Acta Biologica Szegediensis

Volume 67(1):75-86, 2023
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs
DOI:10.14232/abs.2023.1.75-86

ARTICLE

Efficacy of some plant resistance inducers against several sunflower downy mildew (*Plasmopara halstedii* (Farl.) Berl. *et* de Toni) isolates

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ABSTRACT *Plasmopara halstedii* (Farl.) Berl. *et* de Toni is the oomycete that causes sunflower downy mildew (SDM). Traditional means of controlling this pathogen are using resistant hybrids, crop rotation and seed coating with fungicides. Disease control strategies that use a variety of approaches are becoming an increasingly essential aspect of pest management strategies. We conducted this exploratory investigation to evaluate whether specific plant resistance inducers might work against *P. halstedii*. In this study we used azadirachtin (AZA) a botanical insecticide; benzothiadiazole (BTH) and *Trichoderma asperellum*. Three-day-old susceptible sunflower seedlings were pre-treated with different doses of inducers for two hours. The seedlings were immediately inoculated of 7 different pathotype of *P. halstedii*. As a control, metalaxyl-M a systemic fungicide was used. Nine-day-old sunflower plant leaves were sprayed with bidistilled water to stimulate sporangial growth. *In vivo* experiments showed that BTH, *T. asperellum* and the highest doses of AZA significantly reduced downy mildew symptoms. The various pathotypes of the pathogen significantly affected the plant height and disease symptoms under experiments.
Acta Biol Szeged 67(1):75-86 (2023)

KEY WORDS
azadirachtin
benzothiadiazole
Plasmopara halstedii
plant resistance inducers
sunflower
Trichoderma asperellum

ARTICLE INFORMATION
Submitted
02 November 2023
Accepted
11 December 2023
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Introduction

The sunflower (*Helianthus annuus* L.) is one of the most essential oil crops globally. The total harvested area forecast is more than 28 million hectares for 2023, with 56 million metric tons yield (National Sunflower Association 2023). The biotroph oomycete, *P. halstedii* (Farl.) Berl. *et* de Toni, is the causal agent of sunflower downy mildew. According to earlier estimates, the global impact of downy mildew on yield is 3.5% of commercial seed production when current control measures are used. However, in fields with high disease infection, the yield loss might reach 30-50 or even 100% (Arribas 2014; Debaeke et al. 2014).

P. halstedii is typically found in soil and seeds (Jocić et al. 2010). It can survive in the soil for up to 10 years with its persistent reproductive structures (oospores) (Tourvieille de Labrouhe et al. 2008). At the same time, its mycelium, which is embedded in the seed, allows it to spread over considerable distances (Ban et al. 2023). Because of the latter, there is a high risk of spreading different pathotypes (virulence phenotypes or pathotypes) of the pathogen. Approximately 50 pathotypes of *P. halstedii* have been identified globally (Bán et al. 2021). Although sunflower hybrids are cultivated that are resistant to all pathotypes of *P. halstedii*, the high variability of the pathogen requires continuous and intensive breeding efforts (Vear 2016).

Once symptoms of sunflower downy mildew appear, there is no effective control against the pathogen. Besides planting resistant sunflower hybrids, seed coating with fungicides is among the most efficient control measures for downy mildew (Bán et al. 2023). Previously, mefenoxam, a phenyl amide active ingredient, provided efficient control against the disease for a long time (Albourie et al. 1998). However, *P. halstedii* rapidly developed mefenoxam-tolerant genotypes in several countries (Albourie et al. 1998; Gulya 2000; Iwebor et al. 2022; Körösi et al. 2020; Molinero-Ruiz et al. 2003). The introduction of new fungicides, therefore, is crucial for effectively managing

downy mildew in sunflowers (Cohen et al. 2019).

In addition to chemical control, alternative methods are essential elements of integrated pest management (Barzman et al. 2015). There is a growing body of research on the use of plant activators, such as chemical inducers (e.g., benzothiadiazole), botanical pesticides (e.g., azadirachtin), and biocontrol agents (e.g., *Trichoderma* spp.) for disease control (Bán et al. 2023; Doshi et al. 2020; Tarigan et al. 2022). One of the underlying processes is induced resistance, whereby a prior treatment with resistance inducers or inoculation with a non-aggressive, or avirulent pathogen can cause the susceptible plant to respond relatively quickly to a subsequent attack (Vallad and Goodman (2004); Walters et al. 2013; Kamle et al. 2020; Yassin et al. 2021). Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are the two types of induced resistance (Kamle et al. 2020). Chemical inducers and necrotroph pathogens may activate the SAR, while ISR is triggered due to plant growth-promoting microorganisms colonizing plant roots (Spoel and Dong 2012). At the cellular level, the accumulation of lignin, callose, phenols, and other antimicrobial compounds can be detected in susceptible induced plants, which finally leads to the development of resistance (Basavaraj et al. 2019).

BTH (also known as benzo [1,2,3] thiadiazole-7-carbothioic acid-S-methyl ester) is one of the longest-used chemical inducers in research and has been adopted in practice (Walters et al. 2013; Yassin et al. 2021). It has also proven efficient against sunflower downy mildew in various trials (Bán et al. 2004; Körösi et al. 2011; Tosi and Zazzerini 2000). However, only a few *P. halstedii* isolates were examined in these studies that were dominant in the early 2000s but are now considered less aggressive.

Unlike chemical inducers, botanical pesticides have been applied for thousands of years (Ngegba et al. 2022) They are now an element of the growing importance of integrated pest management (Ngegba et al. 2022). Of these, an extract of the neem tree (*Azadirachta indica* A. Juss), contains more than 140 biological active components. such as azadirone, azadirachtin, flavonoids etc., with AZA the most efficient active ingredient, is effective against several plant diseases (Kumar et al. 2020; Adusei and Azupio 2022). Preliminary studies showed that neem leaf extract and AZA performed well against an aggressive isolate of *P. halstedii* (Doshi et al. 2020).

Besides chemical inducers and botanical pesticides, plant growth-promoting fungi (PGPF) have been used in various host-pathogen systems to elicit systemic induced resistance in crops (Hossain et al. 2017). *Trichoderma* spp. are the most commonly used PGPFs as biocontrol agents (Guzmán-Guzmán et al. 2023). These fungi directly attack pathogens in the soil or induce resistance by upregulating host defenses (Zin and Badaluddin 2020). The secondary metabolites of *Trichoderma* species activate the host system's defense response by stimulating the accumulation of enzymes, secondary metabolites, and signaling molecules such as jasmonic acid (JA) and ethylene (ET) (Nawrocka and Małolepsza 2013; Waqas et al. 2014). Moreover, seed coating with *T. harzianum* has been proven effective at controlling *P. halstedii* under greenhouse and field conditions (Nagaraju et al. 2012; Özer et al. 2023).

As indicated in the above works, few results exist on the effect and efficacy of chemical inducers, botanical pesticides, and *Trichoderma* spp. against sunflower downy mildew, though primarily a single isolate of *P. halstedii* was tested. The goal of this study, therefore, was to assess the efficacy of a chemical inducer (BTH), a botanical pesticide (AZA), and seed treatment with *T. asperellum* against seven isolates of sunflower downy mildew, differing in virulence and aggressiveness.

Materials and methods

Plant and pathogen materials

Seven compatible host-pathogen combinations were examined using with one sunflower genotype (cv. *Iregi szürke csíkos*) and seven *P. halstedii* isolates. *Iregi szürke csíkos* is a Hungarian open-pollinated sunflower cultivar with no dominant resistance genes (*Pl* genes) against sunflower downy mildew.

P. halstedii isolates originated from the collection of the Department of Integrated Plant Protection (Hungarian University of Agriculture and Life Sciences, MATE). Previously, *P. halstedii* isolates were collected from sunflower hybrids with the *Pl*6 resistance gene against sunflower downy mildew between 2014 and 2019. Isolates were stored in a deep freezer at -70 °C. The sign and source of the isolates are displayed in Table 1.

Treatment of sunflower seedlings with inducers and inoculation by P. halstedii

Sunflower seeds were germinated before treatment and inoculation with *P. halstedii* (except for mefenoxam treatment and the first *Trichoderma* spp. treatment, see below). For germination, the seeds were soaked in a 1.5% NaOCl solution for 3 min, rinsed in tap water, wrapped in moist filter paper, and kept in the dark at 21 °C for three days. Then, the three-day-old sunflower seedlings were treated with the examined inducers, such as BTH and AZA. Seedlings were soaked in an aqueous solution of BTH (20, 40, and 80 ppm) using the chemical inducer Bion 50 WG (Syngenta-Hungary) for 2 h. NeemAzal T/S (Trifolio-M; Germany) was used as a botanical pesticide with concentrations of 0.01, 0.1, and 0.2% AZA with a similar treatment period to BTH. Mefenoxam served as positive

Table 1. List and characterization of *Plasmopara halstedii* isolates collected in different regions of Hungary.

Isolate code	Location	Collection year	CVF of the isolate*
I1	Abony	2014	704
I2	Körösladány	2014	710
I3	Doboz	2014	704
I4	unknown	unknown	700
I5	Csanytelek	2014	730
I6	Tiszafüred	2014	730
I7	Borsod-Abaúj-Zemplén County	2019	704

*CVF: coded virulence formula (virulence phenotype)

control by coating the ungerminated seeds with Apron XL 350 FS (350 g/l mefenoxam; Syngenta, Switzerland) according to the EU-registered rate of 3 mg/kg seeds. Mefenoxam-treated seeds were coated homogeneously and kept at 24 °C for three days for drying.

For *Trichoderma* spp. treatment, seven-day-old *T. asperellum* cultured on PDA was flooded with 10-15 ml of bidistilled water, and conidia were removed by shaking or using a sterile brush under aseptic conditions. The concentration of the conidial suspension was adjusted to 3 x 10^7 or 3 x 10^8 conidia/ml using a hemocytometer. Gum arabic (5%) was added to the suspension to aid conidia in adhering to seeds. *Trichoderma* spp.-treated seeds were incubated at 25 °C for 3 h before placing into a growth chamber at 19 °C for three days for germination. After germination, the seedlings were re-treated with *T. asperellum* (with one of the above concentrations) before inoculation with various *P. halstedii* isolates.

Inoculation of seedlings by *P. halstedii* was carried out by the whole seedling immersion (WSI) method (Cohen and Sackston 1973; Sedlářová et al. 2016). Using a hemocytometer, the concentration of the inoculum was adjusted to 5 x 10^4 sporangia/ml. Inoculation was carried out at 16 °C overnight.

Experimental setup

The experiment was conducted at the Department of Integrated Plant Protection (Plant Protection Institute, MATE, Gödöllő, Hungary), and the following treatments were included:

- Seeds immersed in bidistilled water (non-treated, non-inoculated)
- Seeds inoculated with *P. halstedii* (non-treated, inoculated)
- Seeds treated with 20 ppm benzothiadiazole (BTH20) (non-inoculated)
- Seeds treated with 20 ppm benzothiadiazole (BTH20) and inoculated with *P. halstedii*
- Seeds treated with 40 ppm benzothiadiazole (BTH40) (non-inoculated)
- Seeds treated with 40 ppm benzothiadiazole (BTH40) and inoculated with *P. halstedii*
- Seeds treated with 80 ppm benzothiadiazole (BTH80) (non-inoculated)
- Seeds treated with 80 ppm benzothiadiazole (BTH80) and inoculated with *P. halstedii*
- Seeds treated with 0.01% AZA (AZA0.01) and inoculated with *P. halstedii*
- Seeds treated with 0.01% AZA (AZA0.01) (non-inoculated)
- Seeds treated with 0.1% AZA (AZA0.1) and inoculated with *P. halstedii*
- Seeds treated with 0.1% AZA (AZA0.1) (non-inoculated)
- Seeds treated with 0.2% AZA (AZA0.2) and inoculated with *P. halstedii*
- Seeds treated with 0.2% AZA (AZA0.2) (non-inoculated)
- Seeds treated with 3 mg/kg mefenoxam (MX) and inoculated with *P. halstedii*
- Seeds treated with 3 mg/kg mefenoxam (MX) (non-inoculated)
- Seeds treated with 3 x 10^7 conidia/ml of *T. asperellum* (T 3 x 10^7) and inoculated with *P. halstedii*
- Seeds treated with 3 x 10^7 conidia/ml of *T. asperellum* (T 3 x 10^7) (non-inoculated)
- Seeds treated with 3 x 10^8 conidia/ml of *T. asperellum* (T 3 x 10^8) and inoculated with *P. halstedii*
- Seeds treated with 3 x 10^8 conidia/ml of *T. asperellum* (T 3 x 10^8) (non-inoculated)

Pretreated and/or inoculated sunflower seedlings (25 plants/treatment) were sown in pots (d = 5 cm, five plants per pot) filled with horticultural perlite (d = 0 - 4 mm) and kept at 22 °C in a growth chamber (12 h photoperiod, light irradiance of 100 $\mu E \cdot m^{-2} s^{-1}$). The plants were grown for 21 days. The trial was set up in a randomized block

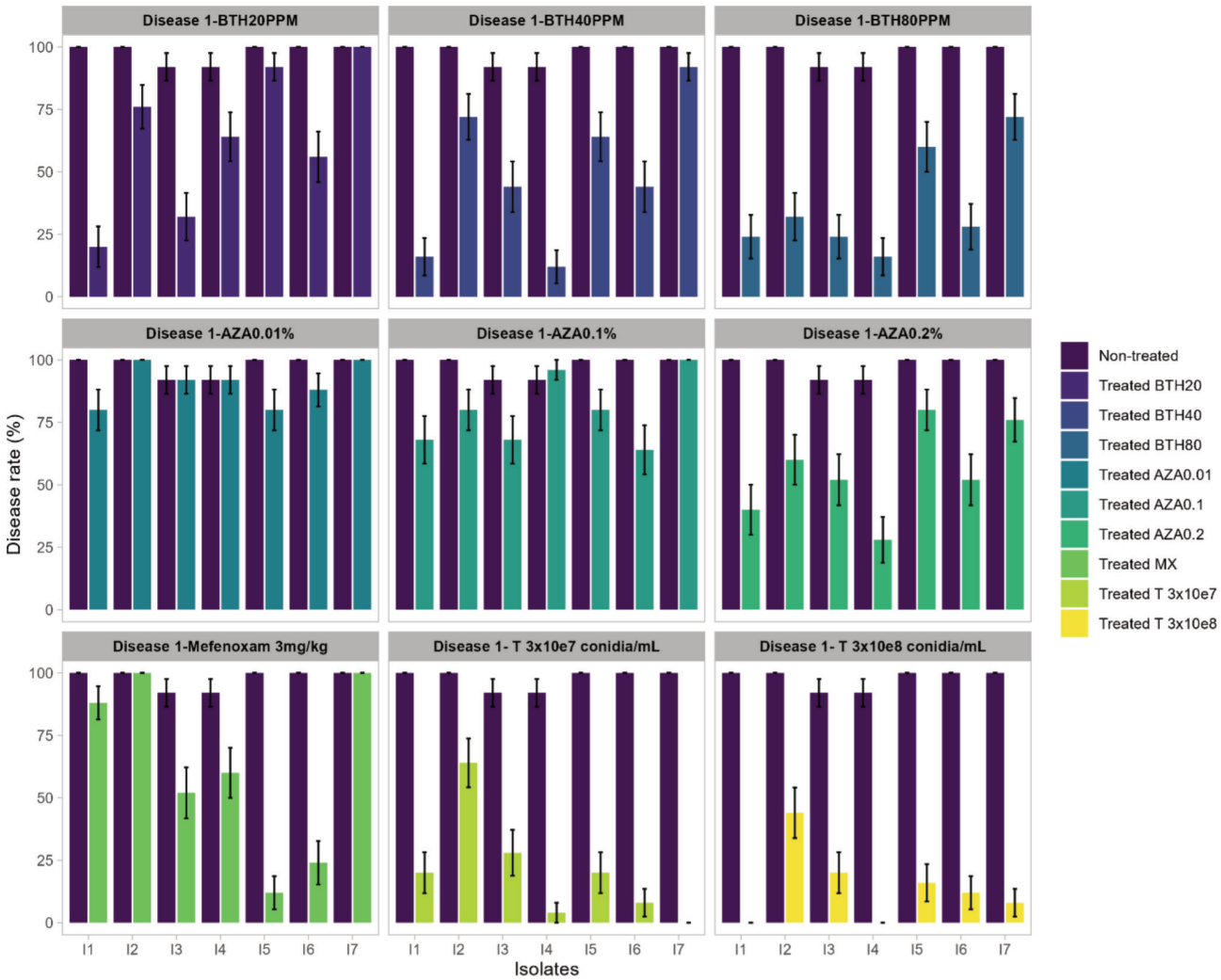

Figure 1. Disease rate 1 (%) of *Plasmopara halstedii* inoculated sunflowers treated with different doses of benzothiadiazole (BTH, 20, 40 and 80 ppm), azadirachtin (AZA, 0.01, 0.1 and 0.2%), mefenoxam (MX, 3 mg/kg) and *Trichoderma asperellum* (3 x 10^7 and 3 x 10^8 conidia) at 10 dpi. Disease rate 1 (%) was determined by the ratio of diseased (sporulating or damped-off plants) and healthy plants. Bars represents standard deviations of five replicates. I1-I7: *Plasmopara halstedii* isolates (see in Table 1).

design with two repetitions per treatment; each repetition consisted of 25 plants per treatment.

Assessment of disease

Nine days after inoculation (9 dpi), seedlings were sprayed homogeneously with bidistilled water to induce sporulation and then covered with dark bags overnight (19 °C). Plants were evaluated twice during the examination period: first just after sporulation (10 dpi,), then at 21 dpi. Disease assessment was made according to the sporulating and damped-off plants at 10 dpi (Disease rate 1 (percentage)), and chlorotic and damped-off plants at 21 dpi (Disease rate 2 (percentage)), calculating the ratio of diseased and healthy plants each time. Plant height was measured twice during the experiment (at 10 and 21 dpi) as *P. halstedii* causes dwarfing on susceptible, non-treated sunflowers.

Statistical analysis

The data were subjected to analysis of variance (one-way and two-way ANOVA) with a p-value of 0.05 for mean separation. Statistical analyses were carried out using the R statistical software.

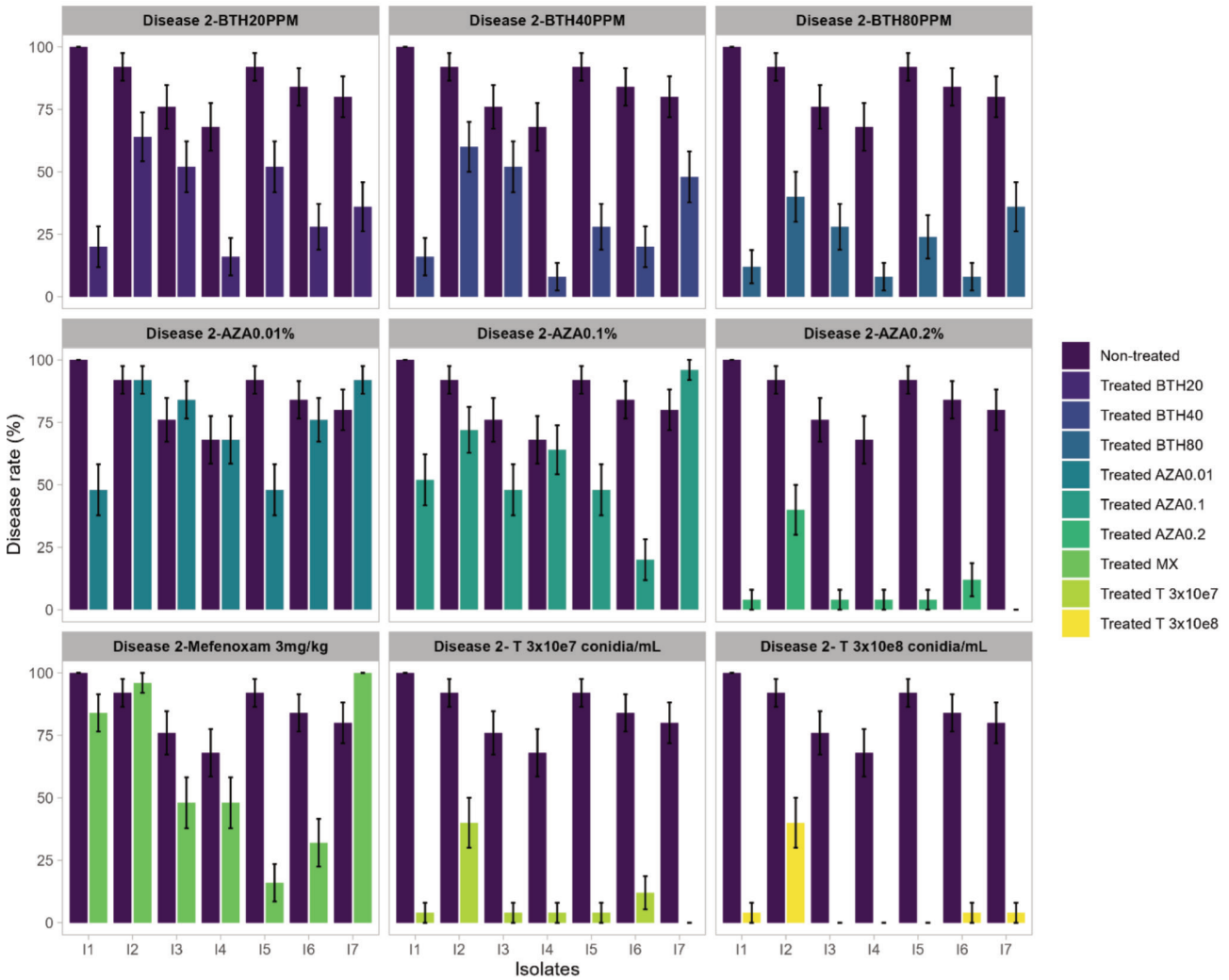

Figure 2. Disease rate 2 (%) of *Plasmopara halstedii* inoculated sunflowers treated with different doses of benzothiadiazole (BTH, 20, 40 and 80 ppm), azadirachtin (AZA, 0.01, 0.1 and 0.2%), mefenoxam (MX, 3 mg/kg) and *Trichoderma asperellum* (3 x 10^7 and 3 x 10^8 conidia) at 21 dpi. Disease rate 2 (%) was determined by the ratio of diseased (chlorotic or damped-off plants) and healthy plants. Bars represents standard deviations of five replicates. I1-I7: *Plasmopara halstedii* isolates (see in Table 1).

Results

Effect of plant inducers on disease rates

Figure 1. shows the average disease rate 1 percentage (sporulation and damping off) of the several *P. halstedii* isolates on mefenoxam-treated and non-treated sunflowers. In mefenoxam-treated and *P. halstedii*-inoculated sunflower plants, five of the seven isolates generated relatively high disease rates (ranging from 12 to 100%) (Fig. 1). The I7 (*Borsod* pathotype 704), I2 (*Körösladány* pathotype 710), and I1 isolates had the highest infection rates (pathotype 704 from *Abony*). Mefenoxam was effective against I5 (pathotype 730 from *Csanytelek*) and I6 downy mildew isolates (pathotype 730 from *Tiszaforet*). In contrast, the reduction in infection rate was observed in both isolates [I3 (*Doboz* pathotype 704) and I4 (pathotype 700)] (Fig. 1).

The lowest dose of BTH (20 ppm), was not significantly different in decreasing disease rate 1 on three *P. halstedii* isolates I2, I5, I7, however, it was significantly different against four isolates from high to low in the following order I1 > I3 > I6 > I4 (Fig. 1). The BTH 40 ppm, on the other hand, showed no significant difference in the isolate (I7) and provided a significant protection rate on the isolates I2 and I5. The BTH, 40 ppm was highly significant and had the lowest infection rate of the isolates I4, I1, I3 and I6 (Fig. 1). The highest dose of BTH (80 ppm), significantly reduced the infection rate of all isolates except isolate I7 (Fig. 1).

On all *P. halstedii* isolates, the lowest dose of botanical inducer azadirachtin 0.01% was not significantly different from untreated-inoculated plants (Fig. 1). Furthermore, AZA 0.1% behaved insignificantly on all isolates except for I1 and I6, which provided less significant protection (Fig. 1). On the other hand, the highest dose of AZA 0.2%, significantly reduced the infection rate of all isolates except two I5 and I7, which had significantly highest infection rates and moderate significant resistance to isolate I2 (Fig. 1). Furthermore, both doses of the biotic inducer *T. asperellum* were significantly effecient against all isolates and provided significant protection against isolate I2 (Fig. 1).

Figure 2. depicts the average percentage disease rate 2 (chlorosis and damping off) of different *P. halstedii* isolates on BTH, *T. asperellum*, AZA, and mefenoxam-treated and untreated sunflowers. In mefenoxam-treated and *P. halstedii*-inoculated sunflower plants, five of the seven isolates generated high disease rates ranging from 16 to 100% (Fig. 2). The infection rates were significantly higher with the I7, I2, I1 and I4. Mefenoxam-sensitive downy mildew isolates were identified I5 and I6. The infection rate in Isolate I3 was significantly lower (Fig. 2).

The lowest dose of BTH (20 ppm), performed less significantly on one *P. halstedii* isolate (I2) and provided significant protection against two isolates I3, I5. The protection was highly significant on four isolates I1, I4, I6 and I7 (Fig. 2). The BTH 40 ppm, on the other hand, was less significant on three isolates I2, I3 and I7. and demonstrated a highly significant protection rate against the isolate I4, I1, I5 and I6 which had the significantly lowest infection rate (Fig. 2). The infection rate of all isolates was significantly lower at the maximum dose of BTH (80 ppm) (Fig. 2).

Except for I1 and I5, the lowest dose of botanical inducer AZA 0.01% showed significantly lower protection rate on all *P. halstedii* isolates (Fig. 2). Furthermore, AZA 0.1% was not significantly effective on all isolates except I6, I3, I5 and I1, which provided significant protection in the following descending order I6 > I3 >I5 > I1 (Fig. 2). On the other hand, the highest dose of AZA (0.2%), significantly reduced the infection rate of all isolates except isolate (I7), which had significantly greater infection rates (Fig. 2). Furthermore, both doses of the biotic inducer *T. asperellum* were highly protective against all isolates and provided a less significant protection rate against isolate I2 (Fig. 2).

Effect of plant inducers on plant height

Due to the essential symptom of *P. halstedii*, which is stunting of the infected plant, plant height was measured twice throughout the investigations (Fig. 3). There was no significant difference in height between non-inoculated, mefenoxam-treated and non-inoculated, nontreated plants at any data collection point in any of the tests. In addition, the mefenoxam-treated and infected sunflowers I3, I4, I5, and I6 were significantly higher than the nontreated-infected sunflowers (Fig. 3) during the initial assessment. Plant heights were considerably more significant for treated sunflowers inoculated with all *P. halstedii* isolates, except for I2, which was significantly shorter than non-treated and infected plants.

Plants inoculated with isolates I4, I5, I6, and I7 and treated with the lowest dose of chemical inducer BTH (20 ppm) were significantly shorter or equivalent to non-treated inoculated plants. Plants treated with BTH 20 ppm and inoculated with isolates I1, I2, and I3 were significantly higher than non-treated and inoculated plants. In the case of BTH 40 ppm, all treated and inoculated plants were significantly higher than non-treated inoculated plants, except for plants infected with isolates I5, I6, and I7, which were not significantly different from nontreated and inoculated plants. The highest BTH dose of 80 ppm significantly reduced stunting signs in all plants treated and inoculated with all isolates except I6, which was not significantly different from non-treated inoculated plants (Fig. 3).

Otherwise, the smallest dose of the botanical fungicide AZA (AZA 0.01%) did not significantly affect plant stunting in any isolates (Fig. 3). The plants treated with 0.1% and inoculated with the isolates I1, I3, and I4 were not statistically significantly different from the non-treated and inoculated plants. In contrast, the other plants inoculated with isolates I2, I5, I6, and I7 were significantly higher than the untreated and inoculated plants (Fig. 3). Plants treated with the greatest dose of AZA (0.2%) and inoculated with all isolates were significantly higher than non-treated and inoculated plants (Fig. 3). Both concentrations of *T. asperellum* significantly reduced the symptoms of stunting in all plants treated with various *P. halastedii* isolates. However, compared to non-treated and inoculated plants, the higher concentration of *T. asperellum* T 3 x 10^8 was the most significant in increasing plant height (Fig. 3).

A two-factor analysis of variance without repeated measures was conducted to test whether there was a difference between the groups of the independent variable isolate concerning the average of disease rate 1, disease rate 2 and the average of plant height and whether there was an interaction between the two variables isolate and treatment concerning the dependent variables. Results showed a significant difference between the two groups, with a significant difference at $p < 0.001$ and an interaction at $p < 0.001$ (Table 2).

The two-factor analysis of variance without repeated measures showed that there is a significant difference

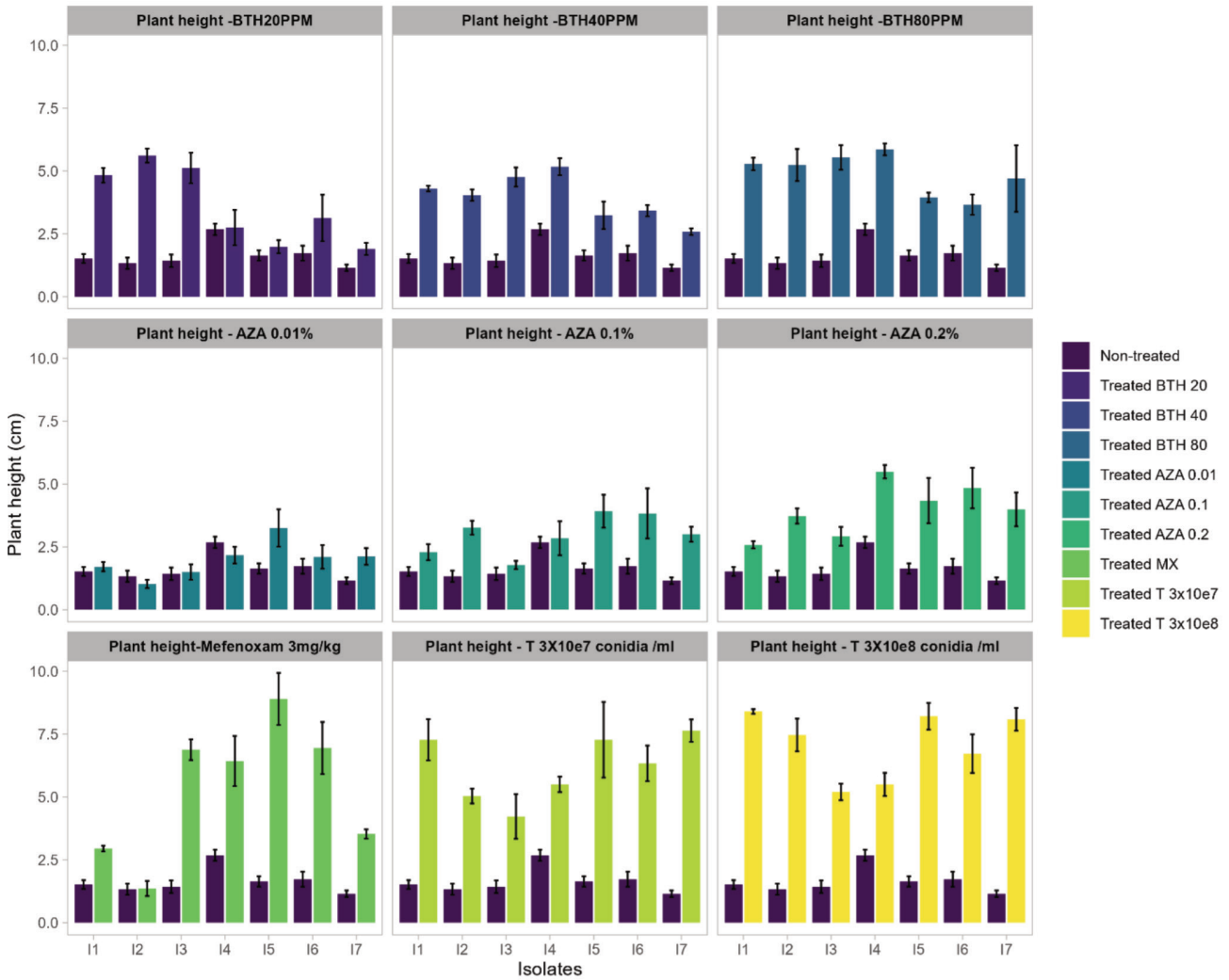

Figure 3. Plant heights of *Plasmopara halstedii* inoculated sunflowers treated with different doses of benzothiadiazole (BTH, 20, 40 and 80 ppm), azadirachtin (AZA, 0.01, 0.1 and 0.2%), mefenoxam (MX, 3 mg/kg) and *Trichoderma asperellum* (3 x 10^7 and 3 x 10^8 conidia) at 10 and 21 dpi. Bars represents standard deviations of five replicates. I1-I7: *Plasmopara halstedii* isolates (see in Table 1).

between the groups of the isolates in relation to the dependent variable's average of sporulation and damping off (Disease 1) and chlorosis and damping off (Disease 2) at $p < 0.001$ and interaction between the two variables isolate and treatment concerning this averages. This difference was significantly higher than the difference between the two groups of the dependent variable treatment, which showed a significant difference in both variables at $p < 0.001$(Table 2).

Additionally, there was a significant difference in the interaction between isolate and treatment, with the two variables, isolates and treatments, having two significant differences at $p < 0.001$. The two-factor analysis of variance showed that there is a significant difference between the groups of Factor1 (isolates) and Factor 2 (treatments) in relation to the dependent variable plant height at $p < 0.001$ and that there is an interaction between the two variables and the average of plant height (Table 2).

Finally, all biological and chemical inducers were significantly more effective than the other treatments at reducing disease symptoms (Disease 1, Disease 2, and plant height). Interestingly, AZA at greater concentrations proved very effective in lowering disease symptoms, however, at the lowest dosage it did not differ much from untreated diseased plants.

Table 2. The result of the two-factor ANOVA.

Disease 1	**Type III Sum of squares**	**df**	**Mean squares**	**F**	**p**
Corrected Model	168.79	69	2.45	15.6	<.001
Intercept	346.77	1	346.77	2211.39	<.001
Isolate	24.15	6	4.02	25.67	<.001
treatment	95.99	9	10.67	68.02	<.001
Isolate x treatment	48.65	54	0.9	5.75	<.001
Error	263.44	1680	0.16		
Total	779	1750			
Corrected total variation	432.23	1749			
Disease 2	**Type III Sum of Squares**	**df**	**Mean Squares**	**F**	**p**
Corrected Model	173.61	69	2.52	16.12	<.001
Intercept	384.23	1	384.23	2462.25	<.001
Isolate	30.4	6	5.07	32.46	<.001
treatment	99.69	9	11.08	70.98	<.001
Isolate x treatment	43.52	54	0.81	5.17	<.001
Error	262.16	1680	0.16		
Total	820	1750			
Corrected total variation	435.77	1749			
Plant height	**Type III Sum of Squares**	**df**	**Mean Squares**	**F**	**p**
Corrected Model	5684.23	139	40.89	28.44	<.001
Intercept	26623.37	1	26623.37	18515.64	<.001
Isolate	83.23	6	13.87	9.65	<.001
treatment	4514.86	19	237.62	165.26	<.001
Isolate x treatment	1086.14	114	9.53	6.63	<.001
Error	805.22	560	1.44		
Total	33112.82	700			
Corrected total variation	6489.45	699			

Discussion

The primary strategy for the control of sunflower downy mildew involves the use of resistant cultivars harboring dominant Pl (polysaccharide lyase) genes, alongside the implementation of crop rotation and the application of selective fungicides, as outlined in studies by (Qi et al. 2016). The emergence of novel pathotypes poses a considerable challenge, undermining the effectiveness of resistance mechanisms integrated into hybrid crops within recurrent crop rotation systems. In addition to genetic resistance and crop rotation strategies, chemical control of the disease can be achieved through the utilization of phenylamide fungicides. Using fungicide seed coatings, such as metalaxyl-M (mefenoxam), and resistant sunflower varieties is currently the most effective method for preventing and reducing damage from sunflower downy mildew (Molinero-Ruiz et al. 2008). Several additional investigations have revealed that these compounds' potency against *P. halstedii* has reduced (Albourie et al. 1998; Gulya 2000).

Metalaxyl is a potent inhibitor of mycelial growth and sporangial development (Farih et al. 1981), although it has a reduced efficacy in inhibiting encysted zoospore germination (Matheron and Porchas 2000). Molinero-Ruiz et al. (2008) reported a decrease on the effectiveness of two distinct active components (Metalaxyl and Metalaxyl-M) for controlling downy mildew in sunflowers is due to the ability of the novel strain to readily overcome *P. halstedii* resistance. Pánek et al. (2022) indicated that the metalaxyl not limiting the pathogen penetration into a host plant but effectively reducing mycelial development. These findings are also consistent with our findings, which reveal that mefenoxam cannot restrict sporangia germination *in vivo*. Moreover, it is unable to prevent pathogen penetration into plant tissue and the development of further symptoms. The amount of infection varies according to the aggressiveness or resistance of the isolate, which may impact or reduce the efficiency of the chemical fungicide, resulting in fungicide tolerance or resistance.

In plants, abiotic as well as biotic stimuli can elicit SAR (Sticher et al. 1997). BTH (benzo (1,2,3)-thiadiazole-

7-carbothioic acid S-methyl ester) activates the plant's defense system but has little antifungal activity (Körösi et al. 2009). Its active component is benzothiadiazole (BTH: benzo (1, 2, 3) thiadiazole -7-carbothioic acid S-methylester), a SA counterpart with a similar structure and method of action (Ryals et al. 1996). BTH, ASM, plant extracts and cell wall fragments can be employed to develop plant resistance. Certain circumstances may induce the plant defense system locally or systemically in response to pathogen invasion (Walters and Fountaine 2009; Walters et al. 2013). In Hungary, the first commercial plant activator was Bion 50 WG, Plant-induced resistance has long been studied. Benzothiadiazole (BION 50 WP), a chemical plant resistance activator, resists wheat powdery mildew (Sticher et al. 1997). Several researches in Italy and Hungary found benzothiadiazole effectively established sunflower Downy mildew resistance in field and greenhouse environments (Bán et al. 2004; Körösi et al. 2011; Tosi et al. 1998).

Neem extracts and products have demonstrated multimodal activity against phytopathogens, beginning with direct growth suppression, inhibiting pathogen establishment and subsequent development on the host plant, and inducing SAR in the pathogens (Goel et al. 2016). Our findings are comparable with those of Doshi et al. (2020). that the maximal concentration of Neem Azal was more effective than the aqueous solution of neem leaf extract in decreasing the infection rate in *P. halstedii* race 704-infected sunflowers. Neem components have been demonstrated to suppress many fungal infections, including pea-powdery mildew (Schmutterer 1988). *Azadirachta indica* and *Reynoutria sachalineusis* aqueous leaf extracts induced cucumber powdery mildew and leaf stripe disease resistance in barley (Daayf et al. 1995; Paul and Sharma 2002). Primarily, Neem Azal extract inhibited pathogenic growth. In pea leaves, Neem Azal reduces germ tubs, haustoria, branches, and colonization to prevent disease growth. Hypersensitivity response (HR) from Neem Azal induces protein accumulation in intercellular fluids (Singh and Prithiviraj 1997). By biochemical changes in the host plant, an aqueous leaf extract of *A. indica* developed resistance in barley against the stripe disease *Drechslera graminea.* The treated leaves displayed considerably increased activity of the enzymes phenylalanine ammonia lyase (PAL) and tyrosine ammonia lyase (TAL), as well as a quick and distinct buildup of fungitoxic phenolic compounds, indicating that soil-borne pathogenic fungal development was effectively inhibited (Paul and Sharma 2002).

The current study also determined the effectiveness of the tested biotic agent *T. asperellum* in inducing resistance against downy mildew in sunflower by seed treatment. Thus, the recent study's findings combine knowledge of the application of abiotic agents for both seedling quality improvement and disease management in a plant-pathogen system in sunflower. Using PGPF (*T. asperelluim*) as seed treatment could be a valuable component of integrated disease control. Apart from their anti-pathogen properties, these fungi are also effective growth boosters, an added benefit for any practical agricultural system. Plant disease control has advanced to new heights in the past several decades. Because of their eco-friendliness, several special crop protection measures have been developed. Plant growth-promoting fungi (PGPF) reinforced the plant cell wall and altered host physiology and metabolism to enhance plant defense chemical production in response to pathogen and abiotic stress (Nowak and Shulaev 2003). Our results agree with Nagaraju et al. (2012), who demonstrated that it was clearly shown that seed treatments with conidial suspension and *T. harzianum* PGPFYCM-14 and PGPFYCM-2 formulations decreased the incidence of sunflower downy mildew disease and afforded moderate to good disease protection and increased the vegetative production for sunflower seedlings under greenhouse and field settings.

According to Cordo et al. (2007), *T. harzianum* induces a biochemical systemic response that protects wheat plants from the *Septoria tritici* causing leaf blotch. All these studies lend credence to the current findings that PGPF: *T. asperellum* could promote growth and induce resistance to sunflower downy mildew disease. These fungi play essential roles as biocontrol agents. However, strains vary significantly in their ability to colonize roots (i.e. to be rhizosphere competent) (Ahmad and Baker 1987), with the most effective strains growing roots and providing advantages for at least the life of annual crops (Matheron and Porchas 2000).

Conclusion

In conclusion, this study aimed to evaluate the efficacy of chemical inducer BTH, botanical pesticide azadirachtin, and seed treatment with *T. asperellum* against seven diverse isolates of sunflower downy mildew. Our findings suggest that natural materials like plant extracts (AZA) and biocontrol agents (*T. asperellum*) hold promise as effective alternatives to chemical treatments for sunflower downy mildew control. *In vivo* investigations further affirm the high effectiveness of *T. asperellum* against different *P. halstedii* pathotype responsible for sunflower downy mildew. Notably, the highest dose of BTH (80 ppm) and the highest dose of AZA (0.2%) were found to be effective in reduce the disease rate. It's worth noting that the response to chemical, botanical, and biological inducers, as well as the disease severity, exhibited variations among the different pathogen isolates.

Acknowledgment

The first and second authors extend their heartfelt gratitude to the Government of Hungary's Tempus Public Foundation for the generous support provided through the *Stipendium Hungaricum* Scholarship. The scholarships, with Project Reference Numbers SHE-19332-002/2018 and SHE-07672-004/2019, have played a pivotal role in facilitating our PhD journeys, enabling us to pursue academic excellence and contribute to our respective fields. We would also like to express our sincere appreciation to Dr. Wajid Umar for his invaluable assistance in statistical analysis and for his expertise in creating graphical representations using the R programming language.

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