Protective role of antifusarial eco-friendly agents (Trichoderma and salicylic acid) to improve resistance performance of tomato plants

Ameena A. Al-surhanee
Biology Department, College of Science, Jouf University, Sakaka 2014, Saudi Arabia

1. Introduction

Under today’s era of increased globalized climatic and natural disturbances, one of the principal daunting challenges for farmers is to produce food and other resources for the burgeoning world population, that has been estimated to accelerate at a rate of about 1.05 % per year (Loudiere and Gourbesville, 2020). Researchers working in the current field proposed that to cope with these pressures by 2050, it is indispensable to maximize the production of important food crops by 87% (Fróna et al., 2019).

Wilt disease caused by F. oxysporum destructively affects plants, as significantly decreasing the crop (Abada and Eid, 2014). It should take into account that from the most dangerous nurse fungus, which negatively affect the production of many plants, such as such as tomatoes, peppers, egg plant, and watermelons and therefore hit the agricultural economy (Bebber and Gurr, 2015; Igiehon and Babalola, 2017; Ortega et al., 2020). Fungal diseases trigger the increase of reactive oxygen species (ROS) such as superoxide radicals, singlet oxygen and hydrogen peroxide (H₂O₂) that limits many important physiological processes, absorption and transport of water and nutrients, as well metabolic products (Aldinary et al., 2021), which harm plant growth. During evolution, plants have established various adaptive strategies to withstand under fungal attack as development of growth and metabolic adaptations (Bai et al., 2018; Zhao et al., 2018).

The impact of ecological resources as valuable strategies to lessen the effect of fungal disease so as to enhance the yield and ability of plants to survive under stress were evaluated. Eco-friendly agents carrying therapeutic nutrition materials are aimed at supplying plants with nutrients, vitamins and hormones which are responsible for growth in addition to inhibiting pathogen attach directly or indirectly (Aldinary et al., 2021; Kumar and Alove,
2020). The physiological immunity is the case of activating the compounds responsible for defence within the plant (Enyedi et al., 1992). The species of **Trichoderma** are considered rhizosphere colonizing fungi, non-pathogenic and has super capacity to inhibit the work of pathogens and at the same time as motivation for plant growth, by producing many antioxidants that protect the plants from the burst conditions that are exposed to the roots due to injury, this is next to the competition with nutrition and production of aromatic compounds as a means of defence of plants, also produce analytical enzymes of the cellular pathogens in soil (Perello et al., 2016). Salicylic acid (SA) is natural derivative and performs an important role in the transfer of defensive signals within cells and is an expressive material for systemic resistance (Mandal et al., 2009). SA performs a vital role and stimulates growth and increases the efficiency of the plant absorption and to carry out the photosynthetic process, which positively affects the anatomical structures of plant leaf, stimulating vegetative growth and resistance against pathogen attack (Emanverdian et al., 2020). This study was aimed for deeply understanding of the physiological immunity and SA in inhibiting the growth traits of **Fusarium oxysporum**. The possibilities of improvement and revitalizing the signals responsible for defence within the tomato plant against the disease of wilt to minimize the use of fungal pesticides that have proven severely damage to public health and the environment.

**2. Materials and methods**

**2.1. Application methods and source of inducers**

**T. harzianum** were collected from Al-Azhar Center for Fermentation Biotechnology and Applied Microbiology (Ferm-bam) Al-Azhar University, Nasr City, Cairo, Egypt and were maintained on slants of PDA and stored at 4°C till further use each treatment using 2 ml/one plants. The preparation of **Fusarium oxysporum** F.sp. **Lycopersici** (pathogen) inoculums was accomplished by following (Al-dinary et al., 2021) method.

**2.2. In-vitro antifungal activity**

The antifusarial activity of SA and **Trichoderma** was established as the technique explained by (He and Wolyn, 2005). MIC of SA was defined by SA concentrations (0.5, 1, 1.5, 2 and 2.5) mM.

**2.3. Dual culture**

According to (Chen et al., 2021), **T. harzianum** was placed 9 cm apart on the opposite side of **Fusarium** on PDA plate supplemented with 2 g/l chloramphenicol at 30 °C for 5 days with three replicates, aggressive activity was documented by the following formula: % PI = [(r1 - r2)/r1] × 100, where r1 is the distance between the end point and cultural point of the **Fusarium** where r2 represents the distance between the sowing point and the edge of the **Fusarium** from **T. harzianum**.

**2.4. Ultra-structure**

The cytological variations generated in **Fusarium** treated with **T. harzianum** and SA were examined with a JOEL JM 100-C electron microscope. The samples were handled and post fixed according to (Lin and Langenberg, 1983).

**3. Experimental setup**

Four weeks old seedlings of tomato (**Solanum lycopersicum** L. var. 023) were achieved from Ministry of Agriculture Al Jouf, Saudi Arabia. Uniform looking seedlings were sown in pots (40 × 40 cm) having mixture of 7 kg sand and clay (1:3), in a plastic greenhouse. The pots were placed in the greenhouse maintained at 22/18 °C day/night T and 70–85% relative humidity. The plants were irrigated normally with tap water for five days. **T. harzianum** and SA were treated for 7 days after injection with **Fusarium oxysporum** F.sp. **Lycopersici**. A complete block design experiment and two controls each consisting of six replicates was used. Each pot contained one plant. The six treatments were; I-healthy control; 2-infected control; 3-infected + **T. harzianum** (through soil); 4-infected + **T. harzianum** (through foliar); 5-infected + SA (through soil); 6-infected + SA (through foliar); 7-infected + (**T. harzianum** + SA) (through soil); 8-infected + (**T. harzianum** + SA) (through foliar).

**3.1. Disease symptoms and disease index**

The disease symptoms were assessed on 60 days old plants and the disease index and % of inducers protection were evaluated according to (Farrag et al., 2017).

**3.2. Vegetative growth and metabolic parameters as resistance indications**

Growth parameters including shoot length (cm), root length (cm) and number of leaves per plant were noted after harvesting the samples.

Assessment of chlorophyll (a and b) and carotenoid were determined according to (Goedheer et al., 1966; Lichtenhaler and Buschmann, 2001) methods.

The total phenolic content was estimated by following method. The content of soluble protein was assessed according to (Kashyap et al., 1980). The method of (Bates et al., 1973) was used for esti-
mation of proline. The soluble sugar content was assessed by anthrone based method and absorbance of reaction mixture was measured at 625 nm (Irigoyen et al., 1992). The Bergmeyer method was employed for peroxidase (POD) activity determination. The polyphenol oxidase (PPO) activity was analysed by the (Lavid et al., 2001) protocol.

3.3. Statistical analysis

The results are the means ± standard error (n = 3). The analysis of variance (ANOVA) and Tukey’s HSD test was used to determine the significance level at p < 0.05 by using Minitab 17.

4. Results

4.1. In vitro studies

A - Dual culture

Results in Fig. 1 indicated that the percentage of inhibition was 25% of *F. oxysporum* by *T. harzianum*, where *Fusarium* singly 2 cm singly but under treatment of *Trichoderma* 1.5 cm. 

B - Antifungal activity of *Trichoderma* and SA on *Fusarium*

Results in figure (1 A) showed that antifungal activity of *Trichoderma* and SA against *F. oxysporum* in vitro, where *Trichoderma* highly showed inhibition zone (10 mm diameter). Also, its SA recorded antifungal activity at MIC 1.5 Mm by 8 mm inhibition zone diameter figure (1B).

C - Ultra-structure responses

Results in Fig. 2 showed that ultra-structure features of mycelium, macroconidia and microconidia of *F. oxysporum* were affected by treatment with SA and *Trichoderma*. There are noticed changes in morphological cell wall and membranes as well as cytoplasmic contents were not distinguished compared with control. Whereas, SA caused deformation of mycelium, macroconidia and microconidia of *Fusarium* through deposition of cytoplasmic components on cell wall compared with control. But *Trichoderma* caused moderate destroyed of *Fusarium* structure through elongated of macroconidia with irregular cell wall and compact and or losing cytoplasmic component compared with control.

4.2. In vivo studies

A - Percent disease incidence (PDI) and percent protection (%)

Table 1 revealed that tested inducer application either (individual or combination) significantly minimized *F. oxysporum* induced wilt PDI in comparison with control plants. Conversely, the data indicated that, the infection percent reached 91.66% in infected control plants. Combination of *Trichoderma* and SA (through foliar and soil) was the best application method and decreased the PDI by 12.5 and 20.83% and resulted in high defense by 86.36 and 72.2%. The treatment by *Trichoderma* (through soil) reduced PDI by 29.16% and caused high protection by 68.18%, and came next *Trichoderma* singly 2 cm.

B - Growth biomarkers

As evident from Table 2, the morphological biomarkers (plant height and number of leaves) were affected by tested inducers at either method application (soil & foliar). It is clear from the Table 2 that *F. oxysporum* induced negative impacts on all tested vegetative traits. In comparison with healthy untreated control, *F. oxysporum* infected plants had a depressive effect on shoot length by 41.50%, root length by 43.88% and leaf number by 63.65%. Concerning, the effect of tested inducers on infected plants, it was noticed that all morphological biomarkers were significantly improved at both method application (soil & foliar), whereas, the best treatment was *Tri + SA* through foliar and soil, respectively versus infected control plants.

C - photosynthetic pigments

The photosynthetic pigments (Chl a and Chl b) exhibited a decline in plants infected with *F. oxysporum* (Table 3). Out of three photosynthetic traits, carotenoid contents showed non-significant increase in comparison to control healthy plants. It was found that clear positive responses in photosynthetically pigments (Chl a and Chl b) through application of elicitors. These effects varied considerably with the mode (foiar or soil) of usage. However, when infected plants treated with (Tri + SA, SA and Tri) through soil were the best treatments that showed a marked increase in chlorophyll a & b, followed by (Tri + SA, SA and Tri) through foliar respectively as compared to control (Table 3). Also, the found results proved that in *Fusarium*-infected plants, carotenoids contents were improved in response to the treatment with (Tri + SA, Tri and SA) through foliar and soil application, respectively.

D - Metabolic indicators

To study the guidelines of the resistance in the infected tomato plants, the contents of protein, carbohydrates and free proline have been measured (Table 4). The data has shown that the control infected plants appeared a sharp decline in the contents of both protein and carbohydrates compared to healthy control. On the contrary, data showed that free proline significantly improved in infected plants as compared with healthy control plants. Interestingly the use of *Trichoderma* and SA either individual or combination through two modes (soil and foliar) enhanced the total soluble sugars and total soluble proteins contents in *Fusarium*-infected plants over infected plants only. The maximum recorded increase was observed in soluble sugars and soluble proteins contents was observed in (Tri + SA through foliar and SA through soil), followed by Tri + SA through soil, respectively. However, the contents of free proline incremented in infected plants and the highest values were noticed in plants treated with (Tri + SA, Tri and SA) and under infection through foliar, then followed by (Tri, Tri + SA and SA) through soil, respectively (see Table 5).

4.3. 3- oxidative stress

It is apparent from Fig. 3 that plants treated with *Fusarium* showed significant increase in total phenol contents of by 15.04% versus uninfected control (Fig. 4). Nonetheless, *Tri + SA, Tri and SA* foliar application was the best treatments and caused an evident rise in the phenol content. And came next, the soil application of *Tri + SA, Tri and SA* showed a marked increment in the phenol content.

For antioxidant enzyme activities it is apparent from Figs. - and -- that there is a marked boost in the activity of POD and PPO under the *F. oxysporum* and/or *Trichoderma* and SA either soil or foliar application. Moreover, highly significant increases and maximum values for POD and PPO were observed due to application of *Tri + SA* on *F. oxysporum* infected plants through (foliar and soil) mode, then followed by SA (foliar), Tri (foliar), SA (soil) and Tri (soil) respectively in comparison to control infected plants. There were marked statistically significant increases (Fig. 3).

E - Isozymes:

Foliar application of *Tri + SA* highly over-expressed the isozymes of POD that showed distinct 7 bands including 4 moderate at RF (0.13, 0.29, 0.82 and 0.92) and 3 high dense band at RF (0.36, 0.44 and 0.76), followed by Tri (foliar), SA (foliar and soil) treatments that showed the same 7 bands, 2 of them were highly dense at RF (0.36 and 0.44) and 4 were moderate at RF (0.189, 0.246, 0.861 and 0.861) and 1 was low at RF (0.92) and came next (Tri and Tri + SA) through soil that gave 6 bands Figs. 4 and 5 and Table 6.
The isozyme of PPO contained 4 PPO isozymes in Fig. 4 and Table 7. Foliar application of (Tri) recorded highly over-expressed PPO that recorded 4 bands including 2 moderates at Rf (0.2 and 0.5) and 2 highly dense at Rf (0.6 and 0.7), followed by Tri + SA (foliar) and SA (soil) that gave 4 bands; 3 of them were moderate at (0.5, 0.6 and 0.7), and 1 was high dense at Rf (0.2). and came next, SA (foliar and soil) treatments that showed the same 7 bands, 2 of them were highly dense at Rf (0.36 and 0.44) and 4 were moderate at Rf (0.189, 0.246, 0.861 and 0.861) and finally 1 was low at Rf (0.92).

5. Discussion

_Fusarium_ wilt disease is respected to be one of the mainly vital constraints across the globe. The developing information of abiotic pressures makes it critical to see other options that are found to be used in an easy way and possible to affect the destructive impacts of _Fusarium_ wilt. The potentiality of myriads of controlling practices has been developed to minimize the deleterious impacts by either eradication of the pathogen or improve plant resistance (Heydari and Pessarakli, 2010; Newton et al., 2010; Ratnadass...
cell production of cell wall-lyzing enzymes including chitinase, glucanase and protease wall as well as alteration of cytoplasmic components (Fig. 2) that is in harmony with (Bates et al., 1973; Dai et al., 1993; Irigoyen et al., 1992; Kashyap et al., 1980). Inhibition of Fusarium growth by Trichoderma through reduced and deformation of mycelium in dual culture method (Fig. 2) as competition mechanism proved by (Heydari and Pessarakli, 2010; Lavid et al., 2012). In this context, the utility of novel and emerging mitigating tools could impart resistance of plant species under biotic stress. It is well known fact that in plants pathogenic infection resistance can be boosted via the biotic or abiotic inducers applied exogenously. It is more preferred to use vital and natural environmentally friendly elicitors for the fungus protection in crop plants rather than altering the whole micro flora of soil and enhanced resistance of plant diseases in order to preserve public health and the environment to suppress wide range of plant pathogens including Fusarium that caused wilt problems (Chaube et al., 2004; Latz et al., 2018; Pascale et al., 2020). This antifusarial activity explained by mycoparasitism way through disruption of fungal

### Table 2
morphological indicators of tomato plant treated with Trichoderma and Salicylic acid:

| Treatments | Method of application | Shoot length(cm) | Root length(cm) | Number of leaves per plant |
|------------|-----------------------|------------------|----------------|---------------------------|
| Tri        | through soil          | 47.25 ± 1.75 bc  | 21.06 ± 0.89 b | 12.44 ± 0.42 b            |
| SA         |                       | 34.12 ± 1.2 d    | 15.04 ± 0.87 d | 7.97 ± 0.22 d             |
| Tri + SA   |                       | 50.16 ± 1.23 b   | 21.9 ± 0.33 b  | 13.16 ± 0.25 b            |
| Tri        | through Foliar        | 47.11 ± 1.6 bc   | 16.38 ± 0.32 a | 9.97 ± 0.41 a             |
| SA         |                       | 46.37 ± 1.75     | 16.09 ± 1.18  | 9.47 ± 3.7                |
| Tri + SA   |                       | 50.72 ± 1.16 a   | 21.33 ± 0.23 b | 16.38 ± 0.12 a            |
| Control infected |               | 30.18 ± 0.86 e   | 13.14 ± 0.05   | 6.52 ± 0.55 d             |
| Control healthy |            | 51.62 ± 0.87 a   | 23.42 ± 1.14 a | 17.94 ± 0.45 a            |
| LSD at 0.05 |                       | 2.495            | 1.314          | 2.365                      |

### Table 3
Photosynthetic pigments (Ch a, Ch b and carotenoid) of tomato plant treated with Trichoderma and Salicylic acid through (soil and foliar) application.

| Treatments | Method of application | Chlorophyll a (mg/ g fresh weight) | Chlorophyll b (mg/ g fresh weight) | Carotenoid (mg/ g fresh weight) |
|------------|-----------------------|-----------------------------------|-----------------------------------|--------------------------------|
| Tri        | through soil          | 6.3 ± 0.03 b                      | 4.88 ± 0.08 b                     | 1.24 ± 0.08 b                   |
| SA         |                       | 6.74 ± 0.25 a                     | 7.81 ± 0.43 a                     | 0.93 ± 0.05                      |
| Tri + SA   |                       | 6.95 ± 0.02 a                     | 7.89 ± 0.16 a                     | 1.84 ± 0.01                      |
| Tri        | through Foliar        | 4.46 ± 0.024 de                   | 3.24 ± 0.024 ed                   | 0.42 ± 0.15 ed                   |
| SA         |                       | 4.72 ± 0.12 d                     | 3.78 ± 0.10 d                     | 0.71 ± 0.01 d                    |
| Tri + SA   |                       | 5.22 ± 0.215 c                    | 4.01 ± 0.15 c                     | 0.49 ± 0.06 c                    |
| Control infected |               | 4.16 ± 0.08 e                     | 2.51 ± 0.01 e                     | 0.26 ± 0.21                      |
| Control healthy |            | 6.17 ± 0.42 c                     | 4.11 ± 0.11 c                     | 0.22 ± 0.07 c                    |
| LSD at 0.05 |                       | 0.344                             | 0.32                             | 0.184                            |

### Table 4
Effect of Fusarium and (soil & foliar) application of Trichoderma and Salicylic acid and their interactions on the content of osmolytes (soluble sugars, soluble proteins and proline) of tomato plants.

| Treatments | Method of application | Total carbohydrate | Total protein | Total proline |
|------------|-----------------------|--------------------|--------------|--------------|
| Tri        | through soil          | 6.78 ± 0.26 c      | 10.86 ± 0.42 c | 2.60 ± 0.01 bc |
| SA         |                       | 8.19 ± 0.03 a      | 13.11 ± 0.04 a | 2.18 ± 0.11 e |
| Tri + SA   |                       | 8.03 ± 0.55 b      | 12.85 ± 0.88 b | 2.45 ± 0.01 d |
| Tri        | through Foliar        | 6.13 ± 0.16 d      | 9.29 ± 0.05 de | 2.81 ± 0.11 b |
| SA         |                       | 5.80 ± 0.03 de     | 9.81 ± 0.25 d  | 2.87 ± 0.01 bc |
| Tri + SA   |                       | 8.76 ± 0.33 a      | 14.02 ± 0.53 a | 3.62 ± 0.13 a |
| Control infected |               | 5.41 ± 0.11 i      | 8.66 ± 0.16 i  | 1.47 ± 0.03 f |
| Control healthy |            | 9.03 ± 0.04 a      | 14.45 ± 0.06 a | 1.25 ± 0.04 d |
| LSD at 0.05 |                       | 0.447              | 0.71           | 0.149          |

### Table 5
Effect of Fusarium and (soil & foliar) application of Trichoderma and Salicylic acid and their interactions on the content of osmolytes (Polyphenol oxidase and Peroxidase) of tomato plants.

| Treatments | Method of application | Polyphenol oxidase | Peroxidase |
|------------|-----------------------|-------------------|------------|
| Tri        | through soil          | 0.68 ± 0.004 a    | 0.55 ± 0.014 d |
| SA         |                       | 0.72 ± 0.01 a     | 0.52 ± 0.003 b |
| Tri + SA   |                       | 1.01 ± 0.03 a     | 0.79 ± 0.030 m |
| Tri        | through Foliar        | 0.80 ± 0.03 c     | 0.61 ± 0.024 c |
| SA         |                       | 0.94 ± 0.06 b     | 0.73 ± 0.050 b |
| Tri + SA   |                       | 1.06 ± 0.005 c    | 0.62 ± 0.0093 a |
| Control infected |               | 0.95 ± 0.003 a    | 0.68 ± 0.004 a |
| Control healthy |            | 0.63 ± 0.01 b     | 0.72 ± 0.01 4de |
| LSD at 0.05 |                       | 0.71              | 0.149        |
of studied plant growth parameters which is in accordance with the results of (Aldinary et al., 2021; Emamverdian et al., 2020; Kumar et al., 2005). This harmful effect in vegetative growth attributes due to *F. oxysporum* can be explained by accumulation of free ROS in cells and disturbances in enzymatic activity and photosynthesis process (Sharma et al., 2019). Photosynthesis plays a main
anabolic role of plants, allowing plants to convert solar energy into biochemical energy which is successively used in all complex cell actions, and it is highly impacted by infection caused by various infections (Botero et al., 2018). In the recent study, *F. oxysporum* triggered a significant decrement in chlorophyll pigment contents, subsequent in a complete growth destroyed. These pigments were negatively affected by *Trichoderma* and SA either (individual or combination), this result became one of the obvious indications of treatment efficiency that can be discussed by *Trichoderma* ability to improve the soil and growth in the plant and supply plant with

Table 6

| Peroxidase groups | Relative Mobility | C1   | C2   | Soil   | Foliar   |
|------------------|------------------|------|------|--------|----------|
|                  |                  | Tri  | Tri  | Tri + | Tri +  |
| PPO1             | 0.2              | 1    | 1    | 1     | 1       |
| PPO2             | 0.5              | 1    | 1    | 1     | 1       |
| PPO3             | 0.6              | 1    | 1    | 1     | 1       |

++ High density Band + Moderate density Band – Low density Band 1 Present Band 0 Absent Band.

Table 7

| Polyphenyl Oxidase groups | Relative Mobility | C1   | C2   | Soil   | Foliar   |
|---------------------------|------------------|------|------|--------|----------|
|                           |                  | Tri  | Tri  | Tri + | Tri +  |
| PPO1                      | 0.2              | 1    | 1    | 1     | 1       |
| PPO2                      | 0.5              | 1    | 1    | 1     | 1       |
| PPO3                      | 0.6              | 1    | 1    | 1     | 1       |

Fig. 5. Effect of Fusarium and (soil & foliar) application of *Trichoderma* and Salicylic acid and their interactions on (A) Polyphenol oxidase isozyme and (B) Ideogram analysis of peroxidase isozyme of tomato plants.
the nutrients (N, P, K) necessary to carry out the vital processes. It is clear from the present results that total phenol and proline content improved in infected plants and the greatest trend for total phenol content was noted in infected plants treated under *Trichoderma* and SA application, then followed by *Trichoderma* and SA, in comparison to control plants. The accumulation of phenolic compounds and proline in plant cells is evidence of the limitation of pathogen development, because these compounds are toxic to the pathogen and the plants use them as biochemical weapons for defense. Also, phenolic compounds may inhibit pathogen disease by enhancing the structural defense (Beckman and Roberts, 1995). In the current experiment, SA alone or with *Trichoderma* improved total phenols and free proline contents significantly in *F. oxysporum* treated plants. So, stimulation of total phenol content with SA and *Trichoderma* could exert a principal function in imparting resistance against *F. oxysporum*. These effects approve the accepted theory, that when infection happens to the plant cells, a change is triggered that shifts the normal primary metabolism into the secondary defense pathways, that results in the stimulation of myriads of genes encoding for defense enzymes (Farrag et al., 2017; Tarkowski et al., 2020). Such enrichment in the activities of antioxidants have been stated by others as well (Alhaithoul et al., 2020; Elkeelish et al., 2020; Zalheer et al., 2020). It was noteworthy to see that POD and PPO activities were found to be improved significantly due to application *Trichoderma* and SA either (individual or combination). SA is a main phenylpropanoid acid that stimulates resistance of plants against various pathogen either (individual or combination). SA is a main phenylpropanoid acid that stimulates resistance of plants against various pathogen (Ojha and Chatterjee, 2012). Both modes of application of *Trichoderma* and SA either (individual or combination) showed significant more beneficial effects. The POD catalyses the H$_2$O$_2$ elimination (Das and Roychoudhury, 2014). Besides this in the present study, *Trichoderma* caused the highest phenolic accumulation which was in direct relationship with boosted activity of PPO. Thus, in the final, the present addendum shows that stimulating the plant's own resistance system by using antifusarial eco-friendly agents (*Trichoderma* and SA) can be an emerging approach in controlling various plant diseases.

6. Conclusion

The results of the experiment showed severe effects, including a decrease in morphological characteristics and metabolic processes, which led to oxidative burst in cells. It is concluded that using treatments *Trichoderma* and SA either (individual or combination) by foliar spray or soil treatment, which led to enhancement in immune responses and stimulation of substances responsible for defense within infected plants, thus reducing disease severity and increasing protection from disease. Therefore, the study recommends more studies on the use of these treatments in strengthening plant immunity and revealing the genes responsible for resistance to try to stay away from the use of harmful pesticides however; additional approaches should be employed to unravel actual underlying mechanisms.

Declaration of Interests

The authors declare that there are no conflicts of interest related to this article.

Acknowledgement

This research work was supported by the Biology Department, College of Science, Jouf University, Sakaka 2014, Kingdom of Saudi Arabia.

References

Abada, K.A., Eid, K.E., 2014. A protocol suggested for management of cantaloupe downy mildew. Am. J. Life Sci. 2, 1–10.

Aldiniry, A.M., Abdelaziz, A.M., Farrag, A.A., Attia, M.S., 2021. Biocontrol of tomato Fusarium wilt disease with a new Moringa endophytic Aspergillus isolates. Mater. Today.: Proc.

Alhaithoul, H.A., Soliman, M.H., Ameta, K.L., El-Esawi, M.A., Elkeelish, A., 2020. Changes in ecophysiology, osmolites, and secondary metabolites of the medicinal plants of Mentha piperita and Catharanthus roseus subjected to drought and heat stress. Biomolecules 10, 43.

Bai, Y., Kissoudis, C., Yan, Z., Visser, R.G.F., van der Linden, G., 2018. Plant behaviour under combined stress: tomato responses to combined salinity and pathogen stress. Plant J. 93, 781–793.

Bates, I.S., Wallden, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207.

Bebber, D.P., Curr, S.J., 2015. Crop-destroying fungal and oomycete pathogens challenge food security. Fungal Genet. Biol. 74, 62–64.

Beckman, C.H., Roberts, E.M., 1995. On the nature and genetic basis for resistance and tolerance to fungal wilt diseases of plants. Adv. Bot. Res. 21, 35–77.

Botero, K., Restrepo, S., Pinzón, A., 2018. A genome-scale metabolic model of potato late blight suggests a photosynthesis suppression mechanism. BMC Gen. 19, 31–44.

Chaub, H.S., Mishra, D.S., Varshney, S., Singh, U.S., 2004. Biocontrol of plant pathogens by fungal antagonists: Historical background, present status and future prospects. Ann. Rev. Phytopath. 2, 1–42.

Chen, J., Zhou, L., Din, U.U., Arafat, Y., Li, Q., Wang, J., Wu, T., Wu, L., Wu, H., Qin, X., 2021. Antagonistic activity of Trichoderma spp. against Fusarium oxysporum in rhizosphere of Radix pseudostellariae triggers the expression of host defense genes and improves its growth under long-term monoculture system. Front. Microbiol. 12, 422.

Dai, G.H., Andary, C., Coxson-Mondolot, L., Boubals, D., 1993. Polyphenols and resistance of grapevines to downy mildew. Int. Sympos. Nat. Phenols Plant Resist. 381, 763–766.

Das, K., Roychoudhury, A., 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front. Environ. Sci. 2, 53.

Elkeelish, A.A., Alhaithoul, H.A.S., Sari, S.H., Soliman, M.H., Hasanuzzaman, M., 2020. Pretreatment with Trichoderma harzianum alleviates waterlogging-induced growth alterations in tomato seedlings by modulating physiological, biochemical, and molecular mechanisms. Environ. Exp. Bot. 171, 103946.

Emamverdian, A., Ding, Y., Mokhberdoran, F., 2020. The role of salicylic acid and giberellin signaling in plant responses to abiotic stress with an emphasis on heavy metals. Plant Signal. Behav. 15, 1773732.

Enyedi, A.J., Yalpani, N., Silverman, P., Raskin, L., 1992. Signal molecules in systemic plant resistance to pathogens and pests. Cell 70, 879–886.

Farrag, A.A., Attia, M.S., Younis, A., Abd Elaziz, A.M.A., 2017. Potential impacts of elicitors to improve tomato plant disease resistance. Al Azhar Bull Sci 9, 311–321.

Fróna, D., Szendérák, J., Harangi-Rákos, M., 2019. The challenge of feeding the world. Sustainability 11, 5816.

Goedheer, J.C., Vernon, L.P., Seeley, G.R., 1966. The Chlorophylls. LP Vernon, GR Seely, Academic Press, New York and London 179.

He, C.Y., Wolyn, D.J., 2005. Potential role for salicylic acid in induced resistance of asparagus roots to Fusarium oxysporum f. sp. asparagi. Plant. Pathol. 54, 227–232.

Heydari, A., Pessaaraki, M., 2010. A review on biological control of fungal plant pathogens using microbial antagonists. J. Biol. Sci. 10, 273–290.

Igehon, N.O., Babalola, O.O., 2017. Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi. Appl. Microbiol. Biotechnol. 101, 4871–4881.

Irigrayon, J.J., Einerich, D.W., Sánchez-Díaz, M., 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativd) plants. Physiol. Plant. 84, 55–60.

Kashyap, M.L., Hynd, R.A., Robinson, K., 1980. A rapid and simple method for measurement of total protein in very low density lipoproteins by the Lowry assay. J. Lipid Res. 21, 491–495.

Kumar, H.D., Aloke, P., 2020. Role of biostimulant formulations in crop production: An overview. Int. J. Appl. Res. Vet. Med. 8, 38–43.

Kumar, T., Rajpui, V.K., Maheshwari, M.K., Kang, S.-C., 2005. Plant growth promotion and suppression of root disease complex due to Meloidogyne incognita and Fusarium oxysporum by fluorescent pseudomonads in tomato. J. Appl. Biol. Chem. 48, 79–83.

Latz, M.A.C., Jensen, B., Collinge, D.B., Jørgensen, H.J.L., 2018. Endophytic fungi as biocontrol agents: elucidating mechanisms in disease suppression. Plant Ecolog. 11, 555–567.

Laviad, M., Schwartz, A., Yarden, O., Tel-Orr, E., 2001. The involvement of polyphenols and peroxidase activities in heavy-metal accumulation by epidermal glands of the waterlily (Nymphaeaceae). Planta 212, 323–331.
Lichtenthaler, H.K., Buschmann, C., 2001. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Curr. Protocol. Food Anal. Chem. 1, F4–F13.

Lin, N.-S., Langenberg, W.G., 1983. Immunohistochemical localization of barley stripe mosaic virions in infected wheat cells. J. Ultrastruct. Res. 84, 16–23.

Loudiere, D., Gourbesville, P., 2020. World water development report-water and climate change. Houille Blanche-Revue Internationale DE L EAU, 76–81.

Mandal, S., Mallick, N., Mitra, A., 2009. Salicylic acid-induced resistance to Fusarium oxysporum f. sp. lycopersici in tomato. Plant Physiol. Biochem. 47, 642–649.

Newton, A.C., Gravouil, C., Fountaine, J.M., 2010. Managing the ecology of foliar pathogens: ecological tolerance in crops. Ann. Appl. Biol. 157, 343–359.

Ojha, S., Chatterjee, N.C., 2012. Induction of resistance in tomato plants against Fusarium oxysporum f. sp. lycopersici mediated through salicylic acid and Trichoderma harzianum. J. Plant Protect. Res. 52.

Ortega, H.E., Torres-Mendoza, D., Cubilla-Rios, L., 2020. Patents on endophytic fungi for agriculture and bio-and phytoremediation applications. Microorganisms 8, 1237.

Pascale, A., Proietti, S., Pantelides, I.S., Stringlis, I.A., 2020. Modulation of the root microbiome by plant molecules: the basis for targeted disease suppression and plant growth promotion. Front. Plant Sci. 10, 1741.

Perello, C., Llamas, E., Burlat, V., Ortiz-Alcaide, M., Phillips, M.A., Pulido, P., Rodriguez-Concepcion, M., 2016. Differential subplastidial localization and turnover of enzymes involved in isoprenoid biosynthesis in chloroplasts. PLoS ONE 11, e0150539.

Ratnadass, A., Fernandes, P., Avelino, J., Habib, R., 2012. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. Agron. Sustain. Dev. 32, 273–303.

Sharma, A., Shahzad, R., Rehman, A., Bhardwaj, R., Landi, M., Zheng, B., 2019. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. Molecules 24, 2452.

Tarkowski, L.P., Signorelli, S., Höfte, M., 2020. γ-Aminobutyric acid and related amino acids in plant immune responses: emerging mechanisms of action. Plant, Cell Environ. 43, 1103–1116.

Zaheer, I.E., Ali, S., Saleem, M.H., Imran, M., Alnusairi, G.S.H., Alharbi, B.M., Riaz, M., Abbas, Z., Rizwan, M., Soliman, M.H., 2020. Role of iron–lysine on morphophysiological traits and combating chromium toxicity in rapeseed (Brassica napus L.) plants irrigated with different levels of tannery wastewater. Plant Physiol. Biochem. 155, 70–84.

Zhao, D., Cheng, M., Tang, W., Liu, D., Zhou, S., Meng, J., Tao, J., 2018. Nano-silver modifies the vase life of cut herbaceous peony (Paeonia lactiflora Pall.) flowers. Protoplasma 255, 1001–1013.