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The Effect of a New Derivative of Benzothiadiazole on the Reduction of Fusariosis and Increase in Growth and Development of Tulips

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Abstract: The use of inducers of systemic acquired resistance (SAR) is widely described in the literature. Such substances have important advantages over plant protection products (PPPs) and, thus, are often indicated as their alternatives. The main risk indicated in the context of the widespread use of SAR inducers is that of yield reduction that may result from the excessive metabolic imbalance of the treated plant. The general aim of the study presented was to check the effect of using a new active substance, namely *N*-methoxy-*N*-methylbenzo(1.2.3)thiadiazole-7-carboxamide (BTHWA), on tulips cultivated in greenhouse conditions. The plant response to BTHWA treatment was also analyzed in terms of the extent to which the growth–immunity phenomena would occur. Surprisingly, the application of BTHWA provided not only efficient protection against fusariosis but also resulted in the stimulation of the growth and development of tomato plants. The results proved very interesting as they stand in contrast to other results on SAR induction. The method of BTHWA application used in this study resulted in SAR induction at a level sufficient to provide effective protection and, at the same time, did not cause disruption to plant metabolism that would result in yield reduction.

Keywords: fungal diseases; fusariosis; systemic acquired resistance (SAR); plant stimulation; benzothiadiazole; BTHWA

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1. Introduction

Effective protection of plants requires the use of fungicides, bactericides and other plant protection products. According to the Food and Agricultural Organization, up to 40% of global crops are lost to pests, with plant diseases and invasive insects costing the global economy USD 220 billion and USD 70 billion, respectively [1]. Even though plant protection products belong to the group of chemicals subject to detailed tests in the context of their potential environmental impact, some reports have indicated their adverse effect on the environment [2,3]. In addition to targeting organisms against which treatments with plant protection products are performed, non-target organisms are also affected, which may damage their populations or ecosystems. For instance, fluazinam, belonging to the 2,6-dinitroanilines group of fungicides, has been reported to inhibit microbial respiration [4]. Other non-target effects may influence signal transduction, as in the case of fludioxonil belonging to fungicides of the phenylpyrrole group of fungicides [5].

The application of pesticides may also lead to the acquisition of resistance by pathogens. One scenario of resistance acquisition is cross-resistance which takes place when the development of resistance to a particular substance leads to acquiring resistance to more compounds that can be made resistant by similar mechanisms [6]. As an example of such a

type of resistance against different compounds that is provided through a single mechanism, the efflux pump is indicated [7]. In turn, co-selection occurs when the selection of one gene responsible for resistance automatically carries with it the selection of another gene responsible for resistance [6]. Although the process of acquiring resistance by microbials, including pathogens, is natural, the extensive and inappropriate use of plant protection products has accelerated it and propelled the emergence of negative consequences [8]. As the number of active substances in pesticides authorized to use will decrease because of the implementation of the EU Commission Implementing Regulation 2015/408/EC [9], additional difficulties in providing efficient protection to plants are expected. The first to be eliminated are the substances most toxic to the natural environment, although they are often the most effective ones [10]. Their elimination has stimulated the search for new and effective methods of plant protection with the use of new active ingredients satisfying legal requirements and societal expectations.

Plants have evolved various defense mechanisms that allow protection against pathogens. One of them is systemic acquired resistance (SAR), which is associated with increased expression of genes coding pathogenesis-related (PR) proteins [11]. The signaling compounds responsible for the activation of SAR are salicylic acid (SA) and its metabolites, such as methyl salicylate (MeSA) [10]. SA is also involved as a signaling molecule in another phenomenon that is related to SAR, which is known as the growth–immunity trade-off [12]. Induction of SAR in plants might incur fitness costs resulting in the reduced size of the plant or obtained yield [13]. Such an excessive disruption of plant metabolism is often caused by the inappropriate application of SAR inducer, which includes performing too many treatments during a growing season, keeping too few intervals between them or its use at a too high concentration. These aspects, which are specific to a given crop, are of key importance in the context of developing the right mode of use for SAR inducers.

When comparing SAR inducers and plant protection products (PPPs), numerous advantages of substances belonging to the former group should be pointed out. The most important is that SAR inducers provide protection against a wide range of pathogens simultaneously, and PPPs are directed towards a specific type or group of pathogens [14]. The use of SAR inducers to control viral pathogens is of high importance, as PPPs can be used only to control insects that are vectors of such pathogens, while their direct control is not possible [15]. In our earlier study, we proved that by using SAR inducers, it is possible to provide efficient protection against both viral and fungal pathogens [16]. Moreover, the state of induction of the plant resistance effect lasts over time, even up to weeks after the last application of the SAR inducer. Some reports indicate the existence of resistance in a form of "plant memory" that can even be carried forward to the next generation [17]. In contrast, the effectiveness of treatments with PPPs is strictly dependent on the time of application. Further advantages are related to the mode of action of SAR inducers. As it is directed toward the stimulation of a plant's natural defense mechanisms and not toward pathogens directly, neither a direct impact on the development of microorganisms, including pathogens, nor the development of pathogen resistance are expected [18]. Thus, SAR inducers are an attractive option for reducing the use of PPPs in various cultivations, including ornamental plants.

Tulips are bulbous ornamental plants belonging to the genus *Tulipa* [19] in the family *Liliaceae*. They are one of the most popular ornamental plants worldwide. The Tulipa genus comprises more than 100 species and thousands of derived cultivars [20]. One of the most serious fungal pathogens affecting tulips is *Fusarium oxysporum* f. sp. *tulipae*, the agent responsible for "bulb rot" and "basal rot" of "fusariosis". The disease is widespread and mainly occurs during storage. The fungus produces dark brown spots on the top or side of tulip bulbs, which results in bulb base or root rot. The infection is manifested by stunted growth, leaf yellowing and overall plant death before flowering [21].

This pathogen is controlled with the use of PPPs, as other agronomic measures, such as the voidance of wounding, removal of diseased bulbs and crop rotation, are insufficient to control this disease [22]. The pathogen itself can survive in the soil for up to 6 years; thus,

extensive use of PPPs is required once a given field is contaminated [20]. Such extensive use poses a threat to the environment and increases the risk of resistance acquisition by the pathogen. Prolonged use of chemicals creates an environmental risk and is likely to lead to the development of fungicide resistance in the pathogen.

The SAR inducer that is the subject of our study is a novel benzothiadiazole derivative named *N*-methoxy-*N*-methylbenzo(1.2.3)thiadiazole-7-carboxamide (BTHWA). This compound was discovered while conducting research on the derivatization of benzo(1,2,3)thiadiazole-7-carboxylic acid, *S*-methyl ester (ASM, BTH), which is an active ingredient of the most popular and widely studied resistance inducer, named BION or Actigard marketed by the Syngenta company [23,24]. The main drawback of BTH in the context of its application in fields is poor solubility in water (only 7 mg/L); thus, our study was aimed at introducing modifications to the chemical structure of this compound that would increase its solubility [25–29]. Selected compounds were also subjected to the determination of their environmental hazard profile [30].

BTHWA proved to be the most effective of the substances we obtained, and, in addition, its solubility was increased compared to the parent compound BTH. Confirmation of the BTHWA activity related to SAR induction was provided by molecular studies indicating the increased expression of marker genes typical of SAR induction. In addition, the level of viral RNA accumulation was also investigated in tobacco plants that were treated or not with SAR resistance inducers prior to TMV infection [31]. The BTHWA-treated plants had higher levels of the SAR marker genes *PAL*, *NPR1* and *PR-1b* at 4 h after treatment, compared to untreated control plants. The aforementioned marker genes are linked to several signaling networks, including the ethylene, jasmonate and salicylic acid pathways, which are all involved in the pathogen defense response. Moreover, the level of viral RNA accumulation in BTHWA-treated plants was significantly lower than that of control plants not treated with SAR inducers before viral inoculation [31]. We have reported the significant effect of BTHWA on strawberries (against *Phytophthora* sp. oomycete and *Colletotrichum* sp. fungus), zucchini (against powdery mildew fungus and WMV, CABYV and ZYMV viruses), ash trees (against *Hymenoscyphus fraxineus* fungus), oak trees (against powdery mildew fungus) and tobacco (against the TMV virus). In our previous studies, we have reported that the use of BTHWA on strawberries, zucchini, ash trees and oak trees [16,28,32–34] resulted in providing efficient protection of these plants against various pathogens. Moreover, in the study on zucchini, we have proved that it is possible to provide protection against both viral and fungal pathogens [16]. The possibility of controlling pathogens of viral origin constitutes a great advantage compared to PPPs, which can only be used to control, e.g., aphids that are vectors of viral diseases.

The general aim of the study presented was to check the effect of using a new active substance, namely *N*-methoxy-*N*-methylbenzo(1.2.3)thiadiazole-7-carboxamide (BTHWA), on tulips cultivated in greenhouse conditions. The plant response to BTHWA treatment was also analyzed in terms of the extent to which the growth–immunity phenomena would occur. This information is of high importance when investigating the possibility of using SAR inducers on a given crop, especially because, as we observed in our previous work describing the use of BTHWA in strawberry cultivation, the effect of treatment with this substance was visible not only in terms of SAR induction but also in plant size and obtained yield [34].

2. Materials and Methods

2.1. Tested Substance

The substance that was the subject of this study is a novel benzothiadiazole derivative designed and synthesized in our group: *N*-methoxy-*N*-methylbenzo(1,2,3)thiadiazole-7-carboxamide (BTHWA) [35], provided by the company Innosil Ltd. (Poznan, Poland). This substance was obtained with 99.9% purity and dissolved in 5% ethanol to obtain working solution. In other studies, BTHWA was formulated as suspension concentrate (SC) type of formulation. Even though the formulation is composed of a polymeric dispersant

and a thickening agent (a polysaccharide) whose presence does not affect microbials, it was decided to use BTHWA in the form of pure active substance. In order to improve its solubility, 5% ethanol was added to working solutions used to prepare growth media for plate tests and spraying liquid for treatment of plants. For each treatment, the stock solution of 1 L was prepared by dissolving appropriate amount of BTHWA in ethanol and adding this solution to distilled water.

2.2. Plate Test

In vitro plate tests were conducted in vitro on potato dextrose agar medium (PDA-Merck). Water solution of BTHWA containing 5% of ethanol was added to the medium after its sterilization and cooling down to 45 °C to obtain the medium containing the following concentrations of BTHWA: 5; 10; 20; 30; 40; 50; 60; 70; and 80 mg/L. The obtained solutions were poured into 90 mm Petri dishes. The medium without BTHWA was prepared as an untreated control (water with 5% of ethanol). Then, 5 mm disks of PDA medium overgrown with a 7-day culture of the isolate of *Fusarium oxysporum* f. sp. *tulipae* were transferred onto solidified medium in the middle of the dishes, and plates were incubated at 25 °C in dark. After 4 and 6 days of incubation, the diameter of mycelium colony was measured. The experiment was conducted in two replicates, with five Petri dishes per variant of treatment in each replicate.

2.3. Experiment with Development of Pathogen

The bulbs of tulip cv. Leen van der Mark were cooled at 5 °C and refrigerated for 12 weeks at 5 °C. After planting into pots, the greenhouse temperature was set to 10 °C and, after rooting the tulip bulbs, to 20 °C. The bulbs were surface disinfected with 50% ethanol solution for 5 min and then washed 3 times with distilled water. After soaking the bulbs in water for 3 days, they were planted into 0.5 L pots filled with peat substrate. In total, 4 treatments with the tested substance were performed. The first treatment with the tested substance was performed right after planting, while the other 3 ones were at bi-weekly intervals. For each treatment, the bulbs were watered with 12 mL of 5% ethanol–water solution containing either 20 or 40 mg BTHWA per liter, while the bulbs of untreated control variant of treatment were watered with 5% ethanol–water solution.

After 7 days from planting of the bulbs, inoculation with *Fusarium oxysporum* f. sp. *tulipae* was performed, using the inoculum of 1.2×10^6 spores per milliliter. Inoculum in a volume of 12 mL was introduced into 4 holes that were manually made in the peat substrate to facilitate access to root systems.

Assessment of the degree of infection was performed according to the 6-point scale, where 0 = no infection symptoms; 1 = first symptoms of infection (infection < 1%); 2 = slightly infected (2% < infection < 6%); 3 = moderately infected (7% < infection < 20%); 4 = moderately severe (21% < infection < 50%); and 5 = heavily infected (infection > 50%).

After 66 days from planting the bulbs, the parameters of tulips were assessed as described in Section 2.5. The experiment was conducted in 2 replicates, with 4 replications on 5 plants each.

2.4. Experiment with Development of Tulip Plant (Biostimulation Studies)

The bulbs of tulip cv. Leen van der Mark were cooled at 5 °C and refrigerated for 12 weeks at 5 °C. After planting into pots, the greenhouse temperature was set to 10 °C and, after rooting the tulip bulbs, to 20 °C. The experimental schema was the same as described above. The bulbs were surface disinfected with 50% ethanol for 5 min and then washed 3 times in sterile water. After soaking the bulbs in water for 3 days, they were planted into 0.5 L pots filled with peat substrate. In total, 4 treatments with the tested substance were performed. The first treatment with the tested substance was performed right after planting, while other 3 treatments were performed at bi-weekly intervals. For each treatment, the bulbs were watered with 12 mL of a 5% ethanol–water

solution containing BTHWA, while the bulbs of untreated control variant of treatment were watered with a 5% ethanol–water solution.

After 66 days from planting the bulbs, the parameters of the tulips were assessed as described in Section 2.5. The experiment was conducted in 2 replicates, with 4 replications on 5 plants each.

2.5. Evaluation of Tulip Growth Parameters

Height of the tulips was measured from the ground to the base of the calyx. Measurements were made for each plant. After the last measurement, the aboveground part of each plant was cut and weighted. Then, each plant was put into a paper bag, dried for 48 h at 75 °C, and dry mass was weighed. The same procedure was applied for root assessment.

2.6. Statistical Analysis

All recorded and calculated data were evaluated using analysis of variance (ANOVA), and the mean differences were compared using post hoc test at a $p < 0.05$ level, according to Duncan test. Statistical analyses were performed using OriginLab 2022 software for Windows, version 9.9 (OriginLab Corp., Northampton, MA, USA).

3. Results

3.1. Plate Test

Statistical analysis of the parameters describing the growth of *Fusarium oxysporum* f. sp. *tulipae* mycelium showed no significant differences between the untreated controls (UTCs) and all tested variants of treatment. A similar dependence was observed both after 4 days of growth and 6 days of growth (Table 1).

Table 1. The effect of growth on *Fusarium oxysporum* f. sp. *tulipae* mycelium on medium with the addition of BTHWA in various concentrations.

Variant of Treatment	Growth of the *Fusarium* f. sp. *tulipae* Mycelium (mm)	
	4 Days of Growth	6 Days of Growth
UTC	6.5 a	19.9 a
BTHWA 5 mg/L	7.0 a	19.6 a
BTHWA 10 mg/L	6.8 a	19.8 a
BTHWA 20 mg/L	6.7 a	19.7 a
BTHWA 30 mg/L	6.6 a	19.8 a
BTHWA 40 mg/L	6.8 a	19.9 a
BTHWA 50 mg/L	6.7 a	19.6 a
BTHWA 60 mg/L	6.6 a	19.4 a
BTHWA 70 mg/L	6.5 a	19.6 a
BTHWA 80 mg/L	6.6 a	19.8 a

Means followed by different letters indicates a statistically significant difference at $p < 0.05$ according to Duncan test.

3.2. Experiment with Development of Pathogen

As the induction of SAR might lead to a reduction in the size of the plant, in this experiment, not only the degree of infection was assessed but also measurements of plant height were performed before the first treatment to indicate the initial height of the plants before each of consecutive treatments and 20 days after last treatment, as indicated in Table 2.

3.2.1. Plant Growth

The bulbs that were grown in a substrate with the pathogen were significantly lower than those grown in the pathogen-free substrate (Table 2). The results clearly indicate that, after the first treatment with BTHWA applied in both concentrations, the growth of plants significantly increased in comparison to the UTCs, which were not inoculated and inoculated. This trend was maintained on all measurements dates.

The fresh mass and dry mass of both the aboveground part of the plant and the roots were also analyzed. As indicated in Table 3, the highest values of the fresh weight and dry mass of the aboveground part of the plant were observed for the plants treated either with BTHWA 20 mg/L or 40 mg/L. These values were significantly higher than those of the plants of the untreated controls that were not inoculated and those that were inoculated (Table 3, Figure 1).

Table 2. Influence of tested variants of treatment on the height of tulips.

Variant of Treatment	Plant Height (mm)				
	Before 1st Treatment	Before 2nd Treatment	Before 3rd Treatment	Before 4th Treatment	20 Days after 4th Treatment
UTC (not inoculated	48.1 a	108.0 b	176.2 b	256.2 b	387.7 b
UTC (inoculated)	48.5 a	89.1 a	135.0 a	189.2 a	284.4 a
BTHWA 20 mg/L	48.3 a	121.1 c	225.3 c	353.2 c	505.2 c
BTHWA 40 mg/L	48.7 a	118.2 c	226.8 c	362.4 c	503.0 c

Means followed by different letters indicates a statistically significant difference at $p < 0.05$ according to Duncan test.

Table 3. Influence of tested variants of treatment on the fresh and dry mass of the aboveground part of tulips and roots and on the length of roots and degree of root infection.

Variant of Treatment	Aboveground Part of Tulips		Root Biomass		Assessment of Roots	
	Fresh Mass (g)	Dry Mass (g)	Fresh Mass (g)	Dry Mass (g)	Length of Roots (mm)	Degree of Root Infection (0–5)
UTC (not inoculated)	345.8 b	32.1 b	78.5 c	8.0 c	82.6 b	0.0 a
UTC (inoculated)	201.5 a	22.6 a	45.9 a	2.9 a	61.5 a	4.75 c
BTHWA 20 mg/L	432.3 c	40.9 c	59.4 b	5.5 b	84.2 b	0.75 b
BTHWA 40 mg/L	438.4 c	41.5 c	61.1 b	5.6 b	84.5 b	0.67 b

Means followed by different letters indicate a statistically significant difference at $p < 0.05$ according to Duncan test.

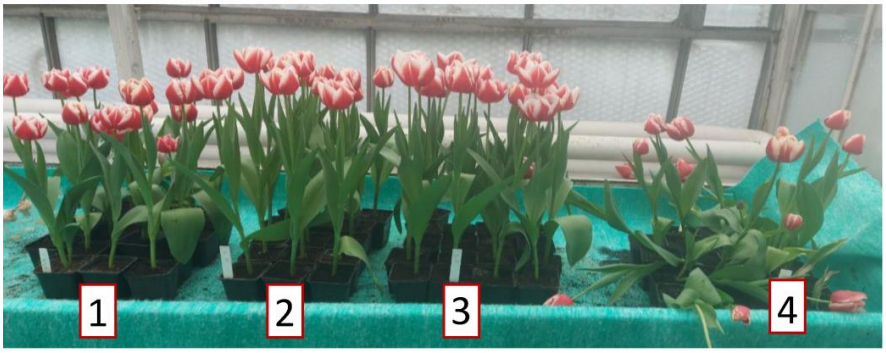

Figure 1. The effect of treatment with BTHWA on the stimulation of tulip height and vigor. (1) UTC (not inoculated); (2) plants treated with BTHWA 20 mg/L; (3) plants treated with BTHWA 40 mg/L; (4) UTC (inoculated).

As for the roots, the highest values of the fresh and dry mass were observed in untreated control plants that were not inoculated. However, the values of the fresh and dry mass of the roots of plants treated with BTHWA in both concentrations were significantly higher compared to those of the untreated control plants that were inoculated (Table 3).

3.2.2. Degree of Root Infection

The efficiency of treatment with BTHWA in terms of providing protection against the disease was assessed on the basis of root infection. The degree of root infection was the highest in the untreated control variant of treatment with pathogen inoculation. The roots of plants treated with BTHWA in both applied concentrations were infected to a significantly lower degree than those of the UTC and inoculated ones. No statistical differences were

observed between the effects of BTHWA in both concentrations (Table 3). Moreover, the length of the roots of plants of the UTC, those not inoculated with *F. oxysporum* f. sp. *tulipae* and those of plants treated with BTHWA either in 20 or 40 mg/L and inoculated was significantly greater compared to that of the roots of the UTC plants that were inoculated.

3.3. Experiment with Development of Tulip Plants (Biostimulation Studies)

The same experimental schema was also used in the experiment aimed to investigate the growth parameters of tulips grown in the pathogen-free substrate. Analysis of the results obtained from the experiment carried out with pathogen infection led us to hypothesize that the application of BTHWA, in the absence of a pathogen, can stimulate growth and development.

The overall pattern of results remains the same as in the case of the previous experiment. Even the first application of BTHWA resulted in a significant increase in plant height compared to that of the plants of the untreated control, and the trend was maintained on all measurement dates. There are no differences between the results obtained after treatment with the two concentrations of BTHWA (Table 4). It should be noted that the tulips treated with BTHWA were stiffer and bloomed for 7 days longer, which is very important for ornamental plants (Figure 2).

Table 4. Influence of tested variants of treatment on the height of tulips.

Variant of Treatment	Plant Height (mm)				
	Before 1st Treatment	Before 2nd Treatment	Before 3rd Treatment	Before 4th Treatment	20 Days after 4th Treatment
UTC	45.1 a	102.1 a	199.0 a	269.2 a	399.7 a
BTHWA 20 mg/L	45.5 a	129.5 b	258.6 b	369.2 b	510.5 b
BTHWA 40 mg/L	46.3 a	132.6 b	269.1 b	375.3 b	517.0 b

Means followed by different letters indicates a statistically significant difference at $p < 0.05$ according to Duncan test.

The stimulation of plant growth is also manifested in the increase in the fresh and dry mass of the aboveground part of the plants. Statistical differences were not observed between the plants treated with 20 mg/L or 40 mg/L of BTHWA (Table 5).

Table 5. Influence of tested variants of treatment on the fresh and dry mass of the aboveground part of tulips.

Variant of Treatment	Aboveground Part of Tulips	
	Fresh Mass (g)	Dry Mass (g)
UTC	363.8 a	36.0 a
BTHWA 20 mg/L	443.7 b	41.6 b
BTHWA 40 mg/L	434.3 b	43.0 b

Means followed by different letters indicate a statistically significant difference at $p < 0.05$ according to Duncan test.

Figure 2. The effect of treatment with BTHWA on the stimulation of tulip height and vigor. (1) UTC; (2) plants treated with BTHWA 20 mg/L; (3) plants treated with BTHWA 40 mg/L.

4. Discussion

In view of the societal expectations and legal measures aimed at limiting the negative effects of plant protection products on natural biodiversity, research aimed at the development of novel active substances providing not only sufficient protection but also satisfying the indicated expectations is of high significance.

As the ornamental plant market is not that large compared to other markets, the number of active substances authorized to use for their protection is limited, and these substances mainly belong to the category of growth regulators that allow the inhibition of scab-like lesions on tulip bulbs caused by *Fusarium oxysporum* [36]. The application of D,L-β-aminobutyric acid (BABA) inhibited the development of fusariosis on tulip bulbs and roots [37]. Among the active substances with fungicidal properties, PPPs containing fenhexamide are reported to provide protection against grey mold [38]. No fungicides are authorized for use on tulips against fusarium [39]. Other reports indicate that carbendazim shows activity against fusarium. However, the extensive applications of carbendazim resulted in the prevalence of carbendazim resistance in most plant pathogens, including fusarium [40] and *Sclerotinia sclerotiorum* [41]. Moreover, it has been reported that carbendazim had an inhibitory effect on soil parameters such as soil respiration, enzymatic activities and soil fungal–bacterial ratios and could impede other fungicides' dissipation in the soil [42].

The proper use of PPPs is related to the observance of appropriate procedures to handle, store, apply and dispose of them. Following these procedures might be difficult in low- and middle-income countries, which has led to an increased rate of population exposure to contaminated food and water and poses a threat to microorganisms [43]. Another group exposed to the adverse effects of PPPs is pollinators. Even some PPPs that are considered to be safe can endanger the existence of bee colonies [44].

Bees play an important role in the pollination of various crops, including ornamental plants. What is more, pollinators contribute to carbon sequestration, the water cycle and water purification, organic matter degradation and the preservation of biodiversity [45]. During pollination, bees can come into contact with PPPs when drifting through spray drift or dusting from an applied product [46]. Another threat is oral intoxication that results from the ingestion of contaminated water, pollen or nectar [47]. Despite the introduction of EU regulations that banned the use of certain active substances, there are still reports indicating their use. For instance, the use of insecticides containing neonicotinoids is forbidden, but their residues were found in honeybee colonies [48]. One possibility to overcome the problem of the contamination of pollen by PPPs is to transform their active substances to ionic forms, which allow their biological function to be maintained while reducing negative impacts on the environment [49]. In our previous study, we reported that the level of the residues of the novel compounds was lower in both the leaves and pods compared to the commercially available fungicide [50]. The level of residues is indicated as one of the components describing the potential impact of active substances on bees [51].

SAR inducers, due to their mode of action which is directed toward the stimulation of a plant's natural defense mechanisms and not toward pathogens directly, are effective in much lower doses than typical pesticides, especially fungicides. The dose of BTHWA in the range of 20–40 mg per liter of solution scales up to 8–16 g of active substance applied per hectare of crop. For comparison, a typical fungicide dose for the same area is around 100–500 g. Thus, a much smaller amount of active substances is released into the environment, which significantly reduces the potential adverse effects of their use in agricultural practice. As follows from our results, BTHWA applied in much higher quantities than those used in agriculture does not influence the growth of pathogens. These results are in line with our preliminary unpublished data indicating that BTHWA has no direct effect on fungi species, such as *Alternaria alternata* and *Trichoderma viride* and the bacteria *Erwinia amylovora*. Moreover, BTH did not show any direct effect on the number of plant pathogens in vitro, and it was classified as not antimicrobial [52]. Thus, it can be concluded that the environmental impact of the application of BTHWA is negligible.

Our results on plate tests, which are in line with those mentioned above, indicate that the action of BTHWA is not directed towards pathogens but at the stimulation of plant natural defense mechanisms.

The SAR-inducing activity of the BTHWA application has been proven in the cultivation of strawberries [31] and zucchini [15]. The effects of the application of this substance on the yield have been examined only for strawberries, for which the BTHWA impact on the multiplicity of the plant was observed depending on its concentration. As for the BTH substance, also belonging to the group of benzothiadiazole, its negative effect on the size of plants and the yield obtained has been reported. This suggests that the application of the concentrations used resulted in the growth–immunity phenomenon.

The results proved very interesting as they stand in contrast to other results obtained in our previous research and by other authors studying SAR inducers. However, to definitely prove that the applied method of BTHWA application resulted in SAR induction at a level sufficient for providing effective protection and, at the same time, did not cause such a disruption in metabolism that would result in yield reduction, it would be necessary to supplement these results with detailed studies at molecular and physiological levels.

Overall, based on the above-presented results, it can be inferred that the application of BTHWA both activates plants' natural defense mechanism as a result of SAR and stimulates the growth of tulips. The chosen doses of BTHWA (20 and 40 mg/L) provided to plants by watering should be considered as optimal as the growth–immunity trade-off did not occur. It is worth noting that the plants treated with BTHWA in both concentrations were significantly higher not only compared to those of the untreated control that was inoculated but also to those of the untreated control that was not inoculated. The same applies to the fresh mass and dry mass of the aboveground part of the plant. As far as the root biomass is concerned, an analogous dependence was observed only when compared to the plants of the untreated control that was inoculated with the pathogen. Moreover, the stimulation effect on the plants was demonstrated in all the parameters studied. As no significant changes in the plant parameters were observed when using BTHWA at concentrations of 20 and 40 mg/L, the most optimal dose for environmental and economic reasons is the lower dose of 20 mg/L.

The observed response was caused by four treatments in total; however, as a significant increase in plant height was observed even after the first application, it is reasonable to consider only a single application in future studies.

The main difference between our previous work and this study is the mode of SAR inducer application. The vast majority of research on SAR inducers is conducted using spraying and not watering. The main reason behind it is that spraying is more reproducible in terms of results as there is not as much variability in the amount of active ingredient administered and the rate at which it gets into the plant. In addition, from the point of view of agricultural practice, the administration of the substance by spraying seems to be easier and cheaper. However, such a mode of application can result in extensive disturbance of the metabolism because the entire amount of active substances is applied at the same time. In the case of watering, some amount of the active substance can be bound in the substrate so the substance will get to the plant in smaller quantities but for a longer time. Thus, the disturbance of plant metabolism might be not that extensive.

Therefore, it seems necessary that in the context of the development of technology for the use of resistance inducers, attention should be paid not only to their appropriate dose, the timing of application and the intervals between them but also to the mode of administration of the active substance. By proving that the application of SAR inducers by watering is also beneficial to plants, new opportunities for using such substances in agriculture are arising.

In future studies, it is reasonable to develop a method for detecting BTHWA in plants and use this method to determine the amount of BTHWA that entered the plant and caused the observed response. It also seems necessary to compare the amount of active substance and the rate of its decay in the plant, depending on the application mode. Another research

topic that would be worth considering is the determination of the potential differences in the active substance uptake depending on the type of substrate or the size of the pot. All these aspects should be considered while developing technology related to the use of such substances in agricultural practice. It should be noted, however, that the implementation of such a technology is much simpler in controlled greenhouse conditions than in the field [53].

5. Conclusions

N-methoxy-*N*-methylbenzo(1.2.3)thiadiazole-7-carboxamide, a new derivative of BTH, has a positive effect on tulips manifested by the limitation of infections with *fusarium oxysporum* sp. *tulipea* and the stimulation of the growth and development of the plants. According to the collected evidence, both these effects were observed for the plants treated with BTHWA either in the concentration of 20 or 40 mg/L. No differences in the effect on the plants were recorded between these two concentrations. The results prove very interesting as they stand in contrast to other results obtained in our previous research and by other authors studying SAR inducers. The application of BTHWA, even in higher concentrations, did not bring about the growth–immunity phenomenon. Moreover, the observed response of plants was caused by four treatments in total; however, a significant increase in plant height was observed even after the first application. The encouraging results described on tulip plants are an incentive for the continuation of studies on the effects of BTHWA and other new SAR inducers on other ornamental plants.

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