



Induction of resistance in Safflower plant against root rot and wilt diseases by certain inducers

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Abstract

The ability of thiamine (vitamin B1) and ascorbic acid to induce resistance against root rot and wilt disease in safflower cultivar Giza1 infected with *Fusarium verticillioides* was studied under laboratory and greenhouse conditions. Infection with *F. verticillioides* caused clear reduction in growth parameters (fresh and dry weight of plants, total photosynthetic pigments), soluble sugars and free amino acids comparing with healthy plants. In addition, it induced the highest accumulation of proline content. On the other side, soaking with two levels of thiamine and ascorbic acid caused reduction in the infected safflower plants and increased dry and fresh weight contents especially in shoots than roots comparing with the infected plants with *F. verticillioides*. Thiamine concentrations (1,3mM) induced the highest stimulating effect on total pigments. The induction of resistance by ascorbic acid and thiamine was associated with some biochemical changes in safflower cultivar by reduction of proline content, enhancement of soluble sugars and free amino acids, this may be relate to reduce the disease and increase plant growth.

Key words: Safflower, *Fusarium verticillioides*, thiamine, ascorbic, root rot-wilt diseases.

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Introduction

Safflower (*Carthamus tinctorius* L.) is one of the oldest crops, traditionally, grown for its seeds, and used for colouring, flavouring foods and for making red and yellow dyes (Zohary and Hopf, 2000). In the last century, the plant has been cultivated mainly for the oil extracted from its seeds (Corleto et al., 1997). Seed oil content is between 35 and 50% (Camas et al., 2007). World production is around 600,000 tons, produced in more than sixty countries worldwide (mainly in India, USA and Mexico) from about 850 000 ha (FAO, 2012). Also, it grows in many locations of Egypt, especially in Assiut, Qena and Aswan Governorates. Safflower root-rot and wilt diseases caused by *Fusarium verticillioides* was very serious disease in Egypt and other countries (Govindappa et al., 2011; Nasehi, 2010). Application of chemical fungicides for controlling plant diseases became improper methods and undesired causing environmental pollution. For managing this pathogen, several alternative measures are being tested. Natural resources as chemical inducers were recently applicable (El-Baz, 2007), resistant cultivars (Mengistu et al., 2007). Induced resistance by chemicals is also a promising approach to prevent diseases caused by soil-borne pathogens (Okubara & Paulitz, 2005). Ascorbic acid (vitamin c) is an important metabolite involved in many cellular processes, including cell division (De Gara et al., 2003). It also participates in anti-oxidation (Aria & Mohammad 2008). In recent years, the importance of vitamins as nutrients and as control agent for different diseases has been demonstrated (Dong & Beer, 2000). Ahn

et al. (2005) demonstrated that thiamine (vitamin B1) induces systemic acquired resistance (SAR) and vitamin B1 function as an activator of plant disease resistance. They reported that thiamine treated rice, *Arabidopsis thaliana* and vegetable crops showed resistance to fungal, bacterial and viral infections. Ascorbic acid has been reported to induce resistance in plants to a number of plants pathogens (Agam et al, 2010). The present study was conducted to investigate the effect of ascorbic acid and thiamine (applied through seeds soaking) to control root rot and wilt diseases of safflower caused by *Fusarium verticillioides* as well as study some biochemical change in plant after treatment.

Materials and methods

Source the causal pathogen: *Fusarium verticillioides* (No. 1) was isolated from safflower plant showing typical symptoms of root-rot and wilt diseases in commercial safflower farms at Assiut Governorate, Egypt. The pathogenicity of this isolate was previously tested and it showed high virulence as recorded by Hoda Ahmed and Ghada Abd Elaziz, (2013).

In vitro investigation of certain chemical resistant inducers on growth of *Fusarium verticillioides*: Laboratory work were carried out to study the effect of various concentrations (0,1,3,6 and 9 mM) of four chemical substances (ascorbic acid, oxalic acid, thiamine and riboflavin) on growth of *Fusarium verticillioides* on linear growth and germination of pathogen *Fusarium*

verticillioides in vitro. It was dissolved in ethanol as described by Galal and Abdou, (1996). Aliquots (10 ml) of the tested solutions were added separately to 990 ml PDA medium. Petri-dishes (9 cm diameter) containing PDA medium were inoculated in the center with discs (5 mm) of *F. verticillioides* taken, from 7 days old culture and incubated at 27 °C. Three Petri-dishes were used for each tested concentration. Diameter of linear growth of each isolate was measured in cm, when fungal growth filled up control Petri-dishes. Percentage of reduction in mycelial growth was calculated using the following formula:

$$\text{Growth reduction (\%)} = (A1 - A2) / A1 \times 100$$

Where, A1= growth in untreated medium (control) and A2 = growth in the treated medium

***In vivo* effect of ascorbic acid and thiamine on incidence of root-rot and wilt diseases of safflower:** Two concentrations (1 and 3 mM) of ascorbic acid and thiamine were used to study their effects on root-rot and wilt diseases of safflower under greenhouse conditions (Experimental outdoor greenhouse at the Faculty of Science, Assiut University (Egypt) under natural field conditions of temperature, humidity, light, and day/night regime) as the following protocol: In 30 cm² diameter pots filled with 4kg sterilized soil. Safflower seeds were soaked for 12 hours Hussain et al., (2006) in the tested concentrations of the above mentioned chemical inducers and some seeds soaked in water (control). The soaked seeds were spread out in a thin layer and left to 24 hours in air

drying. Seven seeds were sown in, pathogen-infested soil. Three pots were used for each treatment as replicates. Root-rot and wilt diseases incidence were estimated at 30 and 90 days from planting date, respectively. The following formulae were used to determine the disease criteria.

$$\text{Root-rot (\%)} = \frac{\text{No. of diseased seedlings}}{\text{Total number of seedlings}} \times 100$$

$$\text{Wilt (\%)} = \frac{\text{No. of wilted plants}}{\text{Total number of plants}} \times 100$$

Biochemical analysis: At 30 days of the treatment fresh and dry weight of roots and shoots were recorded, Total photosynthetic pigments content of safflower fresh leaves were spectrophotometrically determined by using Unico UV-2100 spectrophotometer according to Lichlenthaler, (1987). Soluble sugars content of safflower leaves and roots were determined using anthrone sulphuric acid method (Fales, 1951). Bates et al., (1973) method was used for determination of proline in roots and shoots. Free amino acids were determined according to the methods of Moore and Stein, (1948). All the measuring parameters were calculated as mg/g DW.

Statistical analysis: The data were analyzed using ANOVA (completely randomized) to determine if significant differences were present among means. Duncan's multiple range tests was carried out to determine if mean difference significant at $P < 0.05$ (SPSS-11).

Results and Discussion

Effect of two chemical resistance inducers on linear growth of *Fusarium verticillioides* in vitro: Linear growth of *F. verticillioides* was significantly reduced at most of the tested concentrations (Table 1). Increasing concentration of the tested chemicals, generally increased the percentage of reduction in fungal growth. The highest reduction in linear growth was pronounced by oxalic acid and riboflavin at all concentrations, while ascorbic acid and thiamine gave the least reduction at all concentration especially concentrations of 1 and 3 mM (4.98% and 6.00%), respectively. Also, mycelial growth and spore germination of *F. verticillioides* was influenced and much reduced by antioxidant compounds in ascorbic acid and thiamine. According to the obtained results, 1 and 3 mM concentration of ascorbic and thiamine vitamins were chosen for testing the possibility of induced resistance in safflower diseases. These results agree with Abd-El-Kareem, (2007) who founded that these compounds have no direct antimicrobial activity against many fungal and bacterial pathogens and also, agree with El-Ganaieny et al., (2002). Hoda Ahmed, (2008) who worked on *Fusarium* diseases on onion and

Macrophomina phaseolina on sesame, respectively.

Effect of ascorbic acid and thiamine on incidence of root-rot and wilt diseases of safflower: Soaking safflower seeds (either in 1 mM or 3 mM of thiamine and ascorbic acid) greatly reduced the numbers of infected plants. Our results showed that thiamine was more effective than ascorbic acid in stimulation of plant to resistance of fungus compared to infected control (Table 2). Thiamine containing several mechanisms that mediate the disease protection induced by different chemicals has been demonstrated, including blocking of disease cycle and the direct inhibition of pathogen growth (Thompson et al., 2000). Also thiamine confers systemic acquired resistance (SAR) on susceptible plants through priming, leading to rapid counterattack against pathogen invasion and perturbation of disease progress (Ahn et al., 2005). Arafa et al., (2009) found that under greenhouse conditions, soaked sorghum grains in ascorbic acid before sowing increased germination percentage compared to control. The role of ascorbic acid and thiamine against certain plant pathogens was widely demonstrated by Ahn et al., (2005), Aria and Mohammad, (2008), Khan, (2010), Hamama and Murniati, (2010) and Abdel-Monaim, (2011).

Table 1: Effect of various chemical resistance inducers on linear growth of *Fusarium verticillioides* in vitro.

Conc. (mM) chemicals	Linear growth reduction (%)					Mean
	0	1	3	6	9	
Ascorbic acid	0.00	10.18	16.66	37.22	39.	20.76
Thiamine	0.00	9.25	15.18	29.44	36.	18.84
Oxalic acid	0.00	11.10	22.21	38.33	55.	25.43
Riboflavin	0.00	13.88	28.51	38.14	61.	28.32
Mean	0.00	11.10	20.64	35.78	48.	

LSD at 5%, inducers (A)= 3.02, concentration (B)= 3.38, interaction (AXB)= 6.75.

Table 2: Effect of Ascorbic acid and Thiamine applications on incidence of root-rot and wilt diseases in 2010/2011 and 2011/2012 seasons on safflower cultivar under greenhouse conditions.

Appli. methods	Conc.	Percentage of infected plants			
		Root-rot		Wilt	
		2010/2011	2011/2012	2010/2011	2011/2012
Ascorbic acid	1mM	14.3	9.53	33.36	23.83
	3mM	19.06	14.3	28.6	33.36
Thiamine	1mM	9.53	4.76	23.83	19.06
	3mM	4.76	0	14.30	4.76
Control		38.03	42.9	66.64	66.66
LSD .5%		9.6	21.18	28.7	31.9

Effect of ascorbic acid and Thiamine applications on growth of infected safflower cultivar under greenhouse conditions: Data of figure 1 indicate that shoot fresh weight of safflower reduced by treatment of soil by *Fusarium* compared with healthy plants. It is noticed that treatment with ascorbic acid and thiamine, increased significantly the shoot fresh weight but in roots slightly increase was reported comparing with the control (fungal treatment) plants. The data in figure 2 indicate that dry weight of shoot and root of safflower have a negatively response to infection of soil with *Fusarium* (root rot and wilt diseases). Soaking of safflower seeds in two levels of thiamine and ascorbic acid causes a significant increase in shoot and root dry weight of safflower except at 3mM ascorbic acid. This results are in agreement with other investigators findings as Abdel-Monaim (2011). Spletzer and Enyedi, (1999) and Hamada and Hashem, (2003). Al-Hakimi et al. (2007) proved that soaking of broad bean seeds in thiamine or salicylic acid did not only alleviate the inhibitory effects of fungal infection but also were of stimulatory effects. Data in figure 3 indicate that *Fusarium* infection to safflower plant caused reduction in total

pigments comparing with the healthy plants (absolute control) data not shown. However treatments of infected plants with ascorbic acid (1mM) and thiamine concentrations (1,3mM) induced significant stimulating effect on total pigments Fig 3. These results agree with Farouk et al 2008, who indicated that application of either thiamine, chitosan or humic acid significantly increased photosynthetic pigments content and activated the synthesis of carotenoids which protect chlorophyll content. Also other results proved these results in tomato plants by Zodape et al, (2011).

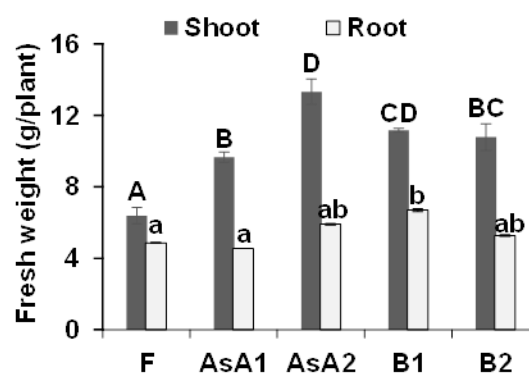


Figure 1: Fresh weight (g/plant) of safflower plants (30-day-old) infected with *Fusarium verticillioides* (F) as affected by two concentrations of ascorbic acid (AsA1, AsA2) and thiamine (B1, B2). The results are means of three replicates (\pm SE). Bars carrying different letters are significantly different at $P < 0.05$.

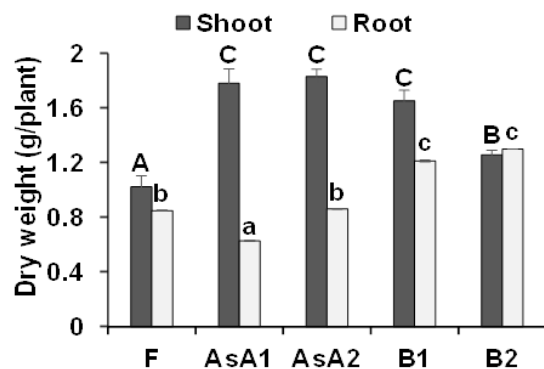


Figure 2: Dry weight (g/plant) of safflower plants (30-day-old) infected with *Fusarium verticillioides* (F) as affected by two concentrations of ascorbic acid (AsA1, AsA2) and thiamine (B1, B2). The results are means of three replicates (\pm SE). Bars carrying different letters are significantly different at $P < 0.05$.

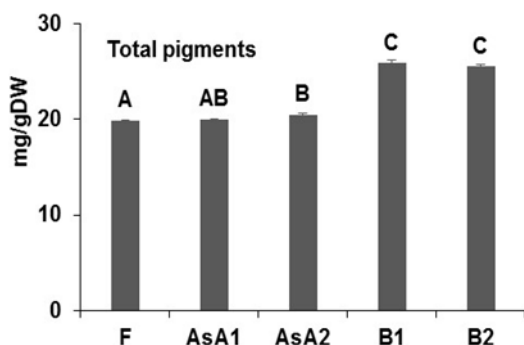


Figure 3: Total photosynthetic pigments (mg/g DW) of safflower plants (30-day-old) infected with *Fusarium verticillioides* (F) as affected by two concentrations of ascorbic acid (AsA1, AsA2) and thiamine (B1, B2). The results are means of three replicates (\pm SE). Bars carrying different letters are significantly different at $P < 0.05$.

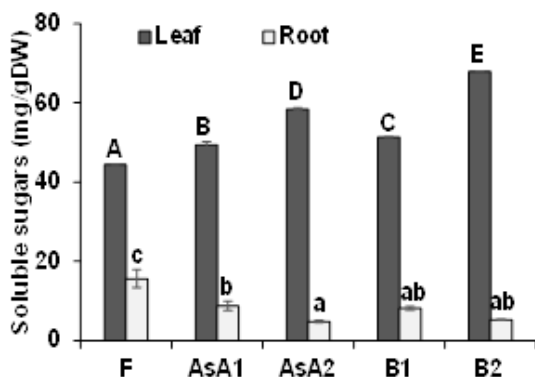


Figure 4: Soluble sugars (mg/g DW) of safflower plants (30-day-old) infected with *Fusarium verticillioides* (F) as affected by two concentrations of ascorbic acid (AsA1, AsA2) and thiamine (B1, B2). The results are means of three replicates (\pm SE). Bars carrying different letters are significantly different at $P < 0.05$.

In the present investigation sowing of safflower plant in the soil infected with *Fusarium verticillioides* exhibit a drastic inhibition in the accumulation of soluble sugars in leaves comparing with the absolute control, (healthy safflower plants). Our results agree with Farouk et al, (2012). It is noticed that significant enhancement in the accumulation of soluble sugars in safflower leaves were recorded with application of ascorbic or thiamine to infected plants (Fig 4). Safflower infested roots showed opposite behavior when treated with ascorbic or thiamine concentrations (1&3 mM). Farouk et al (2012) proved that treatments of tomato plants with thiamine caused a significant effect on total carbohydrates comparing with plants infected by *Alternaria solani*. Accumulation of soluble sugars in infected tomato plants- especially treated with bio agent and hormonal elicitors indicated the relationship between sugar regulation and activation of the systemic resistance (El-Kallal, 2007). On the other hand the increase in soluble sugar in plants grown in pathogenized soil may be due to retarded rate of translation under the influence of cell wall degrading enzymes or toxins produced and transfer via phloem elements (Heiser et al., 1998). There are many reports on the importance of sugar levels in plant resistance to diseases caused by fungal pathogens and oomycetes, but their role as signal molecules in defense responses has only been described in recent publications (Morkunas et al. 2011; Bolouri Moghaddam & Van den Eden, 2010). Many plant responses to the attack of a fungal pathogen are closely connected with the pathways regulating the level of sugar in the plant cell and

ensuring energy homeostasis (Hey et al., 2010). Data obtained herein show that the soaking of safflower infected seeds in 1 or 3mM ascorbic or thiamine concentrations are significantly enhanced the biosynthesis of total amino acids in leaves (Fig.5). Sayed and Gadallah, (2002) proved that the proteolytic digestion of thiamine-binding protein to give amino acids could be an alternative explanation for higher amino acids contents in thiamine-treated plants. On the other hand application of ascorbic only caused a stimulatory effect on the safflower roots compared with the control, this results in accordance with the data of El-Khallal, (2007).

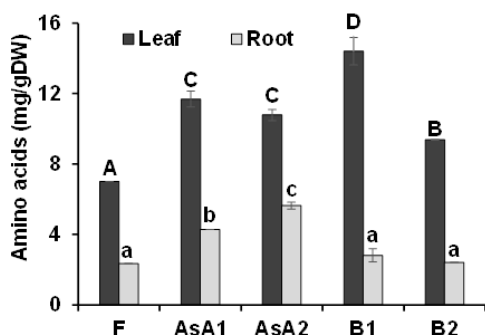


Figure 5: Total free amino acids (mg/g DW) of safflower plants (30-day-old) infected with *Fusarium verticillioides* (F) as affected by two concentrations of ascorbic acid (AsA1, AsA2) and thiamine (B1, B2). The results are means of three replicates (\pm SE). Bars carrying different letters are significantly different at $P < 0.05$.

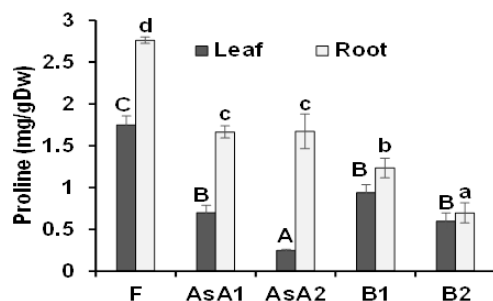


Figure 6: Proline content (mg/g DW) of safflower plants (30-day-old) infected with *Fusarium verticillioides* (F) as affected by two concentrations of ascorbic acid (AsA1, AsA2) and thiamine (B1, B2). The results are means of three replicates (\pm SE). Bars carrying different letters are significantly different at $P < 0.05$.

In general, free amino acids content reduced, as disease infection in the susceptible cultivars and the resistant cultivars at all infection stage (Shukla 2001; Gowily et al. 1995; Mandavia et al., 1990). Bhut, (2005) who have reported in both normal and infected plots, free amino acids content in root tissues decreased as growth of the plants from pre infection (S1) to post infection (S3) in chickpea plant parts at different stages of disease development or growth of the plants. Reddy et al., (2005) proved that *Fusarium solani* infected of turmeric roots resulted in marked increase in all the nitrogen fractions including total nitrogen, protein nitrogen, soluble nitrogen and amino nitrogen. The effect of (Ascorbic acid or thiamine) in safflower root and leaf proline accumulation was shown in Fig.6. Results reveal that an accumulation of proline in infected safflower plants in comparison with the healthy plants. Treatment of safflower infected plants with either ascorbic acid or thiamine (1&3mM) improve and retard proline accumulation to its original value in the absolute control. The highest value was recorded in the *Fusarium* infected plants. Proline is an osmoprotectant in plants, able to balance drought stress (Farghaly et al., 2013; Yashiba et al., 1997) and salt stress (Gadallah, 1999). In a variety of plants, stresses such as cold, heat, salt, drought, UV, and heavy metals significantly increase endogenous proline concentrations. Rathod and Vakharia, (2011) reported that root tissues obtained from infected plot of chickpea that is, inoculated with *F. oxysporum* showed significantly higher proline content as compared to the plants from normal plot at pre infection. Several investigators

reported increase level of proline during disease development (Yurong et al., 2005; Mandavia et al., 1990). In conclusion, ascorbic acid or thiamine are considered as efficient to alleviate the harmful effect of fungal infection on the safflower plants through induced stimulation of dry weight, total photosynthetic pigments, enhancement of soluble sugars, amino acids and reduction of proline accumulation. Thiamine and ascorbic acid application is ecofriendly than fungicides to use for overcoming root rot and wilt diseases of safflower plant. It induced resistance of safflower plant.

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