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NEW PERSPECTIVES OF USING POPULIN AS PLANT EXTRACT AGAINST LATE BLIGHT (*PHYTOPHTHORA INFESTANS* MONTAGNE) RESISTANT STRAIN

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During this study were tested if 1 v/v% and 5 v/v% concentration of populin in comparison with commercially used fungicides may be useless against a resistant phytophthora strain. No development of hyphae was detected during the whole experiment when populin 5 v/v%, Valis and Ranman were applied. Inhibition effect of populin 1 v/v% was detected in a first day, while the hyphae growth started and increased during the experiment. The highest growth was detected at Infinito treatment, followed by control. According to the results it can be concluded that populin 5 v/v% can be a good alternative biological method to control phytophthora. Its effect can be compared with synthetic fungicides and according to the zero-growing rate of hyphae, we recommend its application.

Keywords: biological control, biological fungicides, synthetic fungicides, oomycetes fungi, hyphae development

1. Introduction

The potato and tomato late blight pathogen, *Phytophthora infestans* (Mont.) de Bary [1], has a broad host range within the Solanaceae family including *Solanum phureja* (yellow potato), *S. betaceum* (tree tomato), *S. quitoense* (naranjilla or lulo), *Physalis peruviana* (cape gooseberry), and other wild species [2, 3]. Since the Irish potato famine in the 19th century, this pathogen has been thoroughly studied because of its severe economic impact on agriculture, causing billion-dollar losses annually [4]. For the establishment of an adequate pest management strategy, the determination of methods to control the entire pathogen population is therefore required. It is crucial to know whether growers are facing one or multiple populations, how these differ from one another, if they are recombining through sexual reproduction, and if the pathogen is restricted to a

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specific host. Mitochondrial and nuclear DNA regions of *P. infestans* have been extensively used in different regions of the world to investigate its evolutionary history and population structure [5–7]. This has led to the definition of lineages on the basis of molecular data, which has allowed the monitoring of populations and resulted in the generation of important epidemiological inferences about the pathogen's migration [5, 6]. Additionally, population studies of *P. infestans* have been conducted to elucidate the origin and diversity of isolates from cultivated species [5, 8–10] and to determine if there are any differences between populations from wild and cultivated hosts [11–13]. Population studies has shown low levels of diversity, and its population structure is strongly influenced by host preference. Each *P. infestans* clonal lineage is associated with a different host group: US-1 lineage with tomato, EC-1 with potato, EC-2 with wild solanaceous species, particularly with the *Anarrhichomenum* section, and EC-3 with *S. betaceum* [14–16]. Additionally, genetic differentiation is found among isolates of *P. infestans* associated with *S. ochranthum* [17].

Interestingly, population genetic analyses conducted in Western Europe have shown a dominance of asexual clones and numerous rare genotypes [18–22]. Since 2005, an A2 lineage designated as 13_A2 has increased in frequency from approximately 12 to 70% in Great Britain over three seasons [23]. The 13_A2 genotype was first recorded in the Netherlands in 2004 [24]. Isolates of the 13_A2 lineage are highly aggressive on cultivated potatoes, they outcompete other aggressive lineages in the field, they have overcome previously effective forms of plant host resistance, and they seem to be resistant to the systemic fungicide metalaxyl [19, 25, 26]. The widespread occurrence of both A1 and A2 mating type strains in the United States and Canada increased the probability of sexual recombination in these countries. Yet, most populations in a single field have been monomorphic for mating type and thus sexual recombination is expected to be rare [27–29]. However, studies conducted by Goodwin et al. (1995) [27] and Gavino et al. (2000) [30] in the 1990s have suggested that some populations in British Columbia and in the Columbia Basin of Oregon and Washington might have contained recombinant genotypes. One of these recombinant genotypes is believed to be lineage US-11 [30].

Alternative to produce biological pesticide is using an extract gained from black poplar bud which contains 48 types(kind) of ingredients, isolated from a 95% concentrate. Among these ingredients can be found populin, a compound which belongs to the group of aromatics, gained after treating the black poplar bud with hot water and is soluble in alcohol. In acid medium the results are benzoic acid, saliretin and glucose, in alkaline the products are benzoic acid and salicin [31]. Previous studies have shown that the extract from black poplar has fungicide effect on few types of ascomycetes species. The laboratory investigations included 3 types of concentrates. Measuring the development of

Phytophthora infestans for 10 days consecutive in agarose gel which contains 0(control), 1, 3 or 6 *v/v%* bio-extract. This investigation was repeated twice [31].

The field investigation included a comparation of the traditional fungicides with the efficiency of the 1% biological bio-extract. The investigation included 11 sprays on 7 days intervals using both processes-traditional, bio-fungicide extract as well [31]. The laboratory investigation results shows that the 1 *v/v%* bio-extract has an inhibitory effect on the growth of *Phytophthora infestans*. The agarose gel, which contains 3 and 6 *v/v%* concentration bio-extract has complete inhibition growth of the germs [31]. The occurrence of symptoms and the intensity of these in field potatoes control group's case was higher than in the treated plant's case. The result of the bio-extract treatment compares with the treatment with synthetic substances was significantly different [31]. The laboratory investigation and field investigations as well show that the black poplar bud bio-extract is an efficient fungicide and can be used against the potato late blight [31].

Although the populin effect on Phytophthora has been detected, still significant resistance of some strains (M16) were detected during our previous experiments [32]. Therefore, we tested if a higher concentration of populin in comparison with commercially used fungicides may be useless against a resistant strain.

2. Material and Methods

The whole lab experiment was carried out in 2020. One strain of *P. infestans*, M16, that was previously considered as highly resistant against any treatments (see Turóczi et al. 2020) [32] were previously maintained on pea-broth agar (PBA) (as described by Erwin and Ribeiro 1996) [1]. PBA test plates containing 1 and 5 *v/v%* of the extract from black poplar buds (here termed *populin*), commercially used fungicides as Infinito 687,5 SC, Valis M WG and Ranman Top SC were prepared by the addition of the required volume of filter sterilized (0.45 µm pore-size mixed cellulose ester filter, Whatman GmbH, Germany), concentrated extract with 20% populin and varying volume of sterile distilled water in a total of 67.5 ml combined volume to the medium after autoclaving. The following concentrations were used from both populin and commercial fungicides for experiment:

- **1 *v/v%*** - 5 ml 20% populin+100 ml pea agar+25 ml sterile distilled water.
- **5 *v/v%*** - 25 ml 20% populin+100 ml pea agar+5 ml sterile distilled water.
- **Infinito** - 29,5 ml distilled water+100 ml pea agar+500 µl Infinito 687,5 SC
- **Valis M** - 30 ml distilled water+100 ml pea agar+0,8g Valis M
- **Ranman** - 28,4 ml distilled water+100 ml pea agar+160 µl Ranman Top
- **Controll** - 0 ml+100 ml pea agar+30 ml sterile distilled water

First, PBA glass plates were inoculated with 8-mm-diameter mycelial agar blocks excised from actively growing colonies of *P. infestans*. Altogether 100 plates were inoculated with each strain, fungicide treatments and control (600 as total), meaning 10 plates / strain / treatment and 10 replicates for each one). Inoculated plates were then incubated for 14 days at 20°C in darkness. Colony growth of *P. infestans* in each plate was measured daily in two directions (plates marked left, right, up and down and measured from left to right and up to down directions) over a 10-day period after the 14 days incubation using a digital calliper and units on mm were collected as quantitative data until 55 mm (plates diameter). The whole experiment was carried out with the same number of replicates and followed each along 10 days period.

2.1. Data analyses

Data were first tested for normality and because the hyphae development were not detected for populin 5 *v/v%*, Valis and Ranman, these data were excluded from data analyses. Data for control, populin 1 *v/v%* and Infinito were compared using ANOVA followed by Tukey test.

3. **Results**

According to the results no development of hyphae was detected during the whole experiment when populin 5 *v/v%*, Valis and Ranman were applied. Inhibition effect of populin 1 *v/v%* was detected in a first day, while the hyphae growth started and increased during the experiment. The highest growth was detected at Infinito treatment, followed by control. In all cases a constant growth rate was detected (Fig. 1, Table 1).

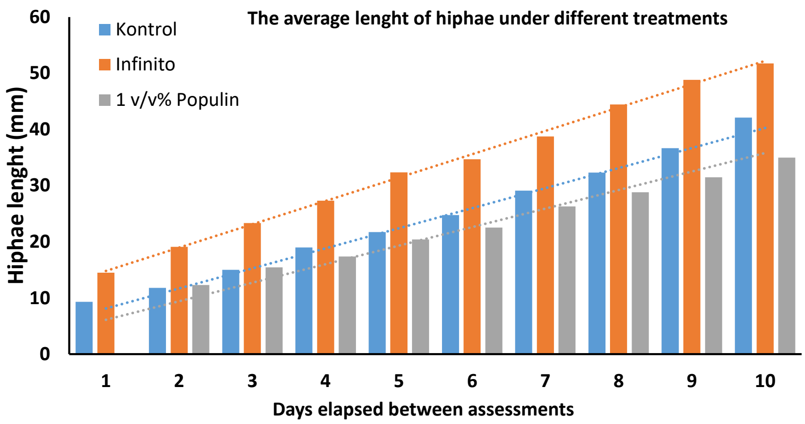

Fig. 1. The growth rate of Phytophthora hyphae during the 10-day period under Infinito and populin 1 *v/v%* treatment and control.

Data in Fig. 1 have been compared to ANOVA followed by Tukey test.

Table 1.

F and p values of ANOVA. Significant p values are bolded.

	Kontrol	Infinito	1 *v/v%*
Kontrol		0.1743	0.801
Infinito	2.61		**0.04969**
1 *v/v%*	0.9016	3.512	

4. Conclusions

According to our results it can be concluded that populin 5*v/v%* can be a good alternative biological method to control phytophthora. Its effect can be compared with synthetic fungicides and according to the zero-growing rate of hyphae, we recommend its application.

During our previous study the effect of poplar bud extract on the potato late blight pathogen *P. infestans* were tested both under lab and field conditions. The populin extract considerably reduced the hyphal growth of six isolates out of seven in culture, one, the M16 were detected as highly resistant one against all treatments. Also we detected that populin was significantly more effective against leaf blight in the field experiment than the two synthetic fungicides used in late blight control [32]. Now we found that the M16 resistant strain is highly sensible to populin 5 *v/v%* (the lower concentration was used in our previous study) and its application can be an effective biological control against *P. infestans.* Altogether we can conclude that during our experiments with populin we found a new and useful biological agent that have not been previously published. Our previous study demonstrated that populin extract considerably reduced the germination of apple scab (*Venturia inaequalis*) conidia and furthermore that infestation levels were lower than in case of conventional treatments [31]. According to results including those of apple scab [31, 33], we conclude that controlling many severe oomycetes diseases with populin may enable partial elimination or at least reduction of synthetic fungicide usage, thereby enabling efficient organic and / or integrated farming in regions of traditional agriculture. Practical application of populin against late blight in potato cultivation needs to be tested under open field condition, however other oomycetes diseases might also be assessed in a future.

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