Limestone dust-high dose (690 kg ha ⁻¹); applied on 2, 22 and 30 June	Limestone dust-low dose (345 kg ha ⁻¹); applied on 2, 22 and 30 June	Actara 25 WG (a.i. thiamethoxam, 25%); applied on 10 and 31 July	
Wireworms (<i>Agriotes</i> spp.)	Brassica pellets (200 g m ⁻²); applied on 2 June	Calcium cyanamide (1000 kg ha ⁻¹); applied on 2 June	Force 1.5 G (a.i. tefluthrin, 1.5%); applied on 2 June	
Early blight (<i>Alternaria solani</i>)	Propolis-high dose (10 ml l ⁻¹ H ₂ O); applied on 2, 22 and 30 June and 10, 17 and 31 July	Propolis-low dose (5 ml l ⁻¹ H ₂ O); applied on 2, 22 and 30 June and 10, 17 and 31 July	Ortiva (a.i. azoxystrobin, 50%) + Shirlan 500 SC (a.i. fluazinam); applied on 30 June and 10 July	
Late blight (<i>Phytophthora infestans</i>)	Propolis-high dose (10 ml l ⁻¹ H ₂ O); applied on 2, 22 and 30 June and 10, 17 and 31 July	Propolis-low dose (5 ml l ⁻¹ H ₂ O); applied on 2, 22, and 30 June and 10, 17 and 31 July	Polyram DF (a.i. metiram, 70%); applied on 2 and 22 June and 21 July. Shirlan 500 SC (a.i. fluazinam) + Ortiva (a.i. azoxystrobin); applied on 30 June and 10 July 2015	

In 2015, a tincture of propolis that was prepared by the beekeeping family Plut from Krvavčji Vrh in the municipality of Semič, Slovenia was applied. In the second year (2016) of the study, a propolis glycolic extract – liquid: water and propylene glycol (B NATURAL, Italy) was used. This preparation contained 20% natural propolis and is also marketed by the manufacturer as a fungicide. According to the producer, the following components have been detected in the product: 0.50 mg ml⁻¹ of quercetin, 1.52 mg ml⁻¹ of apigenin, 0.69 mg ml⁻¹ of pinobanksin, 17.00 mg ml⁻¹ of chrysin, 1.30 mg ml⁻¹ of pinocembrin and 11.94 mg ml⁻¹ of galangin.

Limestone dust was obtained from the local manufacturer (Apnenec Ltd., Slovenia). The dust contained 97.70% CaCO₃. Lime nitrogen (Bird d.o.o., Slovenia) and *Brassica carinata* pellets (BioFence®, Italy) were applied to the soil during the earthing up in

both years of the experiment, namely, on 2 June 2015 and 4 June 2016. The lime nitrogen consisted of 19.80% total nitrogen, 1.5% nitrate nitrogen, > 15% cyanamide nitrogen, approx. 0.5% dicyanamide nitrogen, >50% calcium oxide (CaO), 12% carbon (C), 10% calcium carbonate (CaCO₃), 2% calcium sulphate (CaSO₄), 2% magnesium carbonate (MgCO₃) and 2% mineral oxides and hydroxides. The Brassica pellets contained 6.0% organic nitrogen, 2.2% phosphorus, 2.0% potassium, 45.0% organic carbon and 4.0% water.

The potatoes were harvested with an IK-1 D back output machine (Tehnos Ltd., Slovenia) with two rolling plates on 13 August 2015 and 24 August 2016. The potatoes were picked manually. On the day of the harvest, the tubers were sorted with a special shaking device Strzelec M637 (Krukowiak, Poland) into three fractions: small (<4 cm), medium (between 4 and 5 cm) and large (>5 cm).

Table 2. The products in the four treatments that were tested against the four organisms that are harmful to potatoes with application dates in 2016

Pest / disease	Treatment 1	Treatment 2	Treatment 3 (positive control)	Treatment 4 (negative control, untreated plots)
Colorado potato beetle (<i>Leptinotarsa decemlineata</i>)	Limestone dust-high dose (690 kg ha ⁻¹); applied on 24 June and 5 July	Limestone dust-low dose (345 kg ha ⁻¹); applied on 24 June and 5 July	Actara 25 WG (a.i. thiamethoxam, 25%); applied on 24 June and 17 July	
Wireworms (<i>Agriotes</i> spp.)	Brassica pellets (200 g m ⁻²); applied on 4 June	Calcium cyanamide (1000 kg ha ⁻¹); applied on 4 June	Force 1.5 G (a.i. tefluthrin, 1.5%); applied on 4 June	
Early blight (<i>Alternaria solani</i>)	Propolis-high dose (10 ml l ⁻¹ H ₂ O); applied on 3, 10 and 23 June and 5, 19 and 29 July	Propolis-low dose (5 ml l ⁻¹ H ₂ O); applied on 3, 10 and 23 June and 5, 19 and 29 July	Ortiva (a.i. azoxystrobin, 50%) + Shirlan 500 SC (a.i. fluazinam); applied on 23 June	
Late blight (<i>Phytophthora infestans</i>)	Propolis-high dose (10 ml l ⁻¹ H ₂ O); applied on 3, 10 and 23 June and 5, 19 and 29 July	Propolis-low dose (5 ml l ⁻¹ H ₂ O); applied on 3, 10 and 23 June and 5, 19 and 29 July	Polyram DF (a.i. metiram, 70%); applied on 10 and 23 June. Shirlan 500 SC (a.i. fluazinam) + Ortiva (a.i. azoxystrobin); applied on 5 and 19 July. Revus (a.i. mandipropanid 25%); applied on 29 July	

Field observations and evaluation. Five plants per plot from each treatment were evaluated for early and late blight symptoms. The infection levels caused by both fungi were evaluated according to the slightly modified 6-grade visual evaluation scale (OEPP/EPPO, 1997) that was used by Bohinc et al. (2015): 1 – non-infected plant,

2 – plant with 1 to 5% infected leaf area, 3 – plant with 6 to 10% infected leaf area, 4 – plant with 11 to 20% infected leaf area, 5 – plant with 21 to 50% infected leaf area, and 6 – plant with more than 50% infected leaf area. We also evaluated the phenological growth stages of the potato plants by using the BBCH-Monograph (2001) (Table 3).

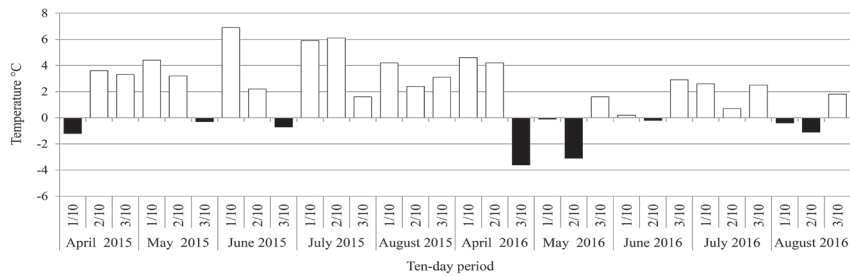
Table 3. List of the evaluation dates of the phenological growth stages (BBCH) of the potato plants in 2015 and 2016

Year	Date	BBCH	Description
2015	2 June	13	3 rd leaf on the main stem is unfolded;
	22 June	33	30% of the plants meet between the rows;
	30 June	39	approximately 90% of the plants meet between the rows;
	10 July	44	40% of the final tuber mass is reached;
	17 July	55	the buds of the first inflorescence extend 5 mm;
	31 July	91	the beginning of leaf yellowing
2016	3 June	13	3 rd leaf on the main stem is unfolded;
	10 June	17	7 th leaf on the main stem is unfolded
	23 June	33	30% of the plants meet between the rows;
	10 July	42	20% of the total final tuber mass is reached;
	17 July	51	the first individual buds (1–2 mm) of the first inflorescence are visible (main stem)

In 2015, the evaluations of the infected leaf area (with early blight only) were performed four times: on 10, 17 and 31 July and 7 August. In 2016, we assessed the leaf areas infected with early and late blight six times: on 20 and 24 June, 1, 10 and 27 July and 5 August. For the CPB, we counted the egg clusters of young larvae (as L1–L2 larvae), old larvae (as L3–L4 larvae) and adults according to Laznik et al. (2010). The counting of individuals of different developmental stages of CPB was performed on five potato plants from the central area of the specific treatment plots. A visual inspection was made on the five successive selected plants in each treatment, and the different CPB developmental stages were counted throughout the experiment. Throughout the entire growing season, the early and late blight monitoring and the CPB counting was always performed on the same plants, because the number of CPB larvae and adults was not large, and consequently, the potato leaves were not severely injured. The injuries caused by the wireworms were counted on the potato tubers in each specific tuber size fraction per treatment. In 2015, a total of 35 tubers per size fraction were examined per treatment, whereas in 2016, 21 tubers were evaluated per fraction. All of the tubers were chosen randomly. On each tuber, the holes (injuries) were counted according to the method of Laznik et al. (2014).

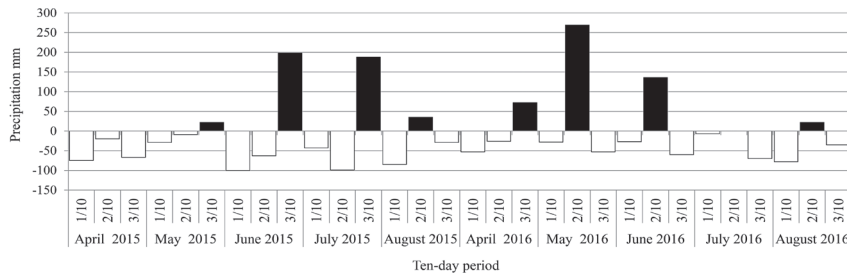
Weather conditions. The climate data were obtained from the bulletin “Naše okolje”, which is published monthly by the Slovenian Environment Agency (2018). An analysis was performed from data period 1981–2010 according to the average values from 1981 to 2010. A value of 0 represented the average temperature from 1981 to 2010. According to the data, 2015 was significantly warmer than the 30-year average value and more temperate than 2016 (Fig. 1). In 21 of the 30 total ten-day periods in the two years of the experiment, the average temperature from 1981–2010 was exceeded; in most cases (16 ten-day periods), the temperature was more than 2°C above the average value for the period.

When both years of the experiment were compared with the 30-year average precipitation, we concluded that both experimental years were drier than the period from 1981–2010 (Fig. 2). In 21 of the 30 total ten-day periods in the two years of the experiment, we recorded less precipitation than that recorded from 1981–2010; in 11 of the ten-day periods, the precipitation was at least 50% lower than the 30-year average. Four precipitation peaks were conspicuous, namely, the 3rd ten-day period in June and July 2015 and the 2nd ten-day period in May and June 2016, when 140 (the 2nd ten-day period in June 2016) to 260% (the 2nd ten-day period



Note. 1/10 indicates the 1st ten-day increment, 2/10 – the 2nd ten-day increment and 3/10 – the 3rd ten-day increment.

Figure 1. Deviations of the ten-day period temperatures from the average temperatures from 1981 to 2010



Note. 1/10 indicates the 1st ten-day increment, 2/10 – the 2nd ten-day increment and 3/10 – the 3rd ten-day increment.

Figure 2. Deviations of the ten-day precipitation periods from the average precipitation from 1981 to 2010

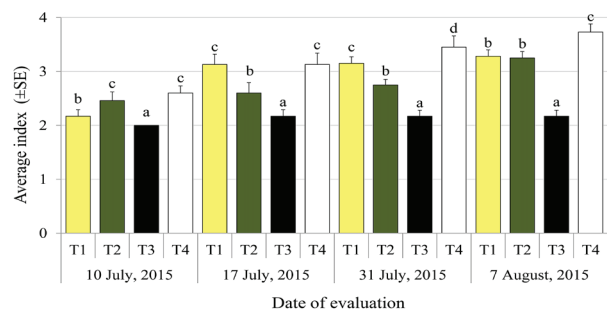
in May 2016) more precipitation than the average for the 30-year period was recorded in Ljubljana, Slovenia.

Statistical analysis. Analysis of variance (*ANOVA*) was conducted to establish the differences among the treatments within the evaluation parameters. Differences in the numbers of CPB in their developmental stages (egg clusters, L1–L2, L3–L4 and adults), infections by early blight and by late blight, and the injuries caused by wireworms among the individual treatments in addition to differences in the yield were analysed with *ANOVA*. Before analysis, each variable was tested for homogeneity of variance, and non-homogenous data were $\log(Y)$ transformed prior to the *ANOVA*. Significant differences ($P \leq 0.05$) between the mean values were identified using Tukey's honestly significant difference (HSD) multiple range test. All statistical analyses were performed using software *Statgraphics Centurion XVI* (Statgraphics Technologies Inc., USA), and the results are presented as the untransformed mean \pm the standard error (SE).

Results

Management of early blight in 2015. Based on the analysis of the experimental results, the infection caused by early blight was influenced by the treatment ($F = 23.15$, $df = 3$, $P < 0.0001$) and evaluation date ($F = 26.33$, $df = 3$, $P < 0.0001$) and their interaction ($F = 7.34$, $df = 9$, $P < 0.0160$). When propolis was applied at the high dose (treatment 1), no more than 6% of the infected leaf area was detected (2.46 ± 0.16) on 10 July (Fig. 3). On 17 July, the infected leaf area in all treatments did not exceed, on average, 11% of the infected leaf area. For example, the level of infection detected in treatment 4 (negative control) was 3.13 ± 0.21 , and the level of infection detected on the plants in treatment 1 was 2.6 ± 0.19 . On 7 August, the plants in treatment 4 (negative control) had the highest infection rate (3.73 ± 0.15).

Management of early blight and late blight in 2016. Based on the analysis of the general results, the infection caused by early blight was influenced by the treatment ($F = 5.31$, $df = 3$, $P = 0.0014$) and evaluation date ($F = 234.53$, $df = 5$, $P < 0.0001$). The effect of the interaction between the evaluation date and the treatment was not significant ($F = 1.54$, $df = 15$, $P = 0.0905$).

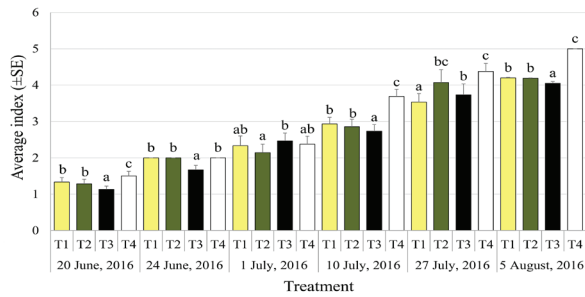


Note. Lowercase letters indicate differences between treatments within a specific date of evaluation); T1 – treatment 1, T2 – treatment 2, T3 – treatment 3, T4 – treatment 4.

Figure 3. Average index of infection caused by early blight in 2015

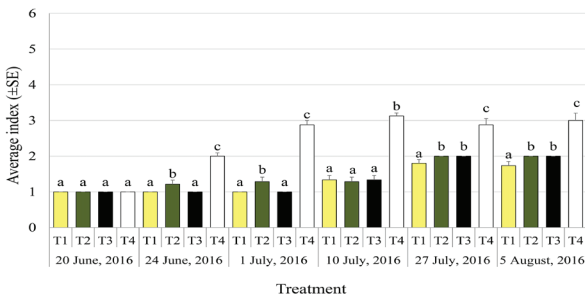
On the first evaluation date, the lowest infection rate was detected in treatment 3 (1.13 ± 0.09). For treatment 1, the level of infection caused by early blight reached 1.33 ± 0.12 . On 1 July, the infection rate reached 2.14 ± 0.23 on the plants in treatment 2. On 27 July, the level of infection caused by early blight was the highest on the plants in treatment 4 (4.37 ± 0.22). On 5 August, the infection by early blight reached 5.00 ± 0.00 in treatment 4, whereas the infection in treatment 4 was 4.05 ± 0.05 . Based on the analysis of the pooled results, the infection caused by late blight was influenced by the treatment ($F = 207.99$, $df = 3$, $P < 0.0001$) and evaluation date ($F = 98.67$, $df = 3$, $P < 0.0001$), and their interaction was significant ($F = 13.22$, $df = 15$, $P < 0.0190$). When the infection rate was evaluated on 1 July, the infection rate on the plants in treatment 4 was 2.87 ± 0.12 . Moreover, the same infection rate was detected on 27 July. On 5 August, the highest infection level by late blight was in treatment 4 (3.00 ± 0.00), whereas the lowest level was detected in the plants in treatment 1 (1.73 ± 0.11) (Figs 4 and 5).

Management of CPB from 2015 to 2016. In 2015, the number of CPB adults was influenced by the evaluation date ($F = 9.68$, $df = 5$, $P < 0.0001$) and the interaction between the exposure date and the treatment ($F = 1.82$, $df = 5$, $P = 0.0303$). The number of CPB adults



Explanation under Figure 3

Figure 4. Average index of infection caused by early blight in 2016



Explanation under Figure 3

Figure 5. Average index of infection caused by late blight in 2016

was not influenced by the treatment ($F = 1.61$, $df = 3$, $P = 0.1859$). Similarly, we did not detect a treatment effect on the egg clusters ($F = 2.15$, $df = 3$, $P = 0.1510$), L1–L2 larvae ($F = 1.01$, $df = 3$, $P = 0.3892$) or L3–L4 larvae ($F = 1.19$, $df = 3$, $P = 0.3136$). We detected an effect of the evaluation date on the egg clusters ($F = 2.44$, $df = 5$, $P = 0.0144$), L1–L2 larvae ($F = 2.39$, $df = 5$, $P = 0.0387$) and L3–L4 larvae ($F = 2.55$, $df = 5$, $P = 0.2592$). Additionally, no effect of the interaction between the evaluation date and the treatment was detected on the egg clusters ($F = 3.00$, $df = 15$, $P = 0.0655$), L1–L2 larvae ($F = 1.18$, $df = 15$, $P = 0.3212$) or L3–L4 larvae ($F = 1.21$, $df = 15$, $P = 0.2599$) (Table 4).

In 2016, the number of CPB adults ($F = 9.68$, $df = 5$, $P < 0.0001$), egg clusters ($F = 25.89$, $df = 5$, $P < 0.0001$), L1–L2 larvae ($F = 2.39$, $df = 5$, $P = 0.0380$) and L3–L4 larvae ($F = 2.55$, $df = 5$, $P = 0.0278$) were influenced by the evaluation date. Based on the results of

the general analysis and the pooled results, no treatment effect was detected on the CPB adults ($F = 1.61$, $df = 3$, $P = 0.1859$), egg clusters ($F = 0.48$, $df = 3$, $P = 0.6978$), L1–L2 larvae ($F = 1.01$, $df = 3$, $P = 0.3892$) or L3–L4 larvae ($F = 1.19$, $df = 3$, $P = 0.3136$). We also detected the effect of the interaction between the treatment and the evaluation date on the CPB adults ($F = 1.81$, $df = 15$, $P = 0.0303$), although no effect of the interaction between the treatment and the evaluation date was detected for the L1–L2 larvae ($F = 1.18$, $df = 15$, $P = 0.2892$), L3–L4 larvae ($F = 1.21$, $df = 15$, $P = 0.2592$) or egg clusters ($F = 0.55$, $df = 15$, $P = 0.9134$) (Table 5).

On the first evaluation date, the highest number of L3–L4 larvae were detected on the plants in treatment 3 (3.47 ± 2.33 larvae plant⁻¹), because no insecticides were used up until that point. The same result was observed for the L1–L2 larvae (2.13 ± 1.17 larvae plant⁻¹). On 27 July (5.25 ± 1.71 larvae plant⁻¹) and 5 August (1.93 ± 0.94 larvae plant⁻¹), the highest number of L3–L4 larvae were on the plants in treatment 4.

Injuries caused by wireworms. In 2015, based on the analysis of the pooled results, the number of injuries (holes) caused by wireworms was influenced by the treatment ($F = 1.68$, $df = 3$, $P = 0.04696$) but not by the distance between the potato plants and the grassland, which was near the field ($F = 2.24$, $df = 4$, $P = 0.0626$). In 2016, based on the analysis of the pooled results, the number of injuries caused by wireworms was influenced by the treatment ($F = 1.65$, $df = 2$, $P = 0.0298$) but not by the distance between the potato plants and the grassland, which was near the field ($F = 3.35$, $df = 4$, $P = 0.0626$). In 2015, the injuries caused by wireworms ranged from 0.07 ± 0.01 holes per tuber in treatment 2 to 0.04 ± 0.01 holes per tuber in treatments 1 and 3. In 2016, the injuries caused by wireworms ranged from 0.56 ± 0.07 holes per tuber in treatment 4 to 0.34 ± 0.07 holes per tuber in treatment 3. All the values are presented in Table 6.

Average yield (2015–2016). The data collected in 2015 showed the effect of the treatment on small tubers ($F = 2.17$, $df = 3$, $P < 0.0001$), medium ($F = 3.17$, $df = 3$, $P = 0.0021$) and large ($F = 4.12$, $df = 3$, $P < 0.0001$) tubers. The average total yield was also influenced by the treatment ($F = 10.18$, $df = 3$, $P < 0.0001$). Based on the results of our survey, we confirmed that the treatment effect of the different formulations was also detected among the small tubers ($F = 3.19$, $df = 3$, $P = 0.0233$). The average total yield was the lowest in treatment 1 (32.21 ± 0.36 t ha⁻¹), whereas no differences were detected among treatments 2 (35.19 ± 0.87 t ha⁻¹), 3 (35.36 ± 0.54 t ha⁻¹) and 4 (34.78 ± 0.95 t ha⁻¹). The

Table 4. The number of Colorado potato beetles in each developmental stage per plant on a specific evaluation date in the various treatments in 2015

	Evaluation date	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Egg clusters	22 June	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	30 June	0.00 ± 0.00 a	0.00 ± 0.00 a	0.13 ± 0.13 a	0.60 ± 0.60 a
	10 July	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	17 July	0.00 ± 0.00 a	0.33 ± 0.19 b	0.40 ± 0.21 b	0.93 ± 0.28 c
	31 July	0.46 ± 0.23 a	0.40 ± 0.21 a	0.20 ± 0.10 a	0.46 ± 0.23 a
	7 August	1.20 ± 0.34 bc	0.93 ± 0.28 b	1.27 ± 0.23 c	0.33 ± 0.15 a
L1–L2 larvae	22 June	0.00 ± 0.00 a	0.00 ± 0.00 a	0.33 ± 0.19 b	0.93 ± 0.28 c
	30 June	0.00 ± 0.00 a	0.00 ± 0.00 a	0.20 ± 0.14 b	2.73 ± 1.84 c
	10 July	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	17 July	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	31 July	0.13 ± 0.13 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.13 ± 0.13 a
	7 August	0.60 ± 0.03 c	0.20 ± 0.10 b	0.00 ± 0.00 a	2.00 ± 1.05 d
L3–L4 larvae	22 June	0.60 ± 0.43 b	0.60 ± 0.60 a	1.93 ± 1.66 b	1.00 ± 0.52 ab
	30 June	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.60 ± 0.60 a
	10 July	0.60 ± 0.60 a	0.00 ± 0.00 a	0.00 ± 0.00 a	1.40 ± 0.84 b
	17 July	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	1.40 ± 0.84 b
	31 July	0.33 ± 0.33 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	7 August	0.00 ± 0.00 a	0.93 ± 0.46 b	0.00 ± 0.00 a	0.46 ± 0.23 b
Adults (imago)	22 June	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	30 June	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	10 July	0.46 ± 0.46 a	0.13 ± 0.13 a	0.06 ± 0.06 a	0.26 ± 0.15 b
	17 July	0.06 ± 0.06 b	0.06 ± 0.06 b	0.00 ± 0.00 a	0.00 ± 0.00 a
	31 July	0.40 ± 0.13 c	0.13 ± 0.09 b	0.00 ± 0.00 a	0.26 ± 0.20 bc
	7 August	0.20 ± 0.05 a	1.06 ± 0.40 c	0.33 ± 0.15 ab	0.66 ± 0.20 b

Note. Lowercase letters indicate differences between the treatments within a specific evaluation date.

Table 5. The number of Colorado potato beetles in each developmental stage per plant on a specific evaluation date in the various treatments in 2016

	Evaluation date	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Egg clusters	20 June	0.66 ± 0.41 b	0.07 ± 0.07 a	0.00 ± 0.00 a	0.06 ± 0.06 a
	24 June	0.20 ± 0.10 a	0.14 ± 0.09 a	0.13 ± 0.09 a	0.12 ± 0.08 a
	1 July	0.20 ± 0.10 b	0.14 ± 0.09 b	0.00 ± 0.00 a	0.31 ± 0.21 b
	10 July	0.00 ± 0.00 a	0.28 ± 0.16 b	0.00 ± 0.00 a	0.00 ± 0.00 a
	27 July	0.00 ± 0.00 a	0.36 ± 0.16 b	0.00 ± 0.00 a	0.06 ± 0.06 a
	5 August	0.00 ± 0.00 a	0.36 ± 0.17 b	0.00 ± 0.00 a	0.06 ± 0.06 b
L1–L2 larvae	20 June	0.00 ± 0.00 a	1.43 ± 0.77 b	2.13 ± 1.17 c	0.00 ± 0.00 a
	24 June	0.00 ± 0.00 a	5.21 ± 4.03 c	0.00 ± 0.00 a	2.87 ± 1.29 b
	1 July	0.60 ± 0.32 b	0.21 ± 0.21 a	0.20 ± 0.20 a	0.50 ± 0.35 b
	10 July	1.47 ± 0.47 b	2.21 ± 1.14 b	0.00 ± 0.00 a	0.87 ± 0.87 a
	27 July	1.06 ± 0.45 b	1.50 ± 0.51 c	0.00 ± 0.00 a	0.31 ± 0.31 a
	5 August	1.06 ± 0.45 b	1.50 ± 0.51 b	0.00 ± 0.00 a	0.31 ± 0.31 a
L3–L4 larvae	20 June	0.00 ± 0.00 a	0.43 ± 0.43 a	3.47 ± 2.33 b	0.00 ± 0.00 a
	24 June	0.07 ± 0.07 a	2.57 ± 1.71 b	0.46 ± 0.40 b	0.31 ± 0.31 a
	1 July	0.40 ± 0.40 a	0.21 ± 0.15 b	0.00 ± 0.00 a	1.00 ± 0.41 c
	10 July	1.20 ± 0.39 b	0.71 ± 0.37 b	0.00 ± 0.00 a	5.68 ± 2.23 c
	27 July	2.26 ± 0.70 b	2.00 ± 1.42 b	0.00 ± 0.00 a	5.25 ± 1.71 c
	5 August	1.73 ± 0.43 b	2.00 ± 1.43 b	0.00 ± 0.00 a	1.93 ± 0.94 b
Adults (imago)	20 June	0.06 ± 0.06 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	24 June	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	1 July	0.00 ± 0.00 a	0.40 ± 0.27 b	0.00 ± 0.00 a	0.00 ± 0.00 a
	10 July	0.33 ± 0.27 b	0.07 ± 0.07 a	0.00 ± 0.00 a	0.06 ± 0.06 a
	27 July	0.33 ± 0.27 b	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a
	5 August	0.33 ± 0.27 b	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a

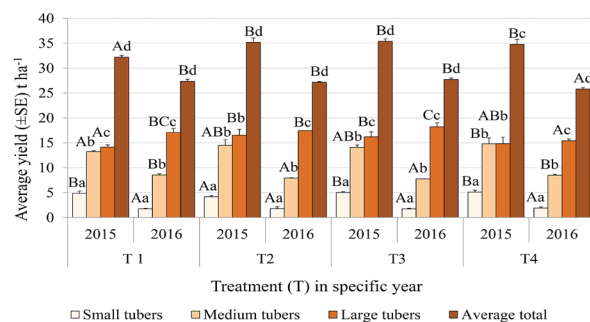
Note. Lowercase letters indicate differences between the treatments within a specific evaluation date.

Table 6. Average number of injuries (holes) caused by wireworms per potato tuber per specific treatment

Treatment	Year 2015	Year 2016
Treatment 1	0.04 ± 0.01 a	0.5 ± 0.08 b
Treatment 2	0.07 ± 0.01 b	0.35 ± 0.07 a
Treatment 3	0.04 ± 0.01 a	0.34 ± 0.07 a
Treatment 4	0.05 ± 0.01 ab	0.56 ± 0.07 b

Note. Lowercase letters indicate differences between treatments within specific year of experiment.

data collected in 2016 showed the effect of the treatment on the medium ($F = 3.17$, $df = 3$, $P = 0.0366$) and large ($F = 4.12$, $df = 3$, $P = 0.0255$) tubers. No treatment effect was detected on the small tubers ($F = 0.99$, $df = 3$, $P = 0.5515$). The average total yield was also influenced by the treatment ($F = 22.15$, $df = 3$, $P < 0.0001$), and the effect was significantly the lowest in treatment 4 (25.82 ± 0.27 t ha⁻¹). All the values are presented in Figure 6.



Note. Uppercase letters indicate differences within the same parameter between treatments regarding one year; lowercase letters indicate differences between different parameters within one treatment regarding one year.

Figure 6. The average yield of potato tubers per treatment per size fraction

Discussion

In our research, we tested the efficacy of three different natural products (limestone dust, calcium cyanamide and Brassica pellets) against insect pests. The efficacy of propolis was tested against two disease agents that cause early and late blight.

The literature reports and agricultural practices (Liška et al., 2017) confirm the efficacy of dust (e.g., diatomaceous earth, zeolites and wood ash) in the suppression of storage pests. Locally accessible dusts are used as insecticides primarily in less developed

countries, and wood ash has been successfully used to reduce CPB populations (Boiteau et al., 2012). In a previous study (Tremblay et al., 2005), applications of limestone dust as a soil additive reduced the extent of soil fungi infections. With a low population of the pest in the first year of the experiment, we established the efficiency of limestone dust in reducing the number of CPB during different developmental stages. With the application of limestone dust at a high concentration and a week after the application, we detected no egg clusters or L1–L2 or L3–L4 larvae on the potato plants treated with the dust. The application of limestone dust at the low concentration was only efficient at reducing the number of egg clusters and L1–L2 larvae; however, this reduction occurred only during the application. Limestone dust can be applied preventively (Olle et al., 2015), whereas thiamethoxam is applied when the adults exceed a critical number – 0.07 beetles per plant (Mailloux et al., 1995). With the increase in the population of the pest in the second year of the experiment, we applied limestone dust twice. Limestone dust at the high concentration proved to be the most efficient at suppressing the L1–L2 larvae. High concentrations of inert dust are known to suppress storage pests (Bohinc, Trdan, 2017).

Ritter et al. (2014) studied the efficiency of lime nitrogen, which prevents injuries due to wireworms feeding on potatoes, as a repellent, although they did not reach any conclusion regarding its recommended application in practice. However, the results of our research recommend the use of these products, because lime nitrogen displayed the effects that were comparable to those of pyrethroid tefluthrin in the year of a massive infestation by wireworms in 2016. The biofumigation method has been demonstrated to be efficient at reducing injuries by wireworms (Furlan et al., 2010) and other harmful organisms (Main et al., 2014) with different species of cultivated plants. Based on the use of Brassica pellets in our two-year experiment, these pellets cannot be recommended for the control of wireworms, because the potato tubers in the Brassica pellets treatment were as damaged as those in the negative control plots.

Our research included the first study of the fungicidal effects of propolis on potatoes in field conditions. To date, laboratory research has demonstrated the effectiveness of propolis in the reduction in infections by plant pathogens, including *Ralstonia solanacearum* (Abo-Elyousr et al., 2017), *Xanthomonas campestris* pv. *vesicatoria* CECT 792 (Ordóñez et al., 2011), *Phytophthora infestans*, *P. capsici* and *P. parasitica* (Yusuf et al., 2005). When we compared the average

daily temperatures in the years of our experiment, the average daily temperature in Ljubljana in 2015 was higher than it was in 2016. In both experimental years, the average daily temperature was also higher than the 30-year average temperature (1981–2010). In 2015, the average temperature in the 1st ten days of June was 23.5°C, and in the 2nd ten days it was 19.8°C; in the 3rd ten days, the temperature was 18.6°C. In 2016, the average temperature in the 1st ten days of June was 18.2°C, and in the 2nd ten days it was 18.6°C; in the 3rd ten days, the average daily temperature was 23°C. The year 2015 was also characterised by a low amount of precipitation. In the 1st ten days of June, Ljubljana received 23.5 mm of precipitation, and in the 2nd ten days, 19.8 mm of precipitation was received; in the last ten days, the precipitation was 18.6 mm (Slovenian Environment Agency, 2018). Therefore, the high average daily temperatures and the low amount of precipitation were the primary reasons why potato blight did not emerge in the first year of the experiment, because the conditions for the spread of the disease were ideal at the time (Becktell, Daughtrey, 2005). The high temperatures in 2015 were also conducive to increasing the efficiency of limestone dust on the CPB, because different dusts are more efficient at high temperatures (Bohinc, Trdan, 2017).

According to Vloutoglu and Kalogerakis (2002), the age of the leaves is an essential factor in the spread of early blight in tomatoes, whereas Olanya et al. (2009) reported the same results for potato. The weather conditions in the years of the experiment also influenced the low level of early blight infection on the potato leaves (less than 10% in the first year and less than 20% in the second year of the study in the negative control plots at the end of the growing period), which resulted in the satisfactory efficiency of propolis. According to Olanya et al. (2009), the early blight index of infection increases with increasing temperatures and a sufficient amount of precipitation. Unfavourable conditions for development only cause a delay in the spread of the pathogen, which was the case in our research.

Glosek-Sobieraj et al. (2018) discovered that environmentally acceptable preparations (they applied growth regulators) could successfully influence the health status of a potato crop. Our research confirmed a similar conclusion in the second year, when many of the harmful organisms emerged and we obtained significantly higher tuber yields in all three treatments with preparations than in the untreated plots. When applying environmentally acceptable preparations for the suppression of harmful organisms, preventive application or applying treatment as soon as possible after the emergence of harmful organisms is important (Olle et al., 2015).

Conclusions

1. Based on the results of the average total potato tuber yield, under low incidences of harmful organisms, the combined use of calcium cyanamide (1000 kg ha⁻¹), propolis (5 ml l⁻¹ H₂O) and limestone dust (345 kg ha⁻¹) is suggested; namely the results in this treatment were comparable to those of synthetic chemical pesticides.

2. In recent years, the number of active ingredients in synthetic fungicides and insecticides has rapidly decreased. As a result, efficient and economic potato tuber production will require their replacement with new, environmentally more acceptable preparations.

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Alternatyvių augalų apsaugos produktų efektyvumas nuo pagrindinių bulvių kenkėjų ir ligų

T. Bohinc, F. Vučajnk, S. Trdan

Liublianos universitetas, Slovėnija

Santrauka

Aplinkai nekenksmingi produktai, skirti žalingų organizmų – Kolorado vabalų (*Leptinotarsa decemlineata*), spragšių (*Agriotes* spp.), sausligės (*Alternaria solani*) ir maro (*Phytophthora infestans*) – kontrolei bulvių pasėliuose buvo tirti 2015–2016 m. Kolorado vabalų kontrolei tirtas dviejų 345 ir 690 kg ha⁻¹ normų kalkakmenio miltelių efektyvumas. Spragšių kontrolei naudota bastutinių (Brassica) augalų šeimos augalų granulės (200 g m⁻²) ir kalcio cianamidas (1000 kg ha⁻¹). Nuo bulvių sausligės ir maro tirta 5 ir 10 ml l⁻¹ H₂O propolio ir propolio glikolio ekstrakto tinktūros (pavadintos propoliu). Tirti keturi alternatyvių augalų apsaugos produktų variantai. Pirmajame variante naudoti kalkakmenio milteliai (690 kg ha⁻¹), Brassica granulės ir bičių pikis (10 ml l⁻¹ H₂O). Antrajame variante naudoti kalkakmenio milteliai (345 kg ha⁻¹), kalcio cianamidas (1000 kg ha⁻¹) ir bičių pikis (5 ml l⁻¹ H₂O). Trečiasis variantas buvo teigiamas kontrolinis – taikytas registruotų fitofarmacinių augalų apsaugos produktų metodas, ketvirtasis – neigiamas kontrolinis – augalai nebuvo apdoroti. Vertinti visi Kolorado vabalų vystymosi tarpiniai: kiaušinėliai, pirmos ir antros stadijos lervos po išsiritimo (L1–L2), trečios ir ketvirtos stadijos lervos po išsiritimo (L3–L4, suaugėliai). Po derliaus nuėmimo nustatytas mažų, vidutinio dydžio ir didelių bulvių gumbų derlius. Taip pat nustatyti ant įvairaus dydžio bulvių gumbų spragšių padaryti pažeidimai. Tyrimo duomenys parodė, kad nuo spragšių kalcio cianamidas buvo efektyvesnis už Brassica granules. Bičių pikio 10 ml l⁻¹ H₂O norma buvo efektyvi alternatyvi priemonė kontroliuojant sausligę ir marą ligos epideminiam protrūkiui nepalankiomis sąlygomis. 2016 m. bulvių gumbų derlius, panaudojus tirtus augalų apsaugos produktus, buvo esmingai didesnis nei laukeliuose, kuriuose šie produktai nebuvo naudoti.

Kuriant ateities bulvių auginimo sistemas, derinant tirtus produktus galima formuoti alternatyvias kenkėjų naikavimo strategijas. Jos bus tinkamos ūkininkaujant kintančio klimato sąlygomis, o tirti produktai papildys dar labai siaurą registruotų fitofarmakologinių augalų apsaugos produktų asortimentą.

Reikšminiai žodžiai: Brassica granulės, bulvės, derlius, kalcio cianamidas, kalkakmenio milteliai, kenkėjai, ligos, pikis.