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RESEARCH ARTICLE

Enhanced defense responses in *Pythium ultimum*-challenged cucumber plants induced by potassium phosphite

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ABSTRACT: *Pythium ultimum* is one of the major causative agents responsible for damping off disease in cucumber plants. In the present study, the effect of potassium phosphite (KPhi) on defense response of *P. ultimum*-inoculated cucumber plants was investigated. Different plant growth parameters as well as chlorophyll a content were studied to evaluate the healing effects of KPhi. Furthermore, the expression pattern changes of a pathogenesis-related chitinase gene was analyzed via qPCR. Results revealed that KPhi treatment significantly increased growth parameters i.e. shoot length, diameter and mean leaf number in cucumber seedlings. KPhi treatment at 1 and 4 gL^{-1} caused 31.37% and 94.48% increase in shoot diameter respectively compared to control plants while shoot length of plant treated with 1 and 4 gL^{-1} KPhi were increased 72.14% and 78.85%, respectively compared to control plants. The chlorophyll a content as well as plant leaf number was significantly increased in plants treated with 1 or 4 gL^{-1} KPhi compared to control plants. It was interestingly revealed that KPhi application decreased Chitinase gene expression compared to control plants. The findings of the present study would be implemented for designing a controlling strategy to decrease the adverse effect of *P. ultimum* on cucumber plants.

KEYWORDS: *Pythium ultimum*, potassium phosphite, chlorophyll a, chitinase, qPCR.

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is one of the most important and widely grown vegetables in the world and is also consumed as a fruit in Iran. Several bacterial and fungal pathogens attack cucumber cultures in various environmental conditions and cause a considerable yield loss (14). Damping off is one of the most devastating cucumber diseases causing considerable economic losses to cucumber culture worldwide. Pythium species are one of the Oomycetes responsible for cucumber damping off which cause severe root and crown rot resulting in wilting and death of infected plants (1).

Different strategies have been implemented to control the damages caused by plant pathogens e.g., cultural practices, development of resistant cultivars, transgenic plants, crop rotation and chemical fungicides of which fungicide application is one the most applied ways to control infection (6, 2, 26, 7). Frequent application of the chemical fungicides with specific modes of action leads to development of resistant strains in fungal population. On the other hand, fungicides not only increase production costs but also would be a real threat to public health and environment (20).

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Exploitation of the natural immune system of the plants is one of the most reliable strategies without negative effects of other controlling methodologies (8). During the evolution process, plants developed various defense mechanisms to inhibit pathogen growth (29). Among them, production antifungal and antimicrobial metabolites (26) extensive physio-biochemical modifications (34), overexpression of pathogenesis-related (PR) genes (5), extensive tissue lignification (4) and induced systemic resistance (ISR) (12) have been subject of several research projects.

The response of the plants against attacking organisms is a complicated process and involves expression of a set of transcription factors and genes encoding different proteins that ultimately induce biochemical and physiological modifications in plants (35). similar to pathogen attack, some biological and chemical agents prime SAR in plants and the mechanism of these reactions have been the subject of numerous studies (25). Chemical inducers are of great attention due to some of their intrinsic features like ease of use, lower price and higher availability.

Phosphites, the alkali salts of phosphorous acid, are generally known to control plant diseases through SAR induction (39). Potassium phosphite (KPhi), the most prevalent form of phosphite salts, is formulated both as fertilizer and as the activator of plant defense system and has been widely used in the world (40, 21, 20). During the two past decades, several studies have been conducted to elucidate the efficiency of KPhi for controlling diseases in various plant species. Phosphite salts have been successfully used for controlling various disease caused by different fungal pathogens including but not limited to Phytophthora spp. (15, 20, 14), Fusarium spp. (10), *Rhizoctonia* spp. (18), *Pseudoperonospora* spp. (37, 33) and *Sphaerotheca* spp. (23).

Ramezani et al. (2018) reported that KPhi triggers disease resistance via enhanced production of antioxidant agents, expression of PR proteins and an increased accumulation of soluble proteins and phytoalexins. It has been also suggested that KPhi improves plant vigor through enhanced root growth and activation of plant defense machinery leading to overproduction of defense enzymes, antioxidants and secondary metabolites after plants were infected with pathogen (27, 22, 32).

Despite of numerous reports of successful application of potassium phosphite for controlling plant diseases, the exact mode of action and the mechanisms underlying the conferred resistance is not fully understood. In the current research, we comprehensively investigated the alterations in KPhi-treated cucumber plants defense machinery upon infection with *Pythium ultimum*. We analyzed changes in plant growth parameters as well as chlorophyll accumulation as indicators of pathogen damage. The expression pattern of an acidic chitinase gene was also investigated to determine the plant defense response against pathogen attack. The main objective of this study was to provide an insight into the mechanisms by which KPhi reduces disease damage in plants.

MATERIALS AND METHODS

plant material

The present study was carried out at the greenhouse and laboratories of Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari Agricultural Sciences and Natural Resources University. Seeds of cucumber, local variety of Isfahan, were used in this research. Seeds were immersed in 70% ethanol for two minutes and were washed with sterile water and planted in a pot filled with sterilized soil mixture (equal volumes of peat, perlite and cocopeat). The pots were kept in a greenhouse with 16/8 h light/darkness photoperiod at a temperature of 24-27 °C. The plants were regularly fertilized with Hoagland solution (11). The experiment was carried out in the form of a factorial design including three treatments and three replications in a pot.

Potassium phosphite preparation and plant treatment

Potassium phosphite (KPhi) stock solution was prepared as described previously (22). Briefly, phosphorous acid (AppliChem, Darmstadt, Germany) was partially neutralized with potassium hydroxide and the pH was adjusted to 6.3. Treatments of the plants was performed with 2 concentrations of potassium phosphite (1 and 4 grL^{-1}). With emergence of the second true leaf, potassium phosphite was applied to plants as foliar spray, while control plants were sprayed with distilled water.

Pythium cultivation, inoculum preparation and plant inoculation

Five days after KPhi treatment, plants were infected with *Pythium ultimum*. The pathogen was isolated from

infected cucumber plants was kindly provided by Agricultural and Natural Resources Research Center of Tehran. For the preparation of inoculum, the method of Ramamoorthy et al. (2002) was used with a few modifications (31). In this method, 200 cm^3 of garden soil, 200 cm^3 of washed sand, 40 gr of corn flour and 80 ml of distilled water were well mixed in a 1000 ml Erlenmeyer flask and were autoclaved at 121 °C twice for 30 minutes. The contents of four *P. ultimum* culture plates on PDA medium were dispersed in distilled sterile water and were added to Erlenmeyer and kept at 27 ± 2 ° C for 3 weeks. Then the Erlenmeyer content was mixed with the soil of the pots at a final ration of 5% as inoculum.

Study of Morphological Parameters

For assessing morphological alteration, the stems height and diameter were measured with ruler and digital calliper respectively. The disease severity in plants was inspected daily.

chlorophyll a measurement

To measure the amount of chlorophyll a, 6 1 cm pieces were removed from cucumber leaves and immersed in 8 ml of methanol and placed in the dark and at room temperature and Chlorophyll a was extracted after 24 hours. The absorbance of the desired solution was read by spectrophotometer at wavelengths of 662 nm (E665) and 652 nm (E652). These absorbance numbers were entered into the formula, [Ch.a] = (16/29.E665/2) - (8/54.E652), and the chlorophylls a were calculated in units of micrograms per milliliter (17).

RNA extraction and cDNA synthesis

About 100 mg of fresh leaf tissues were finely ground in liquid nitrogen and total RNA was extracted using RNAxplus solution (CinnaGen Inc, Iran) and were then treated with DNase I to eliminate residual genomic DNA contaminants. RNA concentration was
determined by measuring the absorbance at 260 nm and its intensity was visualized in 1% agarose gels. The RNA samples (2 µg) were used for first-strand cDNA synthesis using oligo (dT) primers, 10 mM dNTPs, and reverse transcriptase according to the manufacturer's instructions (Thermo Scientific, USA).

Gene expression analysis

Gene expression analysis was performed using Quantitative Real-Time PCR (qPCR) technique. Gene-specific primers for an acidic chitinase gene were picked using online Primer3 software. Actin gene of cucumber was used as an internal control (Table 1). The qPCR reactions were performed using Maxima SYBR Green/ROX qPCR Master Mix (2X) kit (Thermo Scientific) in a BIO-RAD real-time PCR machine (CFX96™ Touch Real-Time PCR Detection System) according to manufacturer's instructions. The reactions were performed as following: 3 min at 95 °C, denaturation at 95 °C for 25 s, annealing at 60 °C for 20 s and extension at 72 °C for 25 s for 40 cycles. Gene expression rate was measured using $2^{-\Delta\Delta Ct}$ method (18). The experiments were performed in three replications and the expressions of the studied genes were finally drawn up by Excel software.

Table 1. Name and sequences of the primers used for qPCR analysis of KPhi-treated cucumber plants inoculated with *P. ultimum.*

primer	Sequence (5´ to 3´)
Chitinase F	GCGGTTTTGGAGGCGTTGAT
Chitinase R	GTCTAGGTGAGCGTCTGGTA
Actin F	GATTCTGGTGATGGTGTGAGTC
Actin R	TCGGCAGTGGTGGTGAACAT

Data analysis

Statistical analysis of data was performed using SAS software and the charts were drawn using Excel software. To compare the mean of data, the Duncan multi-domain test was used at the probability level of 1%.

RESULTS

Effect of KPhi on Growth Factors

As shown in Fig. 1, treatment with Potassium phosphite (KPhi) increased the height and the diameter of the cucumber plants inoculated with *P. ultimum.* The highest stem growth was observed for KPhi treatment at a concentration of 4 grL^{-1} (78.85% increase compared to control) while a 72.14% increase was observed in plants treated with 1 grL^{-1} of KPhi.

In general, the height of cucumber plants treated with KPhi and inoculated with *P. ultimum* was higher than that of control plants. The control plants which were inoculated with pathogen, exhibited the lowest growth rate, indicating the adverse effect of fungi in absence of

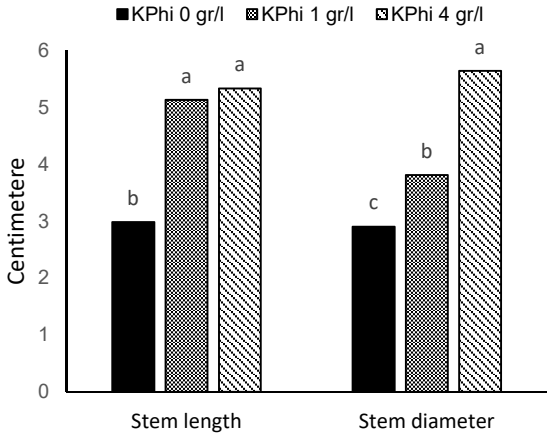

Fig 1. Effect of potassium phosphite on length and stem diameter of cucumber plants inoculated with *P. ultimum*.

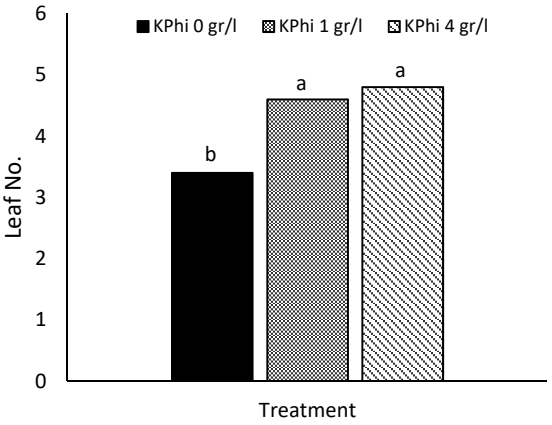

Fig 2. Effect of different concentrations of Potassium Phosphite on leaf number in cucumber plants inoculated with *P. ultimum*.

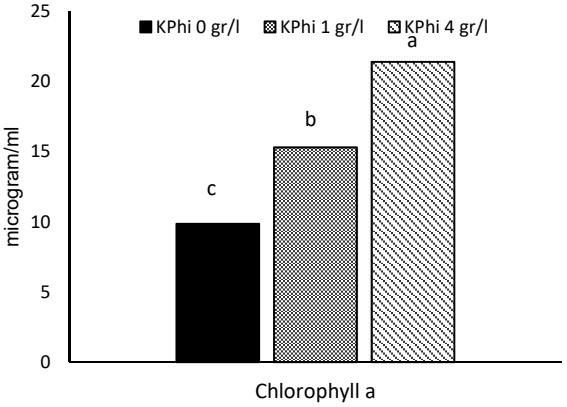

Fig 3. Effect of different concentrations of potassium phosphite on the content of chlorophyll a in cucumber leaves infected with *P. ultimum*. There was a quite direct relationship between KPhi concentration and chlorophyll a contents.

KPhi treatment. Fig. 1, shows the stem diameter in the cucumber plant treated with various concentrations of potassium phosphite under the stress of *P. ultimum*.

The results showed that the highest stem diameter was observed in KPhi 4 grL^{-1} (94.48% increase compared to control), which has a statistically significant difference with other treatments. Then KPhi 1 grL^{-1} treatment with an increase of 31.37% increase compared to control while the plants treated with distilled water as control exhibited the lowest stem diameter compared to other treatments. According to Fig. 2, the number of leaves in different concentrations of KPhi was higher than those that were solely inoculated with *P. ultimum*.

Effect of KPhi on chlorophyll a. content

Figure 3 shows the chlorophyll a content in the leaves of cucumber plants treated with various concentrations of KPhi before being inoculated with *P. ultimum*. As it can be observed, chlorophyll a content in plants treated with 4 grL^{-1} KPhi 4 showed a 60.78% increase compared to control plants and other treatments.

The effect of KPhi on chitinase gene expression

Gene expression analysis indicates an incremental trend in expression of chitinase gene (Fig. 4). The highest expression of the chitinase gene was observed in the two treatments of KPhi1 and KPhi0, 24 hours after inoculation. In the case of KPhi4, the maximum expression was achieved 12 hours after inoculation. Among these treatments, the chitinase gene expression in KPhi0 treatment (control plants) was 2.37 times higher than that of KPhi1 and 5.34 times higher than that of KPhi4. The lowest level of chitinase gene expression was observed in KPhi1 treatment.

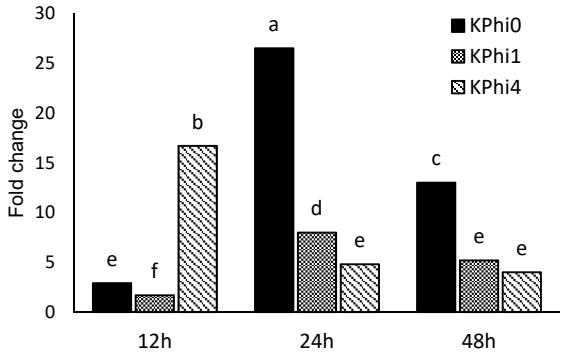

Fig 4. Effect of different concentrations of potassium phosphite on chitinase gene expression in cucumber plants infected with *P. ultimum*.

DISCUSSION

In this study, the effect of two concentrations of potassium phosphite (KPhi) on *P. ultimum*-treated cucumber was investigated and three morphophysiological traits, including stem height and diameter, leaf number, chlorophyll a and the expression of chitinase class III, were analyzed.

From 3 decades ago, it was found that phosphorous acid salts these possess significant quantitative and qualitative effects on agronomic traits such as growth rate, non-biological stress tolerance and product quality (3). Reduced growth rate is one of the clearest consequences of fungal invasion which in turn causes other negative effects. The results of this study showed that the cucumber plants treated with KPhi exhibited quite normal growth rate when they were inoculated with *P. ultimum*.

While the foliar parts of the control plants were inoculation with *P. ultimum*, they exhibited a significant decrease in growth. Generally, plants which were treated with KPhi showed increased height and stem diameter compared to plants which were solely inoculated with *P. ultimum*. By introducing various KPhi compounds in the mid-1990s, researchers found that these compounds produced significant quantitative and qualitative effects on crop products, including increased wet and dry weight, enhanced root growth and fruit size, premature aging, and improved postharvest life (19).

The effects of phosphite salts or phosphonates on plant growth have also been studied by other researchers. The findings of Abbasi and Lazarovits (2006) on the effects of phosphite treatment in cucumber seeds inoculated with *P. ultimum* were similar to those of the present study. They showed that phosphorous acid-treated plantlets had significantly higher growth rates and higher fresh weight compared to control plants (1).

A study by Avila et al., (2011) showed that phosphite inhibits the absorption of phosphate in maize, regardless of the plant's phosphorus status (3). On the other hand, Jackson et al. (2000) reported a significant decrease in the root growth of the eucalyptus plants, which was more significant at the initial growth stage. They suggested that this initial decline may be due to decreased root growth, which may reduce the sensitivity of the root to attacking the pathogens (12). Puerari et al., (2015) examined manganese phosphite for controlling root nematode rootstocks in resistant and susceptible maize cultivars. They reported that manganese phosphite does not affect plant growth and development (30).

In a study by Manna et al. (2015), increasing levels of phosphite resulted in a significant reduction in stem and root mass in rice seedlings compared with control plants (21). Other researchers previously reported the negative effects of phosphite on plant growth, in which phosphite was used as a source of phosphorus rather than phosphate ions. For example, in the case of leaves and roots, phosphate had negative growth effects in plants that were severely deficient in phosphorus, and the stem dry weight and root growth were significantly decreased (39). Another study showed that the rate of root development in *Lupinus angustifolius* inoculated with *P. cinnamomi* treated with phosphate was significantly reduced (8). These findings were in contrast with our results, which led to increased height and higher stem diameter in cucumber plants treated with potassium phosphite and inoculated with *P. ultimum*.

This argument can be attributed to the fact that in our study the increase in the length and diameter of the stem is not due to increased plant growth, but there can be a positive result of reduced disease damage which maintained the normal growth of the plant.

Varadarajan et al., (2002) examined KPhi in the presence and absence of phosphate ions. Results showed that phosphate-treated tomatoes exhibited increased growth rates. Furthermore, root to stem ratio was higher in plants treated with potassium phosphite and phosphate compared to control plants (41). Another study on phosphorous acid as a fertilizer showed that when the plant was treated with it, it had the highest vegetative growth that was consistent with the present study. In this study, there was a significant difference between treatments using potassium phosphate and treatments that were only inoculated with *P. ultimum* (36).

Antifungal and antibacterial effects of phosphorous acid salts is the most important feature of these compounds in Agriculture. It has been proven that these compounds can increase the resistance of plants to biological stresses directly with antimicrobial activity and indirectly by activating the plant's defense system (20, 35). Various biochemical compounds have been identified in plant species that interact with pathogens in the direct or indirect reaction of the plant. Pathogenesis-related proteins such as chitinase, defensin, etc. are the most important compounds that are produced in plants and play a key role in protecting the plant against pathogens.

These compounds may either be present in tissues or be produced in plants upon pathogen attack (13).

The use of phosphite compounds that are environmentally compatible and can increase resistance to plant diseases, have been also used to improve the performance and quality of agricultural products. In a study, the effect of KPhi was investigated as a pretreatment prior to ultraviolet stress on apple trees. The use of KPhi showed a positive effect on two photosynthetic parameters, including leaf chlorophyll content and expression of psbA gene (28). In another study, different salts of phosphite were used on apple plants. These salts included: copper phosphite, zinc phosphite, silicon phosphite, potassium phosphite and calcium phosphite. The results indicated that all phosphite compounds could increase the content of leaf chlorophyll and fruit yield at 5% probability (9). In our study, fungal stress without using KPhi treatment, reduced leaf chlorophyll content in cucumber plants, while with the use of KPhi, this damage was minimized.
Zhang et al. (2011) found that phosphite salts can increase the number of cells and increase the amount of chlorophyll a until there is sufficient phosphate content in the plant. Analyzing the maximum performance of photocysteine II revealed that phosphite stimulates the process of photosynthesis in plant cells (42). In a study by Moor et al. (2009), another comparison was made between phosphate (phosphoric acid salts) and phosphite (phosphorus acid salts) compounds. The results showed that the content of ascorbic acid, anthocyanins and chlorophyll was increased by foliar phosphite application. Also, it was revealed that phosphate leaf foliar application could stimulate the defense mechanisms of the plant (24).

Chitinases are among the most important types of pathogenesis-related proteins (PRP) which in vitro, show the highest in vitro antifungal activities compared to other PRPs and play a very important role in plant resistance to fungal pathogens (13). The expression of chitinase gene in two ways (direct and indirect) causes resistance to fungal diseases. In the direct method, chitinase enzymes control the growth of chitin and disturb the wall synthesis of fungal cells, or in other words, it can decompose the structure of the fungus wall. On the other hand, indirectly by stimulating the plant's defense system, it stimulates the defense mechanism in the plant and produces other defense compounds leading to increased resistance.

Sood et al., (2013) reported high expression of chitinase gene in salicylic acid treated rice plants during their studies. These pretreated plants showed a high expression rate of chitinase gene after contamination with *Rhizoctonia solani* (38). Chitinase gene was induced in pumpkin in response to fungal residues and chitin oligomers. The maximum gene expression was observed within one hour after treatment with fungal residues and its transcripts disappeared within 6 hours. While this gene was induced 3 hours after chitin treatment, its expression gradually decreased over the next 24 hours (15).
In a study, the effect of KPhi was investigated as pretreatment prior to ultraviolet stress on apple trees. The use of KPhi increased the accumulation of glucanase and chitinase enzymes indicating a general defense role for chitinase and glucanase enzymes (28). These results, similar to our findings, showed the effect of phosphite salts on overexpression of PRP genes.
In the present study, it was also observed that KPhi increased the chitinase gene expression level. The highest expression of the chitinase gene was observed in the two treatments of KPhi1 and KPhi0, 24 hours after inoculation with *P. ultimum*. Considering KPhi4 treatment, the highest expression rate was recorded 12 hours after inoculation, which was the highest expression in comparison to other. Among these treatments, expression rate in KPhi0 treatment was 2.37 times higher than KPhi1 treatment and 5.34 times higher than the KPhi4 24 hours after inoculation of the fungus. The lowest rate of gene expression was observed in KPhi1 treatment. According to the results, it can be concluded that the highest expression in different KPhi concentrations was achieved about 12 hours after infection, indicating the effective role of this gene in the plant defense response against the fungal pathogen. In other words, the high expression rate of chitinase at early stages of pathogen attack, led to indirect stimulation of plant defense machinery to produce defense reactions that could cause resistance. In plants that were inoculated with Pythium only, as well as KPhi1, 48 hours after inoculation, chitinase enzyme reached its highest level.

CONCLUSION

In this study, we treated the cucumber plants by KPhi followed by inoculation with *P. ultimum*. We used two different concentrations of KPhi (1 and 4 grL^{-1}) to

evaluate the protective effect of phosphite against *P. ultimum*. The overall results of this study showed that KPhi application reduced the damage caused by *P. ultimum* in cucumber plants. Different concentrations of KPhi induced defense responses in the plants in a dose-dependent pattern. It was also revealed that chitinase gene expression at early stages of pathogen attack is a defensive strategy to activate other plant defense aspects to inhibit pathogen expansion. It was indicated that KPhi can mitigate adverse effects of the disease by maintaining plant physiological procedures. These findings would increase our knowledge of the mechanism of action of KPhi upon pathogen attack which eventually would be used for intelligent application of phosphite salts for plant disease control.

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REFERENCES

[1] Abbasi, P.A. and Lazarovits, G. 2006. Seed treatment with phosphonate (AG3) suppresses Pythium damping-off of cucumber seedlings. Plant Dis.,90: 459-464.

[2] Alexandersson, E., Becker, J.V., Jacobson, D., Nguema-Ona, E., Steyn, C., Denby, K.J. and Vivier, M.A., 2011. Constitutive expression of a grapevine polygalacturonase inhibiting protein affects gene expression and cell wall properties in uninfected tobacco. BMC Res. Notes, 4, 488–493.

[3] Avila, F.W., V. Faquin, J.L. Araujo, D.J. Marques, P.M.R. Junior, A.K.D.S. Lobato, S.J. Ramos and D.P. Baliza. 2011. Phosphite supply affects phosphorus nutrition and biochemical responses in maize plants, Aust. J Crop Sci., 5: 646-653.

[4] Barzegargolchini, B., Movafeghi, A., Dehestani, A. and Mehrabanjoubani, P. 2017. Increased cell wall thickness of endodermis and protoxylem in *Aeluropus littoralis* roots under salinity: the role of LAC4 and PER64 genes. J Plant Physiol., 218: 127-134.

[5] De Lorenzo, G., D Ovidio, R. and Cervone, F. 2001. The role of polygalacturonase-inhibiting

proteins (PGIPs) in defense against pathogenic fungi. Annual Rev Phytopathol., 39: 313-335.

[6] Dehestani, A., Ahmadian, G., Salmanian, A. H., Jelodar, N. B. and Kazemitabar, K. 2009. Transformation efficiency enhancement of Arabidopsis vacuum infiltration by surfactant application and apical inflorescence removal. Trakia J Sci., 8: 19-26.

[7] Dolatabadi, B., Ranjbar, G., Tohidfar, M. and Dehestani, A. 2014. Genetic transformation of Tomato with three pathogenesis-related protein genes for increased resistance to *Fusarium oxysporum* f. sp. Lycopersici. J Plant Mol.r Breed., 2: 1-11.

[8] Groves, E., Howard, K., Hardy, G. and Burgess, T. 2015. Role of salicylic acid in phosphite- induced protection against Oomycetes; a *Phytophthora cinnamomi Lupinus augustifolius* model system. Eur. J. Plant Pathol., 141: 559-569.

[9] Hailey, A., Luke., E. and Percival, c. 2014. Comparative Assessment of Phosphite Formulations for Apple Scab (*Venturia inaequalis*) Control. Arboric. Urban For., 40: 237-243.

[10] Heidarzade, S., Gharanjik, S., Dehestani, A. and Shahriari, D. 2018. Study on enzymatic and biochemical changes in cucumber plant treated with potassium phosphite under *Fusarium oxysporum* f. sp. radicis-cucumerinum stress. Iranian J Hort. Sci., 48: 601-611.

[11] Hoagland, D.R. and Arnon, D.I. 1950. The water culture method for growing plants without soil, University of California, Berkeley, CA, USA.

[12] Jackson, T., Burgess, T., Colquhoun, I. and Hardy, G. 2000. Action of the fungicide phosphite on *Eucalyptus marginata* inoculated with *Phytophthora cinnamomi*. Plant Pathol., 49:147-154

[13] Kasprzewska, A. 2003. Plant chitinases: Regulation and Function. Cell. Mol. Biol. Lett., 8: 809-824.

[14] Khosravi, F., Gharanjik, S. and Dehestani, A. 2017. Molecular responses of *Phytophthora capsici*-challenged cucumber (*Cucumis sativus* L.) plants as influenced by resistance inducer application. J Plant Mol. Breed., 5: 1-10.

[15] King, M., Reeve, W. and Vander, A. 2010. Defining the phosphite-regulated transcriptome of the plant pathogen *Phytophthora cinnamomi*. Mol. Genet. Genom., 284: 425-435.

[16] Lavott, C.J. 1999. Timing citrus and avocado foliar nutrient application to increase fruit set and size. Hort. tech, 9: 607-612.

[17] Lichtenthaler, H.K. and Wellbum, A.R. 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Transac., 11: 591-592.

[18] Livak, K. J. and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the (Delta-Delta C(T)) Method. US National

Library of Medicine National Institutes of Health. 4: 402-8.

[19] Lobato, M.C., Olivieri, F.P., Daleo, G.R. and Andreu, A.B. 2010. Antimicrobial activity of phosphites against different potato pathogens. J Plant Dis. and Protec., 117: 102-109.

[20] Machinandiarena, M.F., Lobato, M.C., Feldman, M.L., Daleo, G.R. and Andreu, A.B. 2012. Potassium phosphite primes defense responses in potato against *Phytophthora infestans*. J Plant Physiol., 169:1417-1424.

[21] Manna, M., Islam, T., Kaul, T., Reddy, C.S., Fartyal, D., James, D. and Reddy, M.K. 2015. A comparative study of effects of increasing concentrations of phosphate and phosphite on rice seedlings, Acta Physiol.Plant., 37: 258-163.

[22] Mofidnakhaei, M., Abdossi, V., Dehestani, A., Pirdashti, H. and Babaeizad, V. 2016. Potassium phosphite affects growth, antioxidant enzymes activity and alleviates disease damage in cucumber plants inoculated with *Pythium ultimum*. Arch. Phytopathol. Plant Protec., 49: 207-221.

[23] Moradi, N., Rahimian, H., Dehestani, A. and Babaeizad, V. 2016. Cucumber Response to *Sphaerotheca fuliginea*: Differences in antioxidant enzymes activity and pathogenesis-related gene expression in susceptible and resistant genotypes. Journal of Plant Molecular Breeding, 4: 33-40.

[24] Moor, U., Poldma., P., Tonutare., T., Karp, K., Starast, M. and Vool, E. 2009. Effect of phosphite fertilization on growth, yield and fruit composition of strawberries. Scien. Hort., 119: 264-269.

[25] Neycee, M. A., Nematzadeh, G.A., Dehestani, A. and Alavi, M. 2012a. Assessment of antifungal effects of shoot extracts in chinaberry (*Melia azedarach*) against 5 phytopathogenic fungi. Intl. J Agron. Plant Prod., 4: 474-477.

[26] Neycee, M. A., Nematzadeh, G.A., Dehestani, A. and Alavi, M. 2012b. Evaluation of antibacterial effects of chinaberry (*Melia azedarach*) against gram-positive and gram-negative bacteria. Intl J Agric. crop scie., 4: 709-712.

[27] Olivieri, F.P., Feldman, M.L., Machinandiarena, M.F., Lobato, M.C., Caldiz, D.O., Daleo, G.R. and Andreu, A.B. 2012. Phosphite applications induce molecular modifications in potato tuber periderm and cortex that enhance resistance to pathogens. Crop Protec., 32: 1-6.

[28] Oyarburo, N.S., Machinandiarena, M.F., Feldman, M.L., Daleo, G.R., Andreu, A.B. and Olivieri, F.P. 2015. Potassium phosphite increases tolerance to UV-B in potato, Plant physiol. biochem., 88: 1-8.

[29] Percival, G.C. and Banks, J.M. 2014. Evaluation of plant defense activators for the potential control of *Pseudomonas syringae* pv. aesculi. Arboric. J, 36:76-88.

[30] Puerari, H.H., Dias-Arieira, C.R., Cardoso, M.R., Hernandes, I. and Brito, O.D.C. 2015. Resistance inducers in the control of root lesion nematodes in resistant and susceptible cultivars of maize, Phytoparasitica, 43: 383-389.

[31] Ramamoorthy, V., Raguchander, T. and Samiyappan, R. 2002. Enhancing resistance of tomato and hot pepper to Pythium disease by seed treatment with fluorescent Pseudomonas. Eur. J. Plant Pathol., 108: 429-441.

[32] Ramezani, M., Rahmani, F. and Dehestani, A. 2017a. Study of physio-biochemical responses elicited by potassium phosphite in downy mildew-infected cucumber plants. Arch. Phytopathol. Plant Protec., 50: 540-554.

[33] Ramezani, M., Rahmani, F. and Dehestani, A. 2017b. The effect of potassium phosphite on PR genes expression and the phenylpropanoid pathway in cucumber (*Cucumis sativus*) plants inoculated with *Pseudoperonospora cubensis*. Sci. Hort., 225: 366-372.

[34] Ramezani, M., Rahmani, F. and Dehestani, A. 2017c. Study of physio-biochemical responses elicited by potassium phosphite in downy mildew-infected cucumber plants. Arch. Phytopathol. Plant Protec., 50: 540-554.

[35] Ramezani, M., Ramezani, F., Rahmani, F. and Dehestani, A. 2018. Exogenous potassium phosphite application improved PR-protein expression and associated physio-biochemical events in cucumber challenged by *Pseudoperonospora cubensis*. Sci. Hort., 234: 335-343.

[36] Rickard, D. A. 2000. Review of phosphorus acid and its salts as fertilizer materials. Jof Plant nutr., 23: 161-180.

[37] Silva, O.C., Santos, H.A.A. and Dalla Pria, L.L. 2011. Potassium phosphite for control of downy mildew of soybean. Crop Protec., 30: 598-604.

[38] Sood, N., Sohal, B.S., and Lore, J.S. 2013. Foliar Application of Benzothiadiazole and Salicylic Acid to Combat Sheath Blight Disease of Rice. Rice Sci., 20: 349-355.

[39] Thao, H.T.B., Yamakawa, T., Myint, A.K. and Sarr, P.S. 2008. Effects of phosphite, a reduced form of phosphate, on the growth and phosphorus nutrition of spinach (*Spinacia oleracea* L.). Soil Sci. Plant Nutr., 54:761-768

[40] Thao, H.T.B., Yamakawa, T. and Shibata, K. 2009. Effect of phosphite-phosphate interaction on growth and quality of hydroponic lettuce (*Lactuca sativa*). J Plant Nutr. Soil Sci., 172: 378-384.

[41] Varadarajan, D.K., Karthikeyan, A.S., Matilda, P.D. and Raghothama, K.G. 2002. Phosphite, an Analog of Phosphate, Suppresses the Coordinated Expression of Genes under Phosphate Starvation. Plant Physiol., 129: 1232-1240.

[42] Zhang, J., Geng, J., Ren, H., Luo, J., Zhang, A. and Wang, X. 2011. Physiological and biochemical responses of Microcystis aeruginosa to phosphite. Chemosphere. 85: 1325-30.

افزایش واکنش های دفاعی گیاهان خیار تلقیح شده با پیتیوم اولتیموم تحت تاثیر تیمار با فسفیت پتاسیم

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چکیده

پیتیوم اولتیموم یکی از مهمترین عوامل ایجاد بیماری بوته میری در گیاهان خیار محسوب می شود. در پژوهش حاضر اثر فسفیت پتاسیم روی واکنش دفاعی گیاهان خیار آلوده شده با این قارچ مورد مطالعه قرار گرفت. شاخص های رشدی مختلف گیاه و میزان کلروفیل آ به عنوان شاخص هایی از اثرات تخفیف دهندگی فسفیت پتاسیم مورد ارزیابی قرار گرفتند. همچنین تغییرات در الگوی بیان کیتیاز به عنوان یک پروتئین دفاعی شاخص مورد بررسی قرار گرفت. نتایج نشان داد تیمار با فسفیت پتاسیم شاخص های رشدی از جمله طول و قطر ساقه و میانگین تعداد برگ درگیاهچه های خیار را بطور معنی داری افزایش داد. در مقایسه با گیاهان شاهد، تیمار با غلظت های ۱ و ۴ میلی گرم بر لیتر فسفیت پتاسیم به ترتیب موجب افزایش ۳۱/۳۷ و ۹۸/۴۸ درصدی در قطر ساقه گیاهان شد در حالی که طول ساقه ها در تیمار های ۱ و ۴ میلی گرم بر لیتر فسفیت پتاسیم به ترتیب ۷۲/۱۴ و ۷۸/۸۵ درصد بیشتر از گیاهان شاهد بود. میزان کلروفیل آ موجود در برگ و تعداد برگ در بوته نیز در گیاهان تیمار شده با ۱ و ۴ میلی گرم بر لیتر فسفیت پتاسیم نسبت به گیاهان شاهد افزایش معنی داری نشان داد. نکته قابل توجه کاهش معنی دار بیان ژن کیتیناز در گیاهان تیمارشده با فسفیت پتاسیم نسبت به گیاهان شاهد بود. نتایج حاصل از تحقیق حاضر می تواند در طراحی راهکارهایی برای کاهش اثرات منفی و خسارت های پیتیوم التیموم در گیاهان خیار مورد استفاده قرا گیرد.

کلمات کلیدی: پیتیوم اولتیموم، فسفیت پتاسیم، کلروفیل آ، کیتیناز، ریل تایم پی سی آر