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Use of Biocontrol Agents and Plant Resistance Inducers for the control of potato late blight

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Abstract

Late blight (*Phytophthora infestans*) is a major disease in potato cultivation, managed primarily with synthetic fungicides. This reliance makes potatoes one of the crops with the highest fungicide load. To reduce chemical dependence and promote Integrated Pest Management (IPM), alternative solutions are needed. This study explores biological control agents (BCAs) and plant resistance inducers (PRIs) as sustainable alternatives. We screened 17 BCAs and 3 PRIs in a detached leaf assay (DLA) and selected the most effective for further trials. The chosen BCAs — Polyversum (*Pythium oligandrum*), Nvs 2 (*Bacillus amyloliquefaciens*), TC 4 (*Trichoderma atroviride*), TC 6 (*T. harzianum*)—and the PRI ChiProPlant (Chitosan hydrochloride) were tested in greenhouse and field trials across five European countries. In greenhouse trials, ChiProPlant and *T. atroviride* significantly controlled late blight. Dosage did not affect the efficacy of the BCAs and PRI, but application timing was crucial. Treatments applied 1 and 5 days before inoculation were most effective, while post-inoculation treatments were least effective. Field trials showed that weekly applications of ChiProPlant, Polyversum, and Nvs 2 significantly reduced late blight infection. However, none matched the efficacy of synthetic fungicides. This study highlights the potential of BCAs and PRIs in late blight management but underscores the need for further research to optimize their integration into IPM strategies.

1. Introduction

Potato late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is a significant disease worldwide, posing a substantial threat to potato production (Haverkort et al. 2008). The disease can lead to complete crop failure under conducive environmental conditions (Dowley et al. 2008). Current management strategies for late blight are dependent on the intensive use of synthetic pesticides (Cooke et al. 2011), which has resulted in the emergence of fungicide-resistant strains of the pathogen (Schepers et al. 2018, Abuley et al. 2023) and which poses a potential risk to the environment and human health (EFSA 2020). To promote Integrated Pest Management (IPM) strategies and reduce reliance on chemical inputs in the agricultural sector, several registered synthetic fungicides have been delisted through restrictions imposed by The Sustainable Use of Pesticides Directive (SUD) by the European Commission (European Parliament & Council, 2009). To promote ecologically sustainable disease control measures, a search for alternative approaches, such as biological control for late blight management, is of utmost importance.

Microbial biocontrol agents (BCAs) and plant resistance inducers (PRIs) could be environmentally sustainable alternatives to combat agriculturally relevant plant diseases (Alexandersson et al. 2016). Several beneficial bacterial, oomycetes and fungal strains have demonstrated biocontrol against *P. infestans in vitro* and under glasshouse conditions, with a few studies also investigating these effects in the field (*in agro*) (as reviewed in Hashemi et al. 2022). Most of the *in vitro* and *in planta* (glasshouse) studies have focused on the biocontrol potential of antagonistic bacteria, oomycetes and fungi from the genera *Bacillus*, *Pseudomonas, Streptomyces, Lysobacter, Pythium, Chaetomium* and *Trichoderma* (Caulier et al. 2018; Yan et al. 2021; De Vrieze et al. 2020; Fu et al. 2022; Lazazzara et al. 2017;

Kurzawińska & Mazur, 2007; Shanthiyaa et al. 2013; Mollah & Hassan, 2023). Moreover, biologically active compounds including chitosan have also been shown to successfully control late blight. Huang et al. (2021) reported a pronounced antimicrobial effect of chitosan against potato late blight in *in vitro* assays and Cooke and Little (2001) demonstrated that foliar sprays of phosphonic acid reduced late blight infection of potato tubers in glasshouse and field experiments.

However, a universal challenge in selecting and employing BCAs for disease control is their susceptibility to various environmental conditions. Microbial agents demonstrating effectiveness *in vitro* often fail to control plant pathogens under field conditions (Dorn et al. 2011). Furthermore, determining the optimal dosage and timing of application can contribute significantly to the ability of biocontrol agents to suppress target pathogens (Raziq and Fox, 2004; Kim et al. 2008).

Reliable data demonstrating efficacy of BCAs under field conditions are necessary for the successful integration of biological control into potato IPM strategies. To the best of our knowledge, no previous large-scale field studies evaluating biocontrol agents against *P. infestans* across multiple regions of Europe have been conducted to date.

In the present study, biological agents (BCAs) and plant resistance inducers (PRIs) were tested for their ability to control late blight in laboratory, glasshouse and field trials on potato cultivars with varying levels of host resistance. The study was aimed at selection of effective BCAs and PRIs in controlling of potato late blight and determination of optimal dosages and timing of applications. The following hypothesis were studied: (1) timing of application is critical for the efficacy of BCAs, and that BCAs are more effective when applied before the onset/arrival of pathogen, (2) BCAs are cultivar dependent, with some BCAs showing more effect on certain cultivars than others.

2. Materials and methods

2.1 Types of experiments

Three types of experiment, including a detached leaf assay (DLA) in petri dishes, whole plant assay in the glasshouse (WPA), and field trials (*in agro*) were carried out to study the effect of BCAs and PRIs on late blight potatoes.

2.2. Products

Seventeen biological control agents (BCAs) and three plant resistance inducers (PRIs) were tested for their ability to control late blight, using DLA (Table 1). The five best-performing BCAs/PRIs from the DLA (TC 4, TC 6, Nvs 2, Polyversum, ChiProPlant) were then selected for testing using whole plant assay (WPA) and *in agro* trials. All the treatments and dosages used are shown in Table 1.

Table 1
List of biological control agents and plant-resistant inducers tested in detached leaf assay

Product	Active ingredient	Type[1]	Dosage	Water volume (l/ha)
Nvs 1	*Bacillus velezensis*	BCA	5kg/ha	500
Nvs 2	*B. amyloliquefaciens*	BCA	5kg/ha	500
Nvs 3	*B. velezensis* *B. amyloliquefaciens*	BCA	5kg/ha	500
Nvs 4	*B. velezensis* (liquid formulation)	BCA	10 l/ha	500
Nvs 5	*B. amyloliquefaciens* (liquid formulation)	BCA	10 l/ha	500
Nvs 6	*B. velezensis* and *B. amyloliquefaciens* (liquid formulation)	BCA	10 l/ha	500
Polyversum	$1x10^6$ oospore/g *Pythium oligandrum* M1	BCA	200 g/ha	300
Seranade ASO	$1x10^{11}$ cfu/l *Bacillus amyloliquefaciens* QST 713	BCA	4 l/ha	300
TC 1*	*T. harzianum*	BCA	10^4cfu/ml	300
TC 2*	*T. capillare* BP-B2-1	BCA	10^4cfu/ml	300
TC 3*	*T. virens* Gv-29	BCA	10^4cfu/ml	300
TC 4*	*T. atroviride* JWB	BCA	10^4cfu/ml	300
TC 5*	*T. atroviride*	BCA	10^4cfu/ml	300
TC 6*	*T. harzianum*	BCA	10^4cfu/ml	300
TC 7*	*T. tomentosum*	BCA	10^4cfu/ml	300
TC 8*	*T. reesei* TMW4.2103	BCA	10^4cfu/ml	300
TC 9*	*T. afroharzianum* T-22	BCA	10^4cfu/ml	300
Resistim	Potassium phosphite: 6.8% phosphonate, 10.9% potassium	PRI	4 l/ha	300

*The pure organisms, not formulated product were used; 1The type of product were (1) biological control agent (BCA), Plant resistant inducer (PRI), and synthetic or conventional fungicide (Fungicide)

Product	Active ingredient	Type[1]	Dosage	Water volume (l/ha)
Fytosol	12.5 g/L COS-OGA	PRI	4 l/ha	300
ChiProPlant	Chitosan (80%) hydrochloride (20%)	PRI	300 g/ha	300
Ranman Top	160 g/l cyazofamid	Fungicide	80 g/ha	300

*The pure organisms, not formulated product were used; 1The type of product were (1) biological control agent (BCA), Plant resistant inducer (PRI), and synthetic or conventional fungicide (Fungicide)

Bacterial products Nvs 1–6 were kindly provided by Novonesis A/S (Denmark). Spore suspensions of the *Trichoderma* species, obtained from Laura Grenville-Briggs Didymus, Swedish Agricultural University, were cultured in Petri dishes containing Potato Dextrose Agar (PDA). The plates were covered, sealed and incubated at 12h light (UV-A)/12 hr darkness at 20–22°C. Once the cultures fully covered the plates and showed the characteristic green color of *Trichoderma* spp., spore suspensions were prepared by flooding with distilled water and scraping the spores off into a beaker after filtering through a sterilized gauze. The solution was adjusted to $1x10^5$ spores/ml with a haemocytometer.

2.2 Inoculum preparation and inoculation

Inoculum for the DLA was prepared in three steps. *Phytophthora infestans* isolates, including EU_43_A1, EU_41_A2 and one unique multilocus genotype (MLG) were grown on rye agar B media and incubated under fluorescent light at 17 ± 2°C for 14 days, as described in Abuley et al. (2023). Detached leaves of the late blight susceptible cv. Bintje were inoculated with a sporangial suspension of the pathogen inoculum and incubated at 17 ± 2°C for 10 days. Sporangia that had formed on the leaves 10 days after inoculation were washed off with sterilized distilled water into a beaker and adjusted to a final concentration of $1x10^4$ sporangia ml^{-1}. The suspension was incubated at 4°C for 4 h before the inoculation to promote zoospore release. Six droplets (10µl) of the sporangial suspension were then placed on the abaxial leaf surface in a humid chamber, covered with lids and placed in an incubation room at 17°C under 12 h/day of fluorescent light.

For the WPA and *in agro* experiments, *P. infestans* isolates were grown on rye agar B at 18°C for 14 days. In the Estonian assays, two isolates with unique multilocus genotypes were used. In the Danish assay, three genotypes, including EU_43_A2, EU_41_A1 and one unique multilocus genotype were used. In Scotland, EU36 genotype was used. The sporangial suspension was prepared by flooding the plates with sterilized distilled water and washing the sporangia off into the beaker via a gauze. The suspension was adjusted to $1x10^5$ sporangia/ml with a haemocytometer and incubated at 4°C for 4 h to promote zoospore release. Each plant was inoculated by 5 mL of suspension.

2.3 Detached leaf assay

Two late blight susceptible ('Folva' and 'Bintje'), one moderately susceptible ('Kuras'), and two resistant ('Sarpo Mira' and 'Nofy') potato cultivars were used in the experiment. Seed tubers of each of the cultivars were planted in 5L plastic pots containing a peat-sandy/clay soil mixture in a ratio of 2:1 v/v. Nutrients were supplied in the following amounts per 100 L soil-peat mixture: 90 g KSO_4, 60 g $(NH_4)_2HPO_4$, 33 g $CaNO_3$, 7 g $MgSO_4$, 7 g $CuSO_4$, 133 g CaMg $(CO_3)_2$. The greenhouse was maintained at 20°C during the day and 18°C at night with a 16h photoperiod. Plants were drip-irrigated at a rate of 1mm/5min. For DLAs, leaves were harvested from 6-week-old potato plants and moistened cotton wool was wrapped around leaf petioles to keep the leaflets alive during the experiment. Leaves were placed in a Petri dish lined with moistened filter paper.

The experiment was a 5x19x2 factorial completely randomized design with 5 levels of cultivars (Kuras', 'Bintje', 'Folva', 'Sarpo Mira', and 'Nofy'), 19 levels of products and 2 product timings (7 days before the inoculation with *P. infestans* or 1 day before the inoculation). The BCAs and PRIs were applied according to the dosages shown in Table 1. In addition to the BCA products, a treatment with Ranman Top (160 g/l cyazofamid) was included as a fungicide reference and an untreated control. Applications were made until the run-off. Inoculation was done by dropping 6 10µl droplets of sporangial suspension on a leaflet. Four replications for each variant were included for each cultivar.

Leaves were monitored visually for late blight symptoms from 24 hours to 7 days after pathogen inoculation. Disease severity was evaluated daily as the percentage of leaf area covered with blight symptoms. The disease severity data taken over time was used to calculate the area under the disease progress curve (AUDPC) using the mid-point method (Shaner & Finney, 1977). The relative area under the disease progress curve (rAUDPC) was calculated by dividing the AUDPC by the maximum potential AUDPC as described by Fry *et al*. 1978. The average rAUDPC value of untreated control was then used to calculate efficacy (i.e., the disease control offered by a product relative to an untreated control), using the method of Abuley and Nielsen (2017).

The performance of products in controlling late blight was ranked based on their efficacy to reduce the severity of disease. Products with the same efficacy were given the same value. This calculation was done separately for each of the three cultivars and two application timings; thus, six values were obtained for a given product. The mean rank of the product across both application timing and all three cultivars was then calculated and assigned as the composite rank, giving a measure of average performance.

2.4 Whole plant assays

Three sub-experiments were carried out in the glasshouse simultaneously:

1. Comparison of the efficacy of BCAs/PRIs with full dose applied one day before inoculation
2. Dose comparison trials with three levels of BCA applied: half, full (normal) and double dose.
3. Timing comparison trials with four application timings: 1) onetime application 1 day before inoculation; 2) one time application 1 day after inoculation; 3) 1 day before inoculation; 3) 1 day

before inoculation with a 5-day interval; and 4) 1 day before inoculation with a 7-day interval.

Glasshouse trials were carried out in Estonia and Denmark for the above-mentioned trials (1–3). The moderately resistant cv. Kuras and susceptible cv. Bintje were used in the WPA. Each cultivar was planted in 1 L plastic pots. The 1 L plastic pots were arranged on greenhouse benches in a randomized design and filled with a commercial limed peat substrate (Biolan) with pH 6.0 and with nutrients in the following amounts 1 L per mixture: 100 mg N, 60 mg P and 200 mg K. Sections of certified seed tubers containing an eye were planted in pots and maintained under controlled conditions at 23°C with a 12 hour photoperiod. The plants were grown for 6 weeks in the greenhouse before products were applied.

Based on the results of the DLAs (Figure S1), TC 4, TC 6, Nsv 2, Polyversum and ChiProPlant were selected as promising candidates for the WPA and *in agro* trials. The products and full dose rates used in the trials are shown in Table 2. Revus Top (250 g/l mandipropamid + 250 g/l difenoconazole) as a chemical reference and an untreated control were included for both cultivars. Each treatment combination was replicated four times. Each plant was sprayed until it ran off (approximately 5ml of foliar application of the fungicide or BCA products per plant) for treatments as well as for inoculation with *P.infestans*.

Late blight progression was monitored visually five times during the experiment (1, 4-, 6-, 8-, and 11-days post-inoculation). The plants were assessed for late blight severity as a percentage of the blighted area. The disease severity data taken over time was used to calculate the area under the disease progress (AUDPC) (Shaner & Finney, 1977). rAUDPC and efficacy were then calculated using methods described in DLA.

2.5 *In agro* trials

In agro trials were carried out in 2022 in Denmark (DK, cultivars ‘Kuras’ (susceptible) and ‘Nofy’ (Resistant)), Estonia (EE, cultivars ‘Kuras’ (resistant)and ‘Triton’ (susceptible)), Finland (FI, cultivars Kuras (resistant) and Mungo (susceptible)), Germany (DE, Kuras (susceptible) and Nofy (resistant)) and Scotland (SC, cultivars Maris Piper (moderately resistant) and Gatsby (resistant)). The experiment was conducted in a randomized complete block design with four replicates and a plot size of approximately 25 m^2. The same BCA’s and PRI tested in WPA were included in the *in agro* trials at the same dosages (i.e., full dose rates), with exception for trials carried out in Finland where the *Trichoderma* treatments were not included. An untreated control treatment and a fungicide treatment were also included. All the fungicide treatments and dosages used in each country are given in S2.

Table 2
List of biological control agents (BCA) and plant-resistant inducers (PRI) tested in glasshouse and *in agro* trials

Product	Active ingredient	Type	Dosage	Water volume (l/ha)
Polyversum	$1x10^6$ oospore/g *Pythium oligandrum* M1	BCA	200 g/ha	300
Nvs 2	*Bacillus amyloliquefaciens*	BCA	5 kg/ha**	500
TC 4*	*Trichoderma atroviride**	BCA	$1,2x10^{12}$cfu/ha	300
TC 6*	*T. harzanium**	BCA	$1,2x10^{12}$cfu/ha	300
ChiProPlant	Chitosan (80%) hydrochloride (20%)	PRI	300 kg/ha	300

*The pure organisms, not formulated product were used; **In Finnish trial, the Nvs 2 dosage was 1 kg/ha, water volume 300 l/ha.

The trials were inoculated by inoculating spreader/infection rows with 1 x 10^4 sporangia/mL of *P. infestans*. The same isolates as in glasshouse trials were also used to inoculate the field trials. In the Finnish field trials, there was natural infection. Treatment applications were started at the first occurrence of a late blight risk period at the individual trial sites, as determined by the local decision support systems (Blight Manager – in Denmark and Finland; Hutton Criteria – in Scotland; BliteCcast – in Estonia; Phytophthoramodel-Weihenstephan in Germany), and continued at weekly intervals for 8–12 weeks. Disease assessments were made as a percentage of infected foliage at a 7-day interval on all plots starting from the onset of late blight and continuing until 2 weeks before harvest.

2.6 Statistical analysis

The R programming language (version 4.3.1) was used for statistical analyses (R Core Team, 2022). The DLA efficacy data was subjected to Gaussian linear models using the 'lm' function in the stats R package (R Core Team, 2022). The effect of factors and their interactions was determined via F-test using ANOVA.

All WPA and *in agro* datasets were checked for normality (Shapiro-Wilk test) and for homogeneity of variances (Levene's test). In case of equal variances, one-way analysis of variance (ANOVA) was performed, followed by Tukey HSD test to identify differences between the treatment variants. For non-normally distributed data, the Kruskal-Walli's test was used. *Post hoc* analysis for treatment differences was conducted using Dunn's multiple comparisons test with Bonferroni correction. P-values < 0.05 were considered statistically significant.

3. Results

3.1 Detached leaf assay

Seventeen BCAs and PRIs were tested in detached leaves in Petri dishes for selection more effective ones in controlling potato late blight with aim to use them in glasshouse and *in agro* studies. No infection was recorded in the resistant cultivars (i.e., 'Sarpo Mira' and 'Nofy'), so these cultivars were excluded from the analyses. Infection was recorded in the untreated susceptible ('Bintje' and 'Folva') and moderately susceptible (Kuras') cultivars, with the first symptoms occurring 3 days post-inoculation. Leaves treated with fungicide had no disease symptoms for the entire duration of the experiment.

An ANOVA test to analyse results from all cultivars showed that all individual factors, product type, potato cultivar, and timing of treatments, had a significant effect on product efficacy ($p < 0.05$). However, potato cultivar had the most pronounced effect on product efficacy to reduce incidence of disease ($F = 946$, $df = 4$, $p < 0.001$), followed by product type ($F = 54$, $df = 21$, $p < 0.001$), and thereafter timing of treatments ($F = 4.5$, $df = 1$, $p = 0.033$).

Results also showed a significant effect of all two-ways interactions between the three experimental factors ($p < 0.05$), whereas the three-way interaction between the three factors was non-significant ($F = 1$, $df = 42$, $p = 0.36$). The product type was the factor with the most effect ($F = 62.7$, $df = 21$, $p > 0.001$), followed by potato cultivar ($F = 7.4$, $df = 2$, $p < 0.001$).

As the previous analysis included both the fungicide treated control and the untreated control, a further analysis was conducted without these treatments. This was done to make a direct comparison of the BCA and PRI treatments. The results showed a strong effect of all three single factors and their two-way interactions ($p < 0.05$), whereas the three-way interaction between potato cultivar, product type and timing of treatment was insignificant ($F = 1$, $df = 38$, $p = 0.4$).

Due to the strong effect of potato cultivar, product type and timing of treatments, we separated the results to avoid biased comparisons. Fungicide treatments consistently showed the highest efficacy in disease reduction. Among tested BCAs and PRIs, potassium phosphite (Resistim) and chitosan hydrochloride (ChiProPlant) were the most effective in disease reduction (Fig. 1). Moreover, the highest efficacy in disease reduction was observed when treatments were applied one day before inoculation. In general, the treatment efficacy in disease reduction was higher in cv. Kuras compared to cv. Folva and cv. Bintje.

Composite ranking

A composite rank value was calculated to rank the performance if the BCAs and PRIs. Results from the untreated leaves and those treated with chemical fungicides were excluded before the analyses of composite ranking of the treatments across the cultivars. The highest-ranked product for late blight control was Resistim, followed by ChiProPlant at both application times. TC 1, TC 2, TC 3, TC 5, TC 6, TC 7, TC 8, Nsv 1, Nsv 2, Nsv 3, Nsv 6 and Polyversum performed better when applied 1 day before inoculation than 7 days before inoculation. Therefore, the composite ranking of the − 1-day treatment

was used. Based on the results of the composite ranking, 4 BCAs (Nvs 2, TC 4, TC 6, Polyversum) and one PRI (ChiProPlant) were selected for subsequent experimentation. Resistim, though the most effective product, was not included because this product is considered a conventional fungicide in some countries.

3.2 Glasshouse experiments

Five best performing treatments (Polyversum [*Pythium oligandrum*], Nvs 2 (*B. amyloliquefaciens*], TC 4 [*T. atroviride*], TC 6 [*T. harzianum*], and ChiProPlant [Chitosan hydrochloride]) selected according to results of DLA were tested in glasshouse grown potato plants to get more reliable results on efficacy of used treatments and to assess the effect of dose and timing on their efficacy in potato late blight control. Data from tests carried out in Estonia and Denmark on cv. Bintje and cv. Kuras were analysed. Infection was successful on bothcv. 'Bintje' and cv. 'Kuras', though in the latter, none of treatments with BCAs or PRIs resulted in significant disease reduction compared with the untreated control. Plants treated with fungicide had no late blight symptoms for the entire duration of the experiment in both cultivars, therefore were excluded from the statistical analyses. The first late blight symptoms occurred 4 days post-inoculation on untreated controls.

3.2.1 Effect of products

According to the Kruskal-Walli's test performed on results of whole plant trials, product type had a significant effect on disease control efficacy across both potato cultivars and trials ($\chi^2 = 14.8$, df = 5, $p = 0.011$). Furthermore, *post hoc* analysis revealed that the application of ChiProPlant and TC 4 resulted in significantly lower late blight severity compared with the untreated control (p = 0.006; $p = 0.021$). Furthermore, the Wilcoxon rank sum test indicated a significant difference in disease severity between the cv. Bintje and cv. Kuras (W = 1975.5, $p = 0.018$) across both countries.

In analysis of data separately for each cultivar, the product type had a marked effect on disease control efficacy in cv. Bintje ($\chi^2 = 14.203$, df = 5, $p = 0.014$), but not on cv. Kuras ($\chi^2 = 5.572$, df = 5, $p = 0.35$) (Fig. 2). *Post hoc* analysis revealed that ChiProPlant ($p = 0.004$), TC 4 ($p = 0.049$), and TC 6 ($p = 0.037$) demonstrated significantly higher disease control compared with the untreated control in the cv. Bintje trial (Fig. 3).

3.2.2 Effect of dose

No dose-response effect was found in the glasshouse trial when analyzing data from both countries and both cultivars across all treatments ($\chi^2 = 2.904$, df = 3, $p = 0.406$). The data for each cultivar was also analyzed separately. No statistically significant differences were found between treatment doses and the untreated control, although the Kruskal-Wallis test revealed a significant effect of dose on disease control efficacy in cv. Kuras ($\chi^2 = 37.06$, df = 5, $p < 0.001$).

We observed a significant product x dose interaction on disease control in cv. Bintje (F = 4.39, $p < 0.001$). Half, full and double dose of ChiProPlant showed significant disease reduction compared with the untreated control ($p < 0.001$; $p < 0.01$ and $p < 0.01$ respectively), but there were no significant differences in disease reduction between the different ChiProPlant doses (Fig. 4). While no differences were observed between the different doses for TC4 in the overall analysis, we observed a significant increase in efficacy with dose when TC4 was analyzed alone where use of double dose resulted in significant disease reduction compared with half dose and untreated control ($p < 0.01$ and $p < 0.05$, respectively).

The product x dose interaction on disease reduction was also significant in cv. Kuras ($\chi^2 = 51.301$, df = 15, $p = 0.001$), but no statistically significant differences were observed between the treatment variants and the untreated control.

3.2.3 Effect of application timing

According to Kruskal-Walli's test, application timing significantly affected disease control efficacy in cv. Bintje ($\chi^2 = 27.96$, df = 5, $p < 0.001$). However, *post hoc* analysis revealed that a statistically significant difference was observed only between the fungicide treatment and the untreated control ($p = 0.001$). When excluding fungicide treatment from the analysis, pre-treatment of plants with the repeated application of BCAs/PRI 5 and 1 day before the inoculation demonstrated significantly higher efficacy in disease control compared with the untreated control ($p = 0.019$). The BCA/PRI treatment of potato plants after pathogen inoculation did not have an effect on disease.

Disease occurrence was significantly affected by product type x timing of BCA/PRI inoculation interaction in cv. Bintje ($\chi^2 = 63.50$, df = 20, $p < 0.001$). Pre-treatment with ChiProPlant 7 and 1 day before the inoculation with *P. infestans* provided comparable efficacy to that of the chemical fungicide Revus Top. Both treatments resulted in significantly less disease compared with the untreated control ($p = 0.018$) (Fig. 5).

A significant effect of product type x timing of BCA/PRI inoculation interaction on disease control efficacy was also observed in the cv. Kuras (W = 73.22, df = 21, $p < 0.001$), but *post hoc* analysis revealed no significant differences between the BCA/PRI treatments and the untreated control.

3.3 *In agro* trials

Nine field trials were established in five countries to assess the efficacy of BCAs and PRI in variable environmental conditions. However, only five trials out of nine had sufficient infection to assess the efficacy of disease control. No disease was observed in the Estonian trials, so data from these trials were excluded from the subsequent analysis. Likewise, data from trials with cv. Nofy in Denmark and Germany were excluded from subsequent analyses due to minimal to no disease symptoms. Data from the Danish and German trials for the cv. Kuras, as well as from the Scottish trials for the cv. Maris Piper (moderately resistant) and cv. Gatsby (resistant), were analyzed separately and in combined analysis. Due to the lower number of products tested in the Finnish trial, the data were not included in the combined analysis.

Product type had a significant effect on disease control in the cv. Kuras in the German field trial (χ^2 = 21.39, df = 6, p = 0.002). ChiProPlant (p = 0.008) and fungicide (p < 0.001) provided significantly higher disease control compared with the untreated control (S3).

In the Danish trials, although there was a significant effect of product type on disease severity in cv. Kuras, (χ^2 = 14.53, df = 6, p = 0.024), only the fungicide treatment showed significant disease reduction (p = 0.002) (S4).

In the trial conducted in Scotland with the moderately blight resistant cv. Maris Piper, there was a significant effect of product on disease control (χ = 21.30, df = 7, p = 0.003); however, only the fungicide treatment resulted in a significant disease reduction (p = 0.003) (S5). Further statistical analysis omitting the fungicide treatment showed that ChiProPlant (p = 0.03) and Polyversum (p = 0.005) significantly reduced disease compared with the untreated control. ANOVA showed a significant effect of product type on disease control on cv. Gatsby (F = 10.09, p < 0.002), however this was only true for the fungicide treatment (p < 0.001) (S6).

Similarly, in the Finnish trials, there was also a significant effect of product on disease control in cv. Kuras (F = 3.58, p = 0.031) with only the fungicide treatment significantly reducing disease (p = 0.038) (S7). A similar trend was observed in cv. Mungo (χ = 10.70, df = 4, p = 0.030), but *post hoc* analysis revealed no significant differences between product treatments and the untreated control (S8).

When comparing data from all experiments, there was a significant effect of product on disease control overall (χ^2 = 62.746, df = 7, p < 0.001), as shown in Fig. 6. Notably, treatments with Polyversum (p = 0.01), Nsv 2 (p = 0.016), ChiProPlant (p = 0.002), and fungicide (p < 0.001) resulted in less disease compared with the untreated control (Fig. 6). However, none of the treatments were as effective as the fungicide.

4. Discussion

Through a series of DLA, glasshouse, and in-agro experiments, we demonstrate the efficacy of BCAs and PRIs against late blight across different agro-climatic areas with varying *P. infestans* populations. We also examine factors such as dose and timing of application, and their influence on the efficacy against late blight.

Detached leaf and whole plant assays conducted in Denmark and Estonia revealed that across both trials, TC 4, TC 6 and ChiProPlant were the most effective products in controlling late blight in the susceptible cv. Bintje. There have been numerous reports of *Trichoderma* showing an inhibitory effect on *P. infestans* from previous studies. For example, *Trichoderma* strain HNA14 significantly reduced late blight in both glasshouse assays and field trials conducted by Yao et al. (2016). In addition, Mollah and Hasan (2023) showed that the *T. harzanium* based biological fungicide “Lycomax” was effective in controlling late blight under field conditions. These authors found that Lycomax reduced late blight severity by more than 90% compared to the untreated control, matching the performance of chemical fungicide Agrizeb (Mollah and Hassan, 2023). The biopolymer chitosan has also been found to exhibit

antifungal properties against *P. infestans* (Yarullina et al. 2024), with its biocontrol properties being attributed to inducing plant defense responses in potato (Zheng et al. 2020), pathogen growth inhibition as well as having a synergistic effect on synthetic fungicides (Huang et al. 2020). However, the efficacy of the aforementioned products was not as high as that of synthetic fungicides in our trials, which consistently achieved 100% efficacy. This is consistent with previous studies in the literature, which have shown that synthetic fungicides generally provide stronger and more consistent control of late blight pathogen (Caulier et al. 2018; Dorn et al. 2011).

We also observed a significant effect of cultivar on product efficacy in DLA and glasshouse trials. Cultivar is known to play a significant role in disease control (Carolan et al. 2017). In contrast to the cv. Bintje where treatments with ChiProPlant, TC4 and TC6 resulted in disease reduction, there was no significant reduction in late blight symptoms in the moderately resistant cv. Kuras. The resistance of the cv. Kuras to late blight, led to lower disease severity in the untreated control and absence of differences between the treatments. Conversely to our results, in a Swedish study involving the plant resistance inducer β-aminobutyric acid (BABA), it was observed that the effect of BABA on late blight symptoms was greater on the partially resistant cultivar than on the more susceptible cv. Bintje in greenhouse and field experiments (Liljeroth et al. 2010). Nevertheless, these findings suggest that it’s important to consider the level of resistance of the cultivar when evaluating the effectiveness of biocontrol products. Furthermore, evaluating product efficacy can be challenging when disease levels are extremely high or very low, as this limits observable differences between treatments.

Our experiments showed a significant dose-response relationship only for treatments with TC 4 in the susceptible cv. Bintje, where treatment with double dose was superior compared with half dose. All treatments of ChiProPlant resulted in significant disease reduction but there were no statistically significant differences between the efficacy of used doses. In contrast, Liljeroth et al. (2010) reported that a higher concentration of PRI β-aminobutyric acid gave a stronger protective effect. Similarly, Liljeroth et al. (2016) observed a clear dose-response effect of phosphite on control of late blight in Swedish field trials, noting that higher doses resulted in increased efficacy.

Our data indicate that product application timing is an important factor in the glasshouse assay. Pre-treatment with ChiProPlant one and seven days before inoculation yielded results comparable to that of fungicide treatment, achieving nearly 100% efficacy. Other pre-treatments with Nsv 2, Polyversum, TC 4 and TC 6 were less effective. On the contrary, post-inoculation treatment of potato plants had no effect on disease severity and may have even increased disease. This highlights the importance of adequate application timing of a biocontrol product. Better efficacy of a bio fungicide is more likely when the antagonist is applied early enough to prevent the pathogen from establishing itself and causing infection. To effectively use PRIs for disease control, they must be applied preventively, before disease establishment, to induce the plant's defense mechanisms (Altamiranda et al. 2008). In a glasshouse experiment, Savchuk and Fernando (2006) observed that applying antagonistic bacteria before, or at the same time as *S. sclerotiorum* inoculation led to complete disease inhibition by day 14, whereas applying the bacteria 24 or 48 hours later resulted in only minimal disease suppression. Studying the effects of

biocontrol agents against *P. infestans*, Stephan et al. (2005) found that applying these products 24 hours before inoculation with the pathogen resulted in greater efficacy compared to applications made 1 hour prior to inoculation. Moreover, despite various treatment applications, none were found to have a curative effect on the disease (Stephan et al. 2005).

In our field experiments, a clear effect of product on disease control was found across all trials. Treatments with Polyversum, Nsv2, ChiProPlant, and fungicide resulted in significant disease reduction compared with the untreated control, although none of the biocontrol products were as effective as the fungicide alone. In the trial conducted in Germany, ChiProPlant and fungicide treatment had a greater effect on disease reduction compared with the untreated control, but in the Danish trial, only the fungicide treatment was significantly more effective compared with the untreated variant. The same trend was observed in separate trials conducted in Scotland with the cvs. M. Piper and Gatsby. This is consistent with the findings of Caulier et al. (2018), who demonstrated that treatments with bacterial antagonists were significantly less effective in reducing disease severity in Belgian field studies compared to the synthetic fungicides.

The limited effect of biocontrol products in field trials might be attributed to the variability in environmental conditions. Biocontrol agents are less likely to achieve successful control at the field level compared to laboratory or glasshouse settings, where temperature and relative humidity can be controlled. Kumbar et al. (2019) and Islam et al. (2022) demonstrated that various bacterial and fungal bioagents effectively reduced late blight disease severity in field trials; however, given the tropical climate, these results may differ from what could be expected in temperate regions. A study by Stridh et al. (2022) reported that the effectiveness of biocontrol products on potato early blight in greenhouse trials did not extend to field-scale trials. In contrast to our studies on potato late blight, none of the tested products successfully controlled another potato foliar disease - early blight in field settings (Stridh et al. 2022).

There are only a few investigations on the efficacy of biocontrol agents against late blight in temperate climate zone in Europe, to the best of our knowledge. In the study by Dorn et al. (2007), various natural products, including products containing microorganisms from the genera *Bacillus*, *Trichoderma*, and *Pythium*, which had previously shown some efficacy in *vivo* assays, were examined in field trials in Switzerland. The efficacy of used natural compounds and microbial preparations was limited in field settings. The authors of this study speculated that the poor performance of natural products could be attributed to their lack of stability in the existing environmental conditions rather than their inherent ineffectiveness (Dorn et al. 2007).

On the other hand, Kurzawinska and Mazur (2009), reported the potential use of Polyversum (*P. oligandrum*) and Biochikol 020 PC (chitosan) in protecting potatoes against late blight based on three-year field experiments. Both products effectively reduced the mean disease index and also the incidence of tuber infection caused by *P. infestans*. It was noted that disease development was influenced by the weather conditions during the vegetative period (Kurzawinska & Mazur). Humidity and cool temperature

are critical factors that contribute to the rapid development of *P. infestans* infection (Fry 2015). Additionally, in the field experiments conducted by Caulier et al. (2018), treatment with *Bacillus subtilis* strain 30B-B6 was shown to significantly reduce late blight severity throughout the crop season.

The results of our study demonstrate that certain biological agents have potential to control *P. infestans* under glasshouse as well as in field conditions. However, further field trials in temperate regions are necessary for a comprehensive evaluation of biocontrol agents and their potential integration into an effective integrated pest management system. Cultivar resistance, timing of application, and environmental conditions must be taken into account when integrating biocontrol products to late blight management strategies.

Declarations

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Competing Interests

The authors declare no competing interests.

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Figures

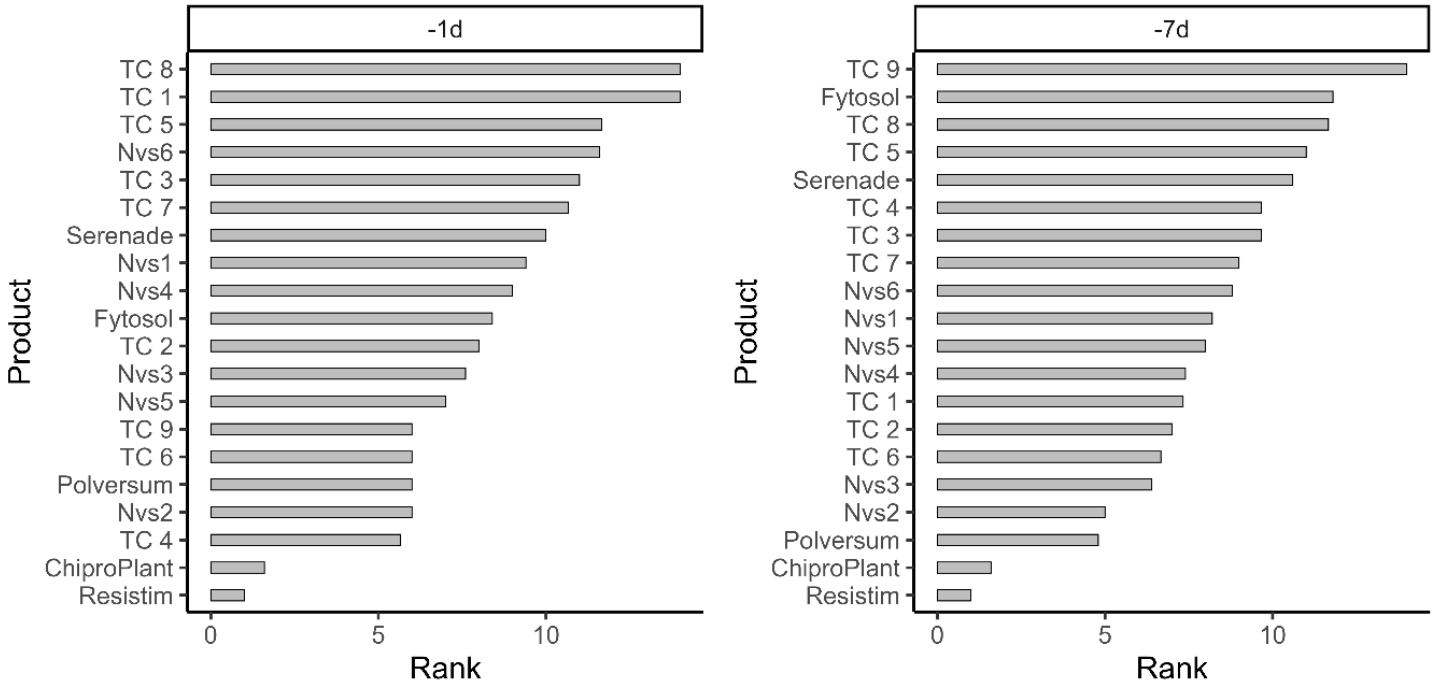

Figure 1

Composite ranking of biological control agents (BCAs) and plant resistant inducers (PRIs) applied as repeated pre-treatments at 1 (a), and 7 (b) days before inoculation of potato leaves with *Phytophthora infestans*. The lower the rank score, the higher the efficacy of the product. The products contain the following active ingredient or organism: Resistim (Potassium phosphite), ChiProPlant (Chitosan hydrochloride), Nvs 1 (*Bacillus velezensis*), Nvs 2 (*Bacillus amyloliquefaciens*), Nvs 3 (*Bacillus velezensis* and *Bacillus amyloliquefaciens*), Nvs 3 (*Bacillus velezensis*, liquid formulation), Nvs 4 (*Bacillus amyloliquefaciens*, liquid formulation), Polyversum (*Pythium oligandrum),* Serenade (*Bacillus amyloliquefaciens)*, TC 1 (*Trichoderma harzianum),* TC 2 *(Trichoderma capillare BP-B2-1),* TC 3 (*Trichoderma virens* Gv-29), TC 4 (*Trichoderma atroviride* JWB), TC 5 (*Trichoderma atroviride)*, TC 6 (*Trichoderma harzianum)*, TC 7 (*Trichoderma. tomentosum)*, TC 8 (*Trichoderma reesei* TMW4.2103), and TC 9 (*Trichoderma afroharzianum* T-22).

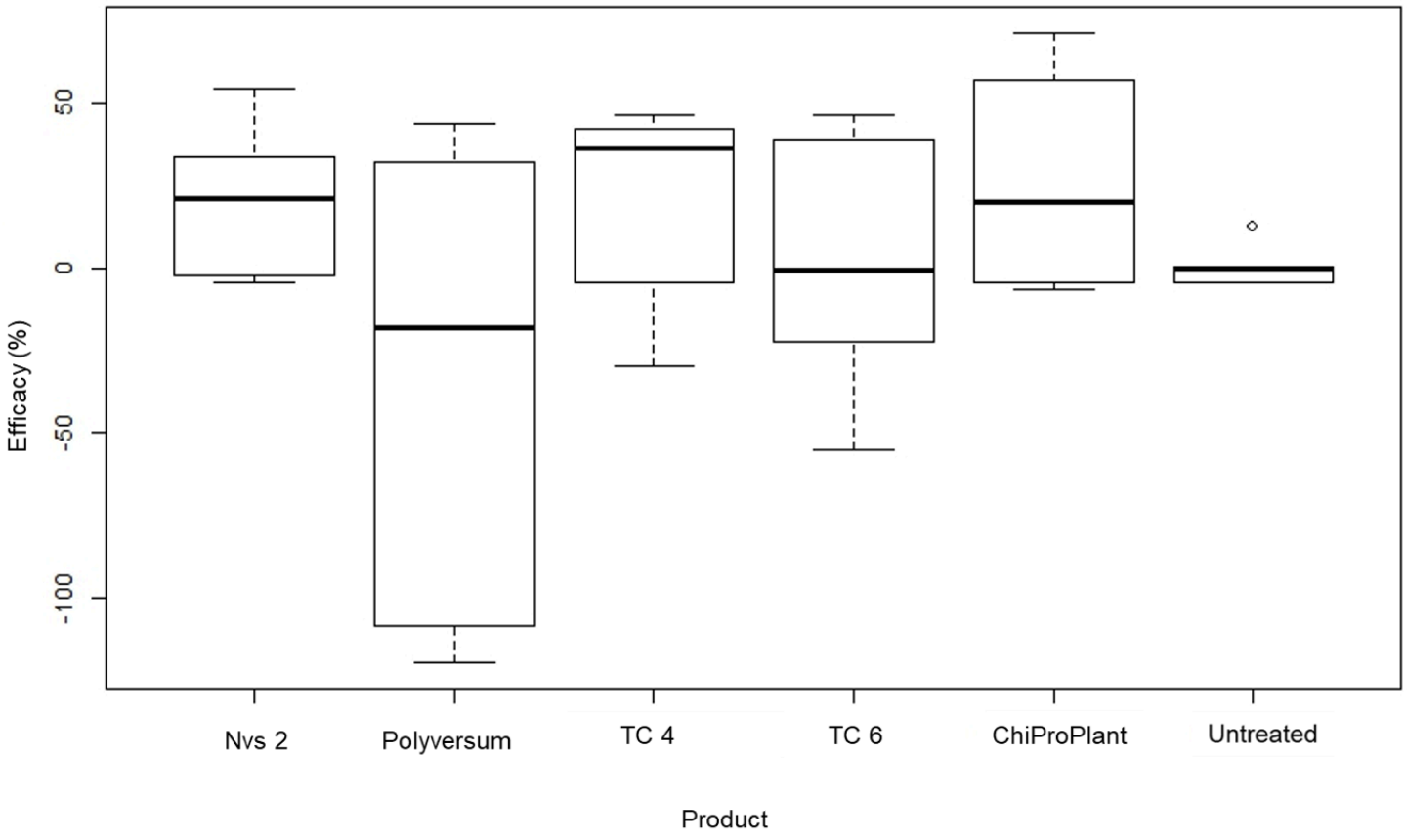

Figure 2

Efficacy of biological control agents and plant resistant inducers against late blight on potato plants in whole plant assay (*in planta*) in cv. Kuras when applied one day before inoculation. Efficacy of the product is expressed as percentage of the relative area under the disease progress curve (rAUDPC) relative to rAUDPC of the untreated control. All differences between the treatments were not statistically significant ($p > 0.05$). The tested products are Polyversum (*Pythium oligandrum*), ChiProPlant (Chitosan

hydrochloride), TC 4 (*Trichoderma atroviride*), TC 6 (*T. harzianum*), and Nvs 2 (*Bacillus amyloliquefaciens*).

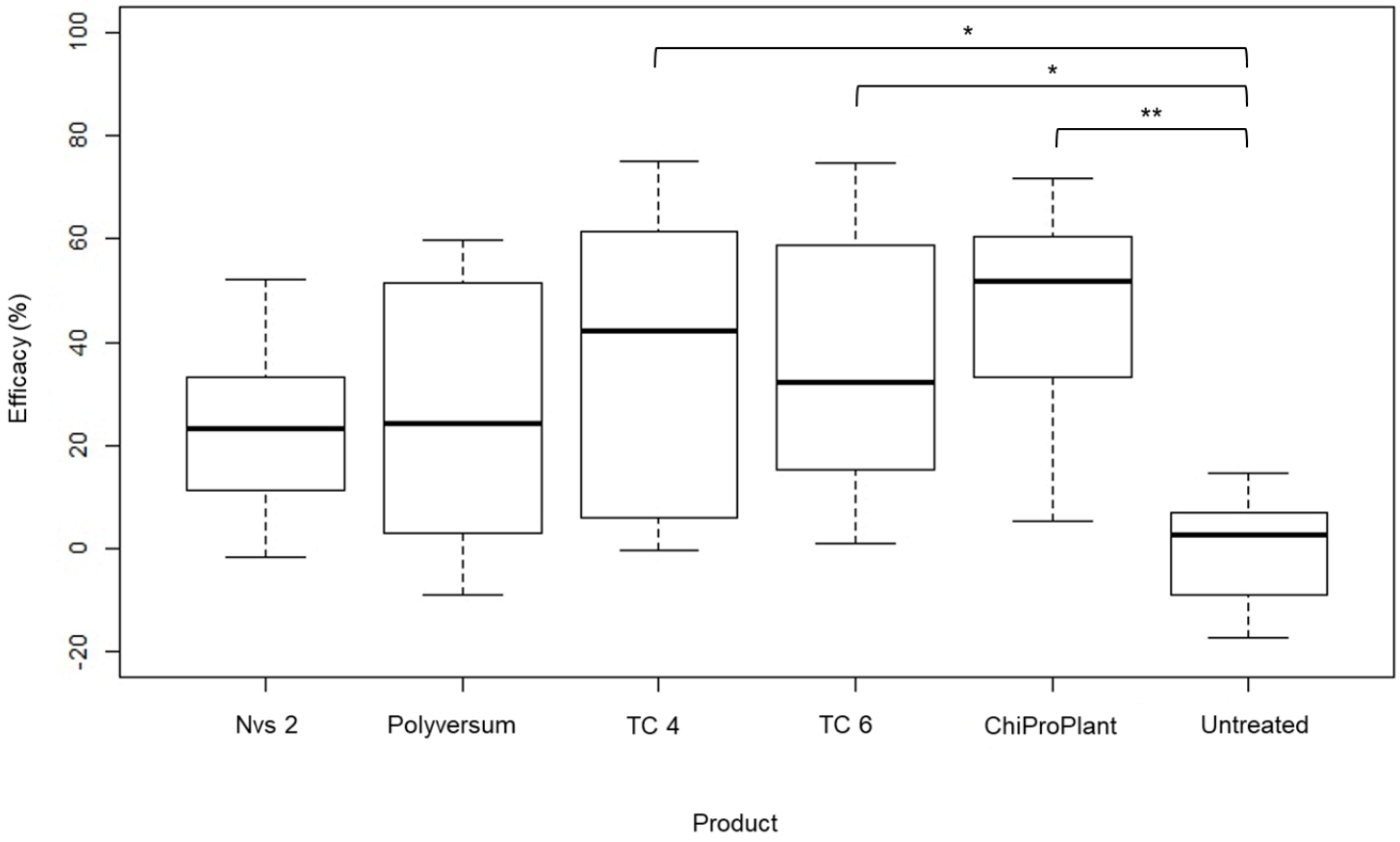

Figure 3

Efficacy of different products (of biological control agents, plant resistant inducers, and fungicide) at different doses in controlling late blight in cv. Bintje when applied one day before inoculation. Efficacy of the product is expressed as percentage of relative area under the disease progress curve (rAUDPC) relative to rAUDPC of the untreated control. Asterisks indicate statistically significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The tested products are Polyversum (*Pythium oligandrum*), ChiProPlant (Chitosan hydrochloride), TC 4 (*Trichoderma atroviride*), TC 6 (*T. harzianum*), and Nvs 2 (*Bacillus amyloliquefaciens*).

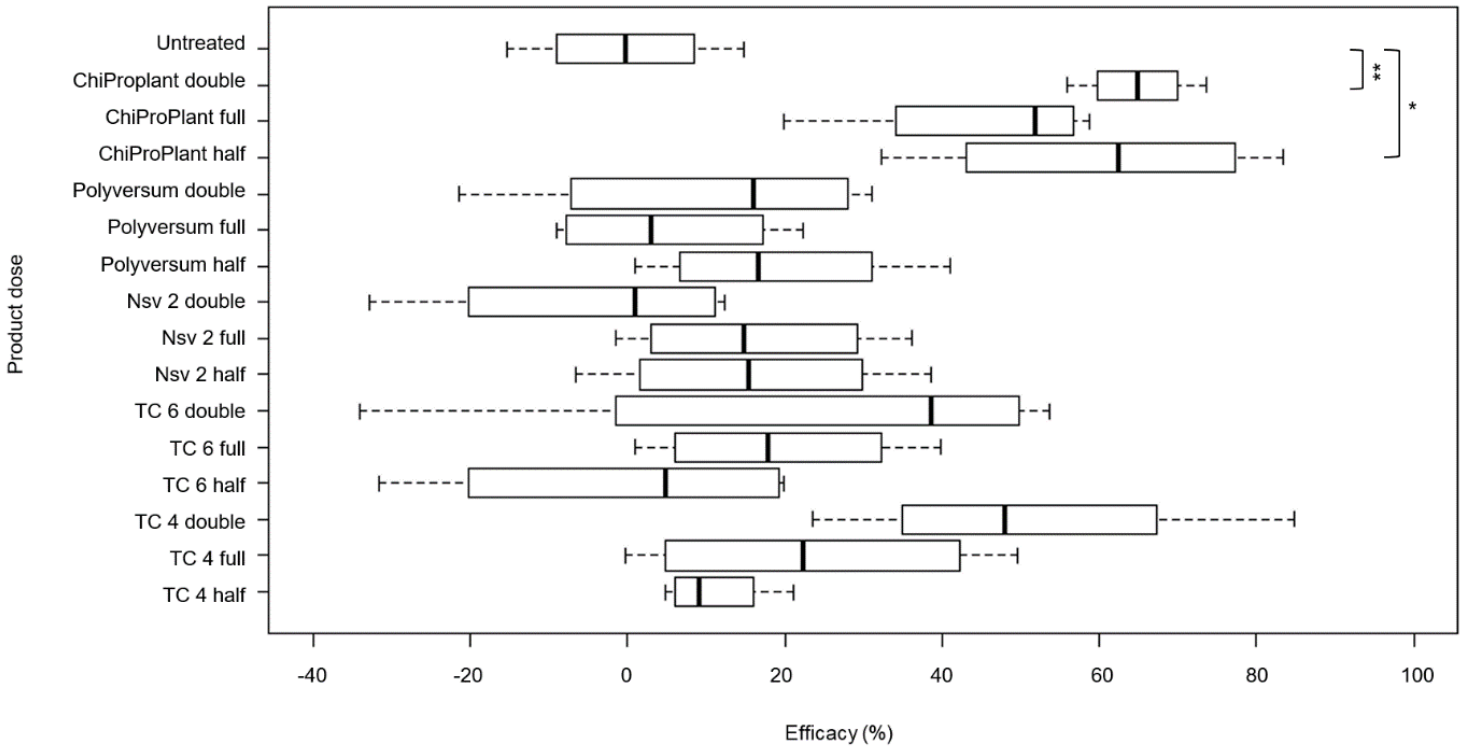

Figure 4

Efficacy of different products (of biological control agents, plant resistant inducers, and fungicide) at different doses (half, normal or full, and double) in controlling late blight in cv. Bintje when applied one day before inoculation. Efficacy of the product dose is expressed as percentage of relative area under the disease progress curve (rAUDPC) relative to rAUDPC of the untreated control. Asterisks indicate statistically significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The tested products are Polyversum (*Pythium oligandrum*), ChiProPlant (Chitosan hydrochloride), TC 4 (*Trichoderma atroviride*), TC 6 (*T. harzianum*), and Nvs 2 (*Bacillus amyloliquefaciens*).

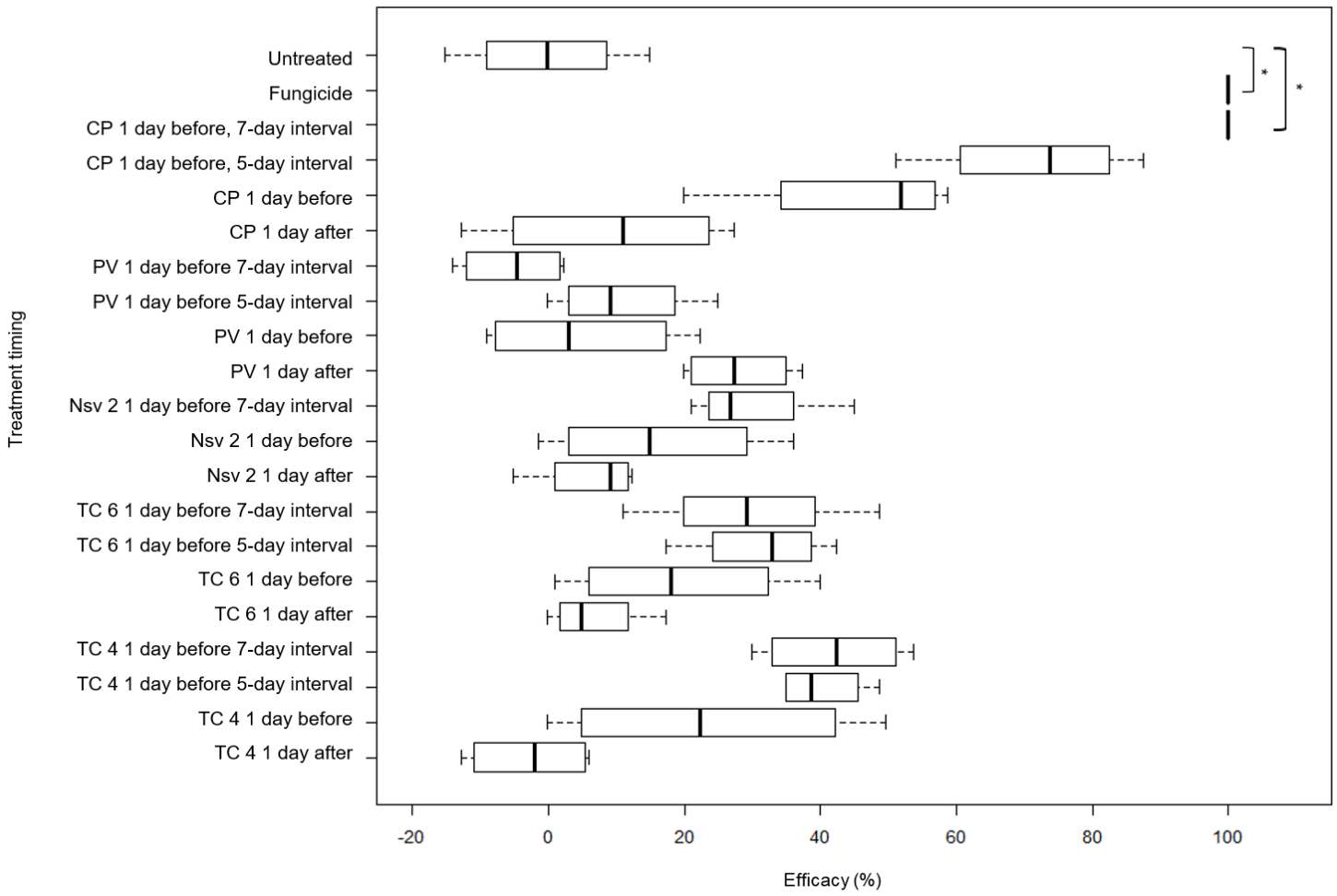

Figure 5

Efficacy of different products (of biological control agents, plant resistant inducers, and fungicide) at different application timings (1 day before inoculation with *Phytophthora infestans*, 1 day before inoculation with *P. infestans* and continued at 7 days interval, 1 day before inoculation with *P. infestans* and continued at 5 days interval, 1 after inoculation with *P. infestans*) on potato late blight in cv. Bintje. Efficacy of the treatment timing is expressed as percentage of rAUDPC relative to relative area under the disease progress curve (rAUDPC) of the untreated control. Asterisks indicate statistically significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The tested products are represented by the following abbreviations PV (Polyversum, containing *Pythium oligandrum*), CP (ChiProPlant, containing chitosan hydrochloride), TC 4 (*Trichoderma atroviride*), TC 6 (*T. harzianum*), Nvs 2 (*Bacillus amyloliquefaciens*), and fungicide.

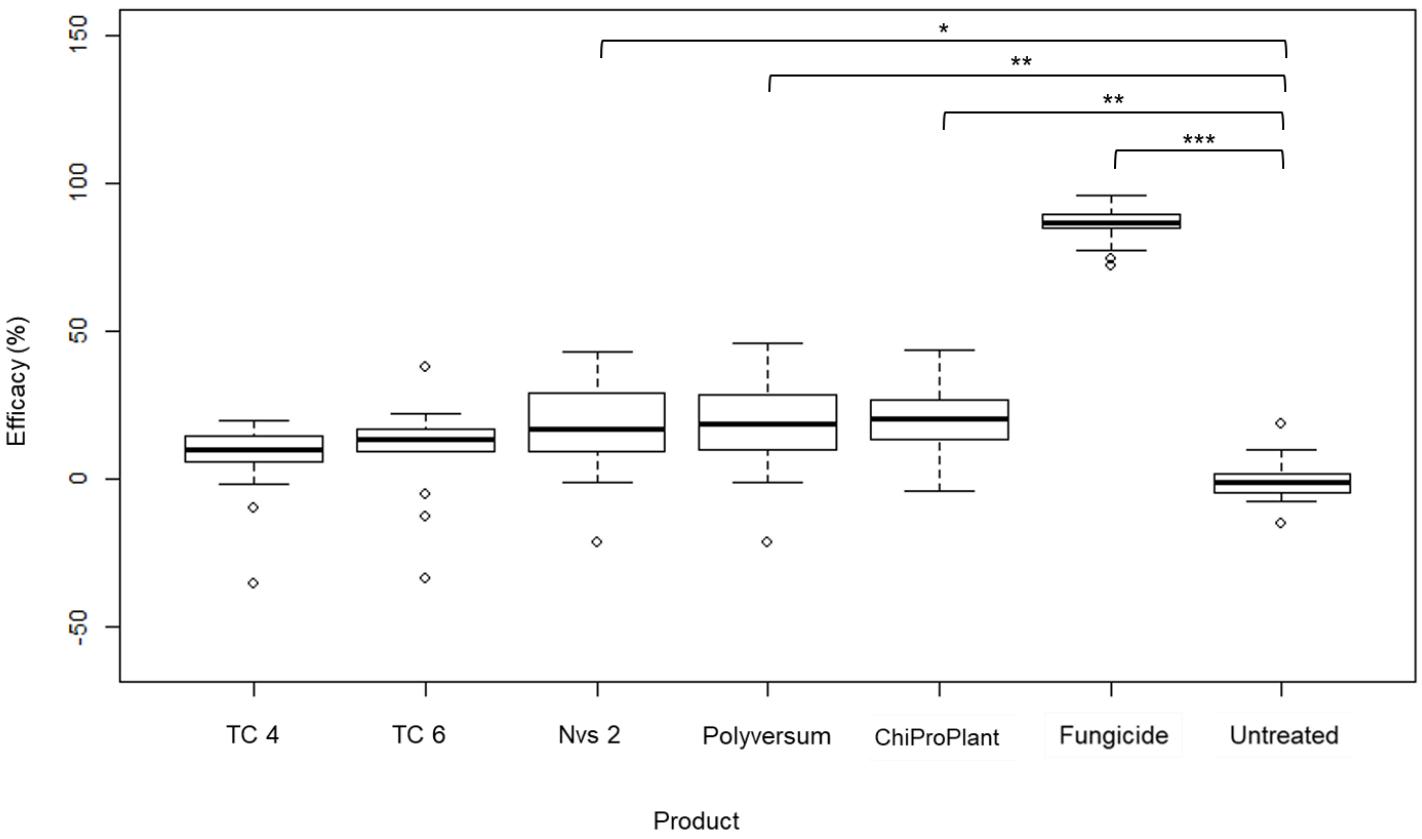

Figure 6

Efficacy of different products (biological control agents, plant resistant inducers, and fungicide) on late blight under field conditions in trials conducted in 3 countries (Denmark, Scotland, and Germany) with a range of cultivars (Kuras, Maris Piper, and Mungo). Efficacy of the product is expressed as percentage of relative area under the disease progress curve (rAUDPC) relative to rAUDPC of the untreated control. Asterisks indicate statistically significant differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). The tested products are represented by the following abbreviations PV (Polyversum, containing *Pythium oligandrum*), CP (ChiProPlant, containing Chitosan hydrochloride), TC 4 (*Trichoderma atroviride*), TC 6 (*T. harzianum*), Nvs 2 (*Bacillus amyloliquefaciens*), and fungicide.

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