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Article

Response of susceptible carnation cultivars during thiamine application as inducer of resistance to vascular wilting caused by *Fusarium oxysporum* f. sp. *dianthi*

Resposta de cultivares de cravo suscetíveis à aplicação de tiamina como indutor de resistência à murcha vascular causada por *Fusarium oxysporum* f. sp. *dianthi*

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Abstract: Colombia is one of the main producers of carnations worldwide. However, its production has been affected by vascular wilting caused by the pathogen *Fusarium oxysporum* f. sp. *dianthi* (*Fod*). In previous studies, thiamine has been proposed as a potential resistance inducer for this disease, under greenhouse growth conditions. However, the specific conditions for its application must be studied when applying it under commercial conditions. In this sense, this study aimed to evaluate the effect of the thiamine foliar spray in different concentrations among some susceptible carnation cultivars in response to *Fod-inoculation*. For this, two *in-vivo* trials were carried out, using foliar spraying of thiamine as a treatment before inoculation with the pathogen *Fod*, then measurements of incidence, severity, and the content of phenolic and flavonoid compounds were made. It was found that thiamine application, in the concentrations evaluated, shows a statistically similar decrease in the disease incidence and severity. These results were verified using multivariate statistical analysis. In addition, it was found that the application of thiamine 1 mmol L^{-1} presents a positive response in reducing the effects of the disease in three susceptible cultivars. The results suggest that thiamine has the potential to be applied as a technique in the integrated management of disease in the productive sector.
Keywords: *Dianthus caryophyllus*, flavonoids, induced resistance, phenolic compounds.

Resumo: A Colômbia é um dos principais produtores de cravos do mundo. No entanto, sua produção tem sido afetada pela murcha vascular causada pelo patógeno *Fusarium oxysporum* f. sp. *dianthi*. Em estudos anteriores, a tiamina foi proposta como um potencial indutor de resistência para esta doença, em condições de cultivo em casa de vegetação. No entanto, as condições específicas para sua aplicação devem ser estudadas ao aplicá-lo em condições comerciais. Nesse sentido, o objetivo deste trabalho foi avaliar o efeito da aplicação foliar de tiamina em diferentes concentrações entre algumas cultivares de cravo suscetíveis em resposta à inoculação *Fod*. Para isso, foram realizados dois ensaios *in vivo*, utilizando pulverização foliar de tiamina como tratamento antes da inoculação com o patógeno *Fod*, a seguir foram feitas medições de incidência, severidade e teor de compostos fenólicos e flavonoides. Verificou-se que a aplicação de tiamina, nas concentrações avaliadas, apresenta diminuição estatisticamente semelhante na incidência e gravidade da doença. Esses resultados foram verificados por meio de análise estatística multivariada. Além disso, verificou-se que a aplicação de tiamina 1 mmol L^{-1} apresenta resposta positiva na redução dos efeitos da doença em três cultivares suscetíveis. Os resultados sugerem que a tiamina tem potencial para ser aplicada como técnica no manejo integrado de doenças no setor produtivo.
Palavras-chave: compostos fenólicos, *Dianthus caryophyllus*, flavonoides, resistência induzida.

Introduction

Colombia is recognized as one of the main flower producers and exporter of cut flowers worldwide. Carnation (*Dianthus caryophyllus* L.) is one of the most relevant products representing more than 25% of the total Colombian flowers production, with 50 thousand tons exported in 2022, representing 50.8% of world exports (Trademap, 2022). However, carnation crops are affected by vascular wilting caused by *Fusarium oxysporum* f. sp. *dianthi* (*Fod*) worldwide. In this disease, the pathogen infects the vascular system of the affected plants, interfering with the absorption of water and nutrients, causing the death of the plant, and significantly affecting production (Pérez Mora et al., 2024; Wolcan et al., 2018).

Traditional disease control methods, in addition to good agricultural practice strategies, focus on the application of synthetic biocidal chemicals to protect plants. In this context, for the management of vascular wilt, fungicides such as captafol have been used, classified by the World Health Organization (WHO) as Extremely dangerous (Wolcan et al., 2018); difenoconazole, classified as moderately dangerous, and azoxystrobin, which is classified as unlikely to present an acute hazard in normal use (Wolcan et al., 2018). In general, this control strategy has negative impacts on human health and the environment, in addition to promoting the development of resistant microorganisms. This situation has increased scientific interest in environmentally safe alternatives, within the framework of sustainable production, to control commercially important crop diseases, such as biological control, the use of resistant cultivars, and the use of resistance-inducing agents (Boubakri, 2020; Sanabria et al., 2020).

It has been reported that certain vitamins can induce resistance to plant diseases and abiotic stressors, suggesting that these substances could be used in production to control these issues. Compared to traditional agrochemicals, the use of vitamins is safer for the environment and human health and is more profitable (Atif et al., 2022; Boubakri, 2020; Singh et al., 2020). Vitamins such as thiamine, riboflavin, and ascorbic acid have been used to control parasitic diseases in plants by inducing innate defense mechanisms (Kheyri and Taheri, 2021; Kheyri et al., 2022; Pérez Mora et al., 2024; Singh et al., 2020). Thiamine, in particular, has been recognized as a plant defense activator and has been successfully used against various pathogens and insects, including fungi, bacteria, viruses, and aphids (Atif et al., 2022; Kheyri et al., 2022; Sathiyabama et al., 2019; Ahn et al., 2007; Torky, 2016; Hamada et al., 2018). However, it is recommended to use these substances only when their effect on each pathosystem is understood, including the mechanisms that determine induced resistance. Therefore, it is necessary to determine the lowest effective dose of the inducer for each pathosystem (Hamada et al., 2018).

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Specifically, in carnation, a previous study evaluated different substances and showed that foliar application of thiamine decreases the incidence and severity of vascular wilt caused by this pathogen in a susceptible cultivar (Pérez Mora et al., 2021). However, it is necessary to confirm if this effect is extrapolated to other susceptible genotypes to suggest its use at the agricultural level. The aim of this study was to deepen the use of thiamine as an inducer of vascular wilting resistance, analyzing the effect of its concentration during the foliar spray and the response of some susceptible carnation cultivars. Therefore, under greenhouse conditions and an appropriate experimental design, monitoring parameters to verify the effectiveness in inducing resistance (e.g. incidence and severity of the disease) and biochemical markers to verify the activation of resistance mechanisms (e.g. the content of total phenols and flavonoids). In general, this approach led us to understand the potential of thiamine as a new inducer of resistance in the control of vascular wilting caused by *Fod* in carnation.

Material and methods

Fungal isolates and plant materials: Certified pathogen-free carnation cuttings (*Dianthus caryophyllus* L.) of four cultivars were used, reported as susceptible to vascular wilting caused by *Fod* (Solex, Mizuki, Brisa, and Voragine). Cuttings were obtained from Florval S.A.S-QFC (Gachancipá, Colombia) and had optimal nutritional conditions with three weeks of rooting.

The inoculum of *Fusarium oxysporum* f. sp. *dianthi* race 2, used in this experiment has previously been isolated from roots of carnation-Mizuki cultivar with typical symptoms of vascular wilting. The pathogen characterization at the species level was verified through PCR amplification to ITS genus-specific region previously describedITS-Fu-f and ITS-Fu-r were designed by comparing the aligned sequences of internal transcribed spacer regions (ITS, and race level was verified through PCR and the transposon differential distribution previously reported (Vanegas-Cano et al., 2022).

Fod inoculum preparation: The fungal isolate was maintained in a solid medium of PDA (potato dextrose agar, Merck) until inoculation. One cm^2 of the medium colonized by the fungus was transferred to liquid medium Czapek-Dox (Fluka) broth, previously sterilized. The resulting suspension remained stirred for five days at 200 rpm, natural photoperiod 12h/12h and 25 °C. The conidial content of the medium was counted in a hemocytometer counting chamber and diluted with sterile water to obtain a suspension of 1.0×10^6 conidia mL^{-1} for immersion inoculation of the carnation roots for 20 s and 1.0×10^3 conidia mL^{-1} for substrate inoculation, for those treatments that will be inoculated with *Fod* (Santos-Rodríguez et al., 2021; Vanegas-Cano et al., 2022).

Effect of thiamine concentration as an inducer of resistance: In a first *in vivo* experiment, rooted cuttings of commercial cultivar Solex were planted in a substrate (black earth: vermiculite in a 75:25 ratio) which previously had been monitored to confirm that the pathogen was not present. The substrate was sterilized at 18 PSI for one hour twice.

The carnation cuttings were kept for 2 weeks in the environmental conditions for acclimatization purposes. After this time, thiamine solutions (Sigma-Aldrich) were applied at concentrations of 0.1 mmol L^{-1}, 1.0 mmol L^{-1}, 10.0 mmol L^{-1} and 50 mmol L^{-1} by spraying the leaves (three applications of 1mL of the respective treatment per plant at 5, 3, and 1 day before inoculation with *Fod*). The treatments were the following: (i) without inducer or inoculum, treated with water (Control); (ii) treatment without inducer inoculated with *Fusarium* (*Fod*); (iii – iv – v - vi) Inductor applied by foliar spray in the 4 working concentrations without the addition of inoculum (B1); (vii – viii – ix - x) Inductor applied by foliar spray at the four working concentrations and subsequently inoculated with *Fod* (B1 + *Fod*). The experimental unit was 10 cuttings with 3 repetitions per treatment.

The inoculation of carnation cuttings with *Fod* was carried out according to the conditions reported by (Romero-Rincón et al., 2021; Vanegas-Cano et al., 2022). All the cuttings (with and without the addition of the inducer) were kept in optimal conditions of humidity (average 60.2%), temperature (average 19.1 °C), photosynthetically active radiation (4.9 mmol m^{-2} s^{-1} on average), and nutrition. Water irrigation was carried out twice a week, maintaining the substrate at field capacity.

Greenhouse experiment: Effect of thiamine as an inducer of resistance in different carnation susceptible cultivars to vascular wilting: Cuttings of the Mizuki, Brisa, and Vorágine carnation cultivars (susceptible to vascular wilt according to personal communication), after three weeks of rooting, were planted in the substrate (black earth: vermiculite, in a ratio of 75:25) previously analyzed to confirm that the pathogen was not present and sterilized at 18 PSI for one hour twice.

The carnation cuttings were kept for two weeks in the environmental conditions of the trial for acclimatization purposes. After this time, a 1.0 mmol L^{-1} thiamine solution (Sigma-Aldrich), a concentration selected according to the results of the previous test, was sprayed on the leaves (three applications of 1mL of the respective treatment per plant at 5, 3, and 1 day before inoculation with *Fod*).

The treatments were the following: (i) Control without inducer or inoculum, treated with water (Control); (ii) treatment without inducer inoculated with *Fusarium* (*Fod*); (iii) Inducer applied by foliar spray without the addition of inoculum (B1); (iv) Inducer applied by foliar spray and inoculated with *Fod* (B1 + *Fod*). The four treatments were applied for each cultivar for a total of 12 treatments in the trial. The experimental unit was 10 cuttings with 3 repetitions per treatment.

The inoculation of carnation cuttings with *Fod* was carried out in accordance with the conditions reported by (Romero-Rincón et al., 2021; Santos-Rodríguez et al., 2021; Vanegas-Cano et al., 2022). All the cuttings (with and without the addition of the inducer) were kept in optimal conditions of humidity (average 59.6%), temperature (average 18.6°C), light, and nutrition. Irrigation with water was carried out twice a week, keeping the substrate at field capacity.

Incidence and severity estimation: For the different treatments, the evaluation of typical vascular wilt symptoms was carried out by visual inspection of the plants during the period from 2 to 8 weeks (Pérez Mora et al., 2021). The incidence was determined by counting the number of plants that have yellowing leaves from the total inoculated plants, expressing this relationship as a percentage. Furthermore, the severity of the disease was estimated for each of the plants in each of the treatments using a scale from 0 to 4, where: 0 = Healthy plant; 1 = Wilting of the lower leaves (1/3 of the plant) (not necessarily due to Fusarium wilt); 2 = Wilting of the lower and middle leaves (2/3 of the plant); 3 = Complete wilting of the plant; 4 = Death of the plant.

The severity of each treatment was evaluated as the area under the disease progress curve (AUDPC) considering the severity scale, according to Equation (1):

$$\boldsymbol{AUDPC} = \sum_{i=1}^{n} \left(\frac{S_i + S_{i+1}}{2}\right) * (t_{i+1} - t_i)$$

where “t” is the time of each reading measured in weeks, “S” is the estimated severity according to the aforementioned scale, and “n” is the number of readings (Pérez Mora et al., 2021, 2024).

Extraction and determination of phenolic compounds and flavonoids: The extraction of phenolic compounds and flavonoids was carried out based on what was described by Pérez et al. (2021). Samples of 20 mg (dry weight) of roots were weighed, macerated using liquid nitrogen, and transferred to an Eppendorf tube, and 1 mL of 80% methanol was added. The mixture was subjected to an ultrasound treatment (42 Hz, 100 W) for 15 minutes. It was centrifuged (12,000 g, 15 min, 4 °C), and the supernatant was stored in the dark at -70 °C for further analysis.

The total content of phenolic compounds (TPC) was measured using the Folin-Ciocalteu method (Martínez-González et al., 2022) with some modifications. The reaction mixture contained 50 mL of the methanol extracts, 100 mL of Folin-Ciocalteu reagent, 100 mL of distilled-deionized H_2O, and 200 mL of distilled-deionized water were added. After 1 h incubation at room temperature (20 °C), the absorbance at 764 nm of the reaction mixture (Thermo Genesys 10 UV spectrophotometer, Madison, Wisconsin, USA). A calibration curve was performed using gallic acid (Sigma ®) as a standard. Total phenol content was reported as mg of gallic acid equivalents per g of dry roots.

The Total Flavonoid content (TFC) was determined using the colorimetric method reported by (Martínez-González et al., 2022) with some modifications. The reaction mixture contained 100 mL of methanol extract, 30 mL of 5% $NaNO_2$ solution, and 100 mL of deionized water. After 5 min 60 mL of 10% $AlCl_3$ solution was added. After incubating at room temperature (approximately 20 °C), for 6 min, 100 mL of 2M NaOH was added and the absorbance at 510nm was measured. A calibration curve was performed using catechin (+) (Sigma ®) as a standard and the results were expressed as mg equivalent of catechin per g of dry roots.

Statistical analysis: The experiments were carried out using three experimental units for each treatment, each of them composed of 10 healthy rooted carnation cuttings. Data were reported as the mean ± standard deviation. Analysis of variance (one-way ANOVA) and significant differences between means (Tukey's test), principal component analysis (PCA), and cluster ranking analysis were performed with the trial version of Minitab software version 19.

Results

Effect of thiamine concentration on the induction of resistance to Fod vascular wilting

The effect of the thiamine concentration on vascular wilting resistance induction was evaluated using 4 concentration levels in the range of 0.1 mmol L^{-1} to 50 mmol L^{-1}. The results showed that all the plants treated with thiamine solutions in the four concentrations, present a better response against the pathogen, considering that the incidence of the disease in these treatments was significantly lower (Fig. 1A). Instead, all the plants treated with the pathogen inoculation (*Fod*), showed vascular wilting symptoms at 8 weeks. In the same sense, the comparison among treatments with different thiamine concentrations does not present significant differences concerning to the absolute control (treatment without inducer or pathogen) (Fig. 1A). It is clarified that control assays show incidence values since observations are being made regarding the presence of yellowing in the leaves. Note that level 1 is classified as "initial but still questionable disease symptoms only (not necessarily due to Fusarium wilt)," a process that may result from normal senescence processes or response to assay conditions.

Consistently, the severity of the disease measured as the AUDPC showed similar results; all the thiamine concentrations used in the experiment caused a decrease in the severity of the disease compared to the inoculated treatment without inducer (*Fod*) (Fig. 1B).

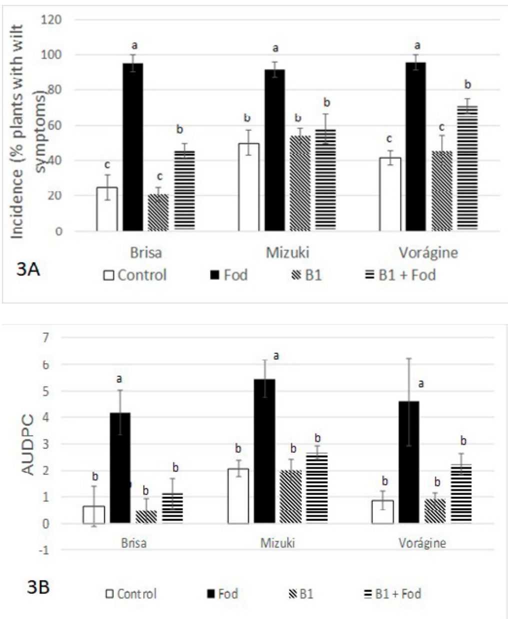

Fig. 1. Incidence and severity of vascular wilt caused by *Fusarium* in carnation: effect of thiamine concentration on resistance induction. 1A. Disease incidence at 8 weeks. 1 B. Disease severity was measured as the AUDPC. Results are presented as the mean ± standard deviation for n=3. Different letters in treatments mean statistically significant differences (p<0.05). Transverse section of the stems from non-inoculated plants on PDA culture medium did not reveal pathogen growth.

In this regard, it is noteworthy that, although the treatments with thiamine and without inoculation with the pathogen were statistically similar in the AUDPC response, the treatments with higher concentrations of thiamine, showed greater variability between replicates for the response variable (*i.e.* AUDPC). These results suggest that, although the resistance induction to the pathogen was similar for the evaluated treatments, lower concentrations of thiamine showed a more consistent response.

The total content of phenolic and flavonoids compounds (TPC and TFC) in carnation roots for the plants obtained from the different treatments were evaluated using colorimetric assays (Table 1). In general, it was found that the thiamine application with a concentration of 1.0 and 50.0 mmol L^{-1}, generated an increase in the total phenolic content (TPC) at root level before to inoculation with *Fod* (0h sample time). Regarding the content of flavonoids (Table 1) (TFC), it was observed a decrease due to the application of the resistance inducer before inoculation with the pathogen. However, when subjecting the plants to the challenge with the pathogen, there was a significant increase in TFC at 12 hours ($p < 0.05$), due to thiamine application at concentrations of 0.1 mmol L^{-1}, 1 mmol L^{-1}, and 10 mmol L^{-1} with and without Fod inoculation compared to the control (Table 1).

To deepen in the biochemical process involved in the thiamine resistance induction, the monitoring parameters and the biochemical measures were analyzed using multivariate analysis. Fig. 2A shows the biplot diagram of principal components analysis (PCA), where principal component 1 (PC1) was positively influenced by the incidence and severity of the disease, explaining 50.1 % of the variance. This component separates the treatment *Fod* (without inducer of resistance and inoculated) from all other treatments, which are more similar to the control treatment. In the same sense, the cluster hierarchization graph (Fig. 2B) classifies the treatments into 4 groups, clearly separating the treatment *Fod* from those treatments where some amount of thiamine was added. (Fig.s 2B-D). In addition, it was found that the lowest thiamine concentrations, 0.1 mmol L^{-1} and 1.0 mmol L^{-1}, were grouped in the same cluster with the control treatment, with and without the addition of inoculum (Fig. 2B-A), which suggests that lower concentrations of thiamine had the greater effect on the induction of resistance.

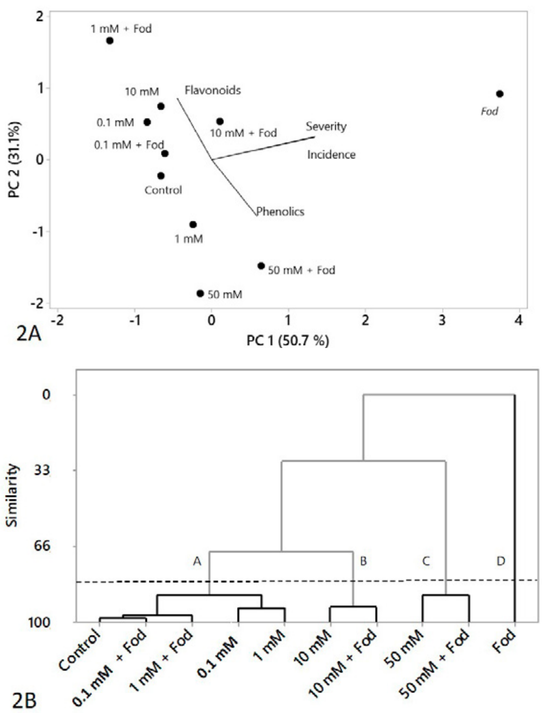

Fig. 2. Multivariate analysis of the effect of thiamine concentration on the induction of resistance to vascular wilt caused by *Fod* in carnation. 2A. Double projection diagram of principal component analysis. 2B. Dendrogram of the analysis of hierarchy by clusters. (mM: mmol*L^{-1}, *Fod*: treatments with an application of the inoculum of *Fusarium oxysporum f. sp. dianthi*)

Table 1. Content of Total Phenolic Compounds (TPC) and flavonoids (TFC) in carnation roots of the Solex cultivar due to the effect of the addition of thiamine in different concentrations and challenges with the pathogen *Fod*. Results are presented as the mean ± standard deviation for n=3. In the same column, different letters mean statistically significant differences between treatments ($p<0.05$).

	Phenolic content (mg gallic acid/g dry root)		Flavonoid content (mg catechin/g dry root)	
Treatment	**0 h**	**12h**	**0 h**	**12h**
Control	13.7 ± 0.7[b]	13.8 ± 0.1[c]	0.540 ± 0.067[a]	0.394 ± 0.017[c]
Fod		15.3 ± 0.2[b]		0.422 ± 0.046[bc]
0,1 mmol L^{-1}	12.3 ± 0.8[b]	15.1 ± 0.6[ab]	0.315 ± 0.097[b]	0.605 ± 0.086[a]
0,1 mmol L^{-1} + *Fod*		14.4 ± 1.0[ab]		0.480 ± 0.015[b]
1 mmol L^{-1}	15.4 ± 0.4[a]	16.6 ± 0.5[a]	0.368 ± 0.083[b]	0.476 ± 0.018[b]
1 mmol L^{-1} + *Fod*		12.3 ± 0.2[d]		0.587 ± 0.060[a]
10 mmol L^{-1}	13.7 ± 1.2[ab]	13.9 ± 0.6[c]	0.252 ± 0.096[b]	0.535 ± 0.057[a]
10 mmol L^{-1} + *Fod*		15.3 ± 0.6[ab]		0.567 ± 0.099[a]
50 mmol L^{-1}	15.6 ± 1.2[a]	15.3 ± 1.2[a]	0.155 ± 0.022[c]	0.243 ± 0.099[d]
50 mmol L^{-1} + *Fod*		17.4 ± 3.0[a]		0.403 ± 0.041[c]

Effect of the thiamine foliar spray on the resistance induction to Fod in some susceptible carnation cultivars: This work also demonstrated that thiamine applied by spray on carnation leaves induces resistance against *Fod* in some cultivars reported as susceptible in commercial production conditions. Fig. 3A shows the effect of the application of thiamine at a concentration of 1 mmol L^{-1}, on the incidence of the disease reported as the percentage of plants with symptoms. The results reveal a significant decrease ($p < 0.05$) in wilt incidence across all three evaluated carnation cultivars following this treatment.

Fig. 3B shows the results of vascular wilting severity measured as AUDPC during this *in vivo* assay. The treatments inoculated without the thiamine spray (*Fod*), for the three cultivars did not present significant differences ($p < 0.05$), which suggests a similar level of resistance to the disease for these three genotypes, as had been previously reported for the commercial conditions (Personal communication). On the other hand, the significant delay of the disease caused by *Fod* in the treatment (B1 + *Fod*) compared to the inoculated treatments (*Fod*), confirmed the role of the thiamine at 1 mmol L^{-1} as a resistance inducer. These results were consistent with the incidence of the disease at 8 weeks after inoculation (Fig. 3A).

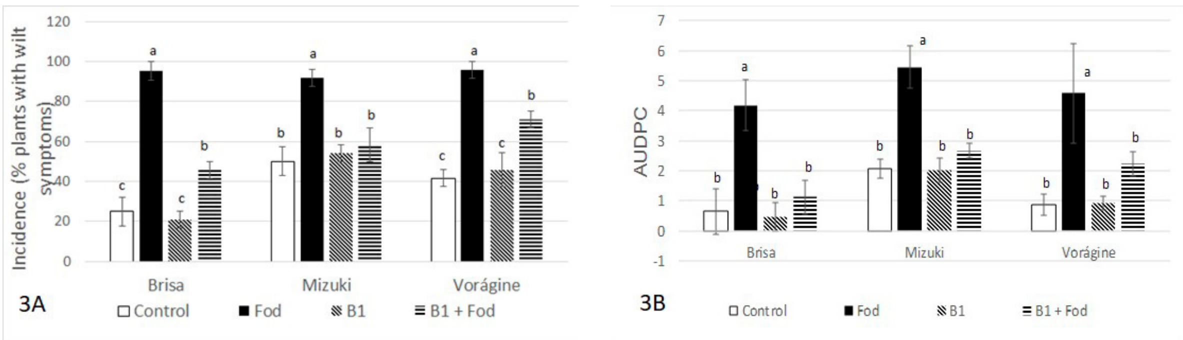

Fig. 3. Incidence and severity of vascular wilt caused by *Fusarium* in susceptible carnation cultivars Brisa, Mizuki, and Voragine. 3A. Percentage of incidence of the disease at 8 weeks. B. Disease severity is measured as the AUDPC. Results are presented as the mean ± standard deviation for n=3. Different letters in treatments mean statistically significant differences ($p<0.05$). (B1: Thiamine). Transverse section of the stems from non-inoculated plants on PDA culture medium did not reveal pathogen growth.

The effect of the thiamine foliar spray in the AUDPC was statistically similar to the controls in the Brisa and Mizuki cultivars, confirming the positive effect of thiamine application. However, the controls in this last cultivar present an unexpectedly high severity, which suggests that the Mizuki cultivar is more sensitive to the specific conditions of the *in vivo* test. Although the Voragine cultivar presented less severity due to the addition of thiamine when compared with the other cultivars, it was found a statistical increase in the severity for the treatment (B1 + *Fod*) comparing to the control treatment and the treatment with the application of thiamine without inoculation (B1).

The total content of phenolic and flavonoid compounds (TPC and TFC) at the root level was evaluated both at the constitutive level and after pathogen inoculation, for the cultivars Mizuki, Brisa, and Voragine. The TPC increases significantly in plants treated with thiamine spray before inoculation (0h), as well as TFC, except for the carnation Voragine cultivar (Table 2). Although, 12 h after inoculation with *Fod* the results were more variable depending on the cultivar. Significant increases in TPC are observed in treatments B1 and B1 + Fod for cultivars Mizuki and Voragine compared to the control and the Fod-inoculated treatment. While, thiamine application (B1 treatment) cause a significant increase in TFC contents, which may suggest a priming process in cultivars Mizuki and Voragine, and the treatment B1 + *Fod* cause a significant increase in TFC contents in cultivars Brisa and Voragine compared with *Fod*-inoculated treatment. These results could be related to the improved response of plants against the pathogen, considering that TPC and TFC are biomarkers of response in cultivars resistant to *Fod*.

The multivariate analysis outlined the most important effects of thiamine foliar spray in the cultivars under investigation (Fig. 4). The Principal Component Analysis PCA (Fig. 4A) showed two principal components explaining 84.5% of the variance.

Table 2. Content of phenolic compounds and flavonoids in carnation roots due to the effect of the addition of thiamine and challenges with the pathogen in different susceptible cultivars of carnation. (B1: thiamine). Results are presented as the mean ± standard deviation for n=3. In the same column and for each cultivar, different letters mean statistically significant differences between treatments (p<0.05).

		Phenolic content (mg gallic acid/g dry root)		Flavonoid content (mg catechin/g dry root)	
Cultivar	**Treatment**	**0 h**	**12h**	**0 h**	**12h**
Brisa	Control	11.6 ± 0.5^{b}	11.6 ± 0.4^{b}	0.44 ± 0.05^{b}	0.36 ± 0.06^{ab}
	Fod		13.9 ± 1.4^{a}		0.36 ± 0.02^{b}
	B1	12.7 ± 0.4^{a}	10.1 ± 0.2^{c}	0.53 ± 0.03 ^{a}	0.32 ± 0.05^{b}
	B1 + *Fod*		10.9 ± 0.7^{b}		0.41 ± 0.03^{a}
Mizuki	Control	8.2 ± 0.9^{b}	7.1 ± 0.6^{d}	0.28 ± 0.03^{b}	0.30 ± 0.07^{c}
	Fod		9.1 ± 0.2^{c}		0.54 ± 0.03^{b}
	B1	11.3 ± 1.0^{a}	13.8 ± 0.7^{a}	0.41 ± 0.06^{a}	0.74 ± 0.11^{a}
	B1 + *Fod*		10.0 ± 0.9^{b}		0.51 ± 0.03^{b}
Vorágine	Control	6.3 ± 0.3^{b}	5.5 ± 0.7^{c}	0.50 ± 0.02^{a}	0.34 ± 0.01^{b}
	Fod		6.4 ± 0.2^{c}		0.36 ± 0.04^{b}
	B1	7.0 ± 0.2^{a}	7.4 ± 0.4^{b}	0.41 ± 0.04^{b}	0.47 ± 0.05^{a}
	B1 + *Fod*		9.4 ± 0.3^{a}		0.49 ± 0.04^{a}

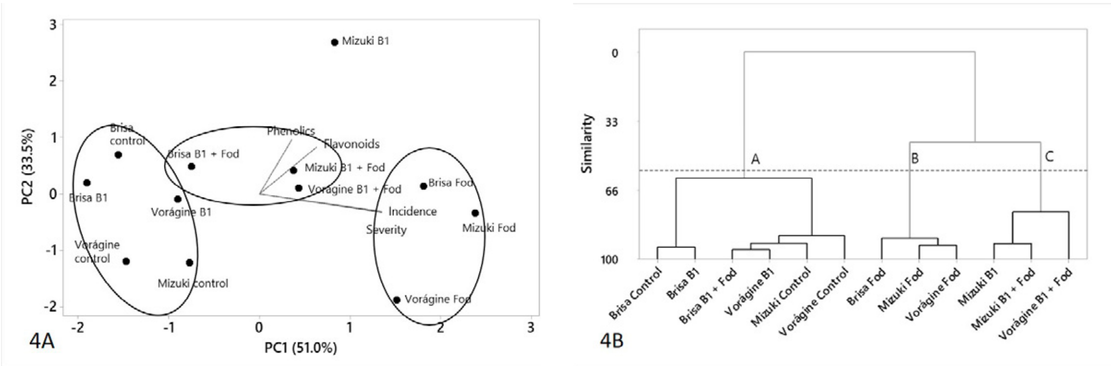

Fig. 4. Multivariate analysis of the effect of thiamine addition on the induction of resistance to vascular wilt caused by *Fod* in susceptible carnation cultivars Brisa, Mizuki, and Voragine. 4A. Double projection diagram of principal component analysis. 4B. Dendrogram of the analysis of hierarchy by clusters. (*Fod*: treatments with the application of *Fusarium* inoculum, B1: treatments with the application of thiamine).

The first component (51.0%) positively influenced by the incidence and severity measurements, separates the non-inoculated treatments (control and B1) from the inoculated treatments (B1 + *Fod* and *Fod*). In the same sense, the treatments inoculated with *Fod* are separated by the first component, showing a positive effect by the application of thiamine. These results are confirmed by the analysis of hierarchy by clusters where the same classification was made for this statistical tool.

Discussion

The phenomenon called Induced Resistance is based on the stimulation of the plant biochemical defense mechanisms previous to pathogen infection (Salwan et al., 2023). The use of natural compounds as inducers of these processes is an alternative to the use of chemical pesticides in the control of plant diseases, due to their environmental safety and harmless effects on health (Boubakri, 2020; Devi et al., 2020; Jia et al., 2020; Singh et al., 2020)bacterial and viral pathogens or any pests. The apprehension has been [a]ppeared, and the system intensifies a resistance to the attack. In this research, efficacy of eight IR chemicals along with standard fungicide hexaconazole was evaluated against French bean rust with reference to alteration in biochemical constituents present in the plant under pot conditions. Minimum disease severity was recorded when plants were challenge inoculated after 20 days of treatment of IR chemicals. Minimum disease severity (8.50 %. In this context, some investigations have deepened in the use of thiamine as a resistance inducer in different plant-pathogen interaction systems (Hamada et al., 2018; Kheyri et al., 2022; Pérez Mora et al., 2021, 2024; Yin et al., 2012)Rhizoctonia solani AG-1A, of rice and compared with that of systemic fungicide, carbendazim (BCM. Specifically, in carnation, it was investigated effects of applying salicylic acid, ascorbic acid, potassium phosphite, and thiamine by spray and drench to induce resistance, and found all four inducers to be effective. Thiamine was identified as the most promising inducer (Pérez Mora et al., 2021) However, further research is needed to better understand the mechanisms behind thiamine's effects and to optimize its use in agricultural production.

An interesting finding in this paper is that the different concentrations of thiamine applied significantly reduced the effects of Fod inoculation. Thiamine has been used in different concentrations as a resistance inducer, showing significant effects in inducing resistance to different diseases. In *Capsicum annuum* plants against *Tobacco mosaic virus*, treated with thiamine in a concentration range between 0.25 mmol L^{-1} and 15 mmol L^{-1}, it was found that the inducing effect increased and reached its maximum at 4 mmol L^{-1} and that higher concentrations did not increase the effect (Torky, 2016). In the same sense, Arabidopsis plants sprayed with thiamine in the range of 1 mmol L^{-1} to 50 mmol L^{-1} showed protection against a bacterial disease from a concentration of 5 mmol L^{-1}, reaching the maximum protection at a concentration of 10 mmol L^{-1} (Ahn et al., 2007)leading to rapid counterattack against pathogen invasion and perturbation of disease progress. Priming reduces the metabolic cost required for constitutive expression of acquired resistance. To investigate the effects of priming by thiamine on defense-related responses, Arabidopsis (Arabidopsis thaliana. In the case of the present study, this maximum protection was not evident since the concentrations evaluated in the range of 0.1 mmol L^{-1} and 50 mmol L^{-1} presented the same effect of decreasing the incidence and severity of the disease caused by *Fod*, which suggest that working at lower concentrations of thiamine is equally efficient than working at high concentrations. To continue with this study, the subsequent experiments were carried out using the lowest concentration evaluated corresponding to 1 mmol L^{-1}.

The effect of a potential resistance inducer in different susceptible genotypes, confirms its role in each pathosystem. For example, a study evaluated the effect of the thiamine application on susceptible and partially resistant cultivars of *Brassica napus*, to the pathogen *Sclerotinia.* In this study the induction of resistance is based on a biochemical process involving antioxidant enzymes activated in both genotypes, proposing a conserved process for thiamine action (Zhou et al., 2013). In our study, the results suggest that the thiamine foliar spray has a positive effect on all the cultivars investigated and could be applied to other susceptible genotypes. It is necessary to carry out more biochemical and molecular studies to determine if it is possible the conservation these strategies used for the thiamine in all the carnation genotypes. However, it is interesting the role of the thiamine-inducing antioxidant process in *B. napus*, because in carnation, it could be similar considering the central role of flavonoid compounds (flavonol glycosides), in regulating the antioxidant process in carnation roots (Romero-Rincón et al., 2021).

According to the results of this study, it is shown that the addition of thiamine 1 mmol L^{-1} modulates the content of phenolic compounds in carnation roots in all cultivars analyzed before inoculation. This can be associated with priming phenomena that stimulate plant defense mechanisms, enabling an enhanced response to pathogen attacks (Jia et al., 2020; Pérez Mora et al., 2024). More of the phenolic compounds in plants are natural antioxidants acting as reducing agents, and free radical scavengers (reducing the content of reactive oxygen species) (Dumanović et al., 2021). In particular, it has been reported that the flavonoid family of compounds can inhibit the development of diseases in plants by mechanisms that include the inhibition of extracellular fungal enzymes necessary for the success of the pathogen, inhibition of fungal oxidative phosphorylation and its antioxidant activity, acting as phytoalexins (Bahuguna et al., 2012; Yong-Hong et al., 2017).

Increases in the content of phenolic compounds have been reported for different plant-pathogen interactions, which are related to the phenylpropanoid pathway (Bahuguna et al., 2012; Boubakri et al., 2013; Torky, 2016). Studies in grapes against the pathogenic fungus *Plasmopara viticola* showed that the application of thiamine positively modulates the phenylpropanoid pathway, where phenolic compounds are generated, increasing the expression of key genes in the biosynthetic pathway such as PAL (Phenylalanine Ammonium Lyase) (Boubakri et al., 2013). Similar results are reported at the protein level for PAL, the first pathway of the phenylpropanoid pathway, and at the level of metabolites (increased content of phenolic compounds), by the addition of thiamine in melon fruits against *Trichothecium roseum* and *Alternaria alternata* (Yong-Hong et al., 2017).

The results for the contents of flavonoid compounds in our investigation show increases that can be compared with those reported for Asian pear against *A. alternata* (Yin et al., 2012), and for melon against *T. roseum* and *A. alternata* (Yong-Hong et al., 2017). Phenolic compounds and flavonoids are considered molecular markers of disease resistance in other systems due to their possible role as phytoalexins and their antioxidant capacity (Boubakri et al., 2013). Considering that in the carnation-*Fusarium oxysporum* system these compounds have been associated with disease resistance (Santos-Rodríguez et al., 2021), the increase in the content of these compounds due to the challenge with the pathogen is an expected response in priming processes (Madany et al., 2020). The results obtained suggest that the addition of thiamine as an inducer has the expected effect of preparing the plant for interaction with the pathogen.

Conclusions

In this study, it was found that thiamine in a concentration range between 0.1 mmol L^{-1} and 50 mmol L^{-1}, decreases the incidence and severity of vascular wilt caused by *Fod* in carnations and lower concentrations are suggested for next studies, considering the cost-benefit ratio and the lower variability in the response. In addition, it was found that the application of thiamine at a concentration of 1 mmol L^{-1} generated a resistance induction response in different cultivars of carnation, which suggests this compound, under working conditions, is a potential inducing agent of resistance to vascular wilting. It is necessary to deepen the study of the biochemical and molecular mechanisms on which this induction of resistance is based, to have tools that allow us to understand the process and finally apply it in production systems.

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Author contribution

All authors contributed to the study´s conception and design. Material preparation, data collection, analysis, and manuscript preparation were performed by **WPM:** All authors commented and analyzed data on previous versions. Project administration. **HAD:** funding acquisition. **HAD:** and **LMM**. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that they have no potential conflict of interest in the submitted work.

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