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Structural, Physiological, and Biochemical Alterations of the Galled Stems of *Tamarix nilotica*

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TAMARIX nilotica (Tamaricaceae) is an important wild medicinal plant. It inhabits semi-arid environments, where it is often one of the most important plants suitable for insect infestation. Thrips (Thysanoptera: Insecta) infect *T. nilotica* and cause the formation of galls, which affect plant growth and development. Therefore, the present work was undertaken to study the morpho-anatomical, biochemical, and physiological changes induced by insect infestation in *T. nilotica*. Zeatin (Zn), gibberellin (GA_3), salicylic acid (SA), 24-epibrassinolide (BR), and abscisic acid (ABA) were increased in the infected stems relative to the healthy ones. Galled stems showed increased thickness of the periderm and phellem layers, whereas a large portion of the vascular cylinder had disappeared, leaving a large C-shaped cavity in which the mature insect nested. Gall sites exhibited decreased chlorophyll content which was associated with a decrease in the emission of chlorophyll a fluorescence. Moreover, total soluble sugars, sucrose, total free amino acids, total soluble protein, and mineral content, as well as the activities of SOD, CAT, PX, PO, APX, and AO, were significantly reduced in the infected *Tamarix* stems, whereas polysaccharides, proline, MDA, and H_2O_2 were increased compared with the healthy stems. Galled stems exhibited increased antiradical activity (expressed as IC_{50}), which was concomitant with the accumulation of phenolics, tannins, alkaloids, and flavonoids and the enhancement of PAL. Six endophytic fungi inhabited *Tamarix* galls. The development of thrips galls on *T. nilotica* stems drastically impacted the host structure and its cellular metabolism and induced oxidative stress. The roles and effects of endophytic fungi on insect-induced galls warrants further exploration.

Keywords: Antioxidant enzymes, Endophytic fungi, Primary metabolites, Stem gall, *Tamarix nilotica*, Thrips.

Introduction

Globally, the larger part of plant biomass losses is attributed to herbivores, particularly insect herbivores, which affect the productivity of ecosystems and the diversity of plant communities (Zvereva et al., 2020). After many years of plant–insect interactions, plants developed advanced defense mechanisms against insect herbivores, which might be either constitutive or induced upon insect infection (Aljbory & Chen, 2018). One of the early events that evolved in response to insect attacks is the formation of galls, which are utilized as nutritional sinks for insects. Economically, gall-inducing insects represent severe threats to agriculture or forestry, as they can suppress plant defenses, consume plant resources, and change the patterns of plant biomass accumulation (Miller III & Raman, 2019).

Plant reactions against herbivore attacks include several signal transduction pathways encompassing a group of phytohormones (War et al., 2020). Plant hormones are involved in all stages of plant–herbivore interactions, starting from the sensing of contact with the insect and pressure and up to the final stages of the process. During the initiation and/or development of a gall induced by an insect, auxins and cytokinins (CKs) are involved in the development of both

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hypertrophy and hyperplasia (Body et al., 2019). Salicylic acid (SA), ethylene, and ABA play a key role in mediating the anti-herbivore plant volatiles that are engaged in the induction of an indirect defense against herbivores (Aljbory & Chen, 2018). These phytohormones may act separately and/or in combination, depending on the herbivore; moreover, the concentration of phytohormones in the galls is highly dependent on gall developmental stage and plant genotype (Mhoswa et al., 2020).

The mechanical wounds and metabolic alterations induced by the insect attack lead to signaling events that are mediated by hormonal changes, thus imposing new morphogenetic modifications (cell hypertrophy and hyperplasia) and resulting in gall formation (Raman, 2011). In addition, the development of galls causes several disturbances in host plant physiology, such as alterations in primary metabolism, nutrient composition, and photosynthetic processes (Costa Rezende et al., 2018), which might be a necessary requirement to tolerate the direct defense machinery and corresponding physiological adaptations (Kerchev et al., 2012a). Correspondingly, the disruption of the host plant physiological processes increases the accumulation of reactive oxygen species (ROS), thus causing oxidative stress (Kerchev et al., 2012b), which damages the homeostasis of gall sites (Isaias et al., 2015) and triggers an overproduction of antioxidants (War et al., 2020).

Insect galls represent stable microenvironmental sinks for plant metabolites, which is favorable for fungal proliferation (Muñoz-Adalia et al., 2019). Fernandez-Conradi et al. (2019) reported that the association between endophytic fungi and insect galls differs from those established with the surrounding tissues, which suggests that the physiological alterations in plant tissues triggered by gall induction are significant determinants of endophyte assemblages. However, detailed studies of the impact of gall makers on endophytic fungi remain scarce (Heneberg et al., 2016; Fernandez-Conradi et al., 2019).

Tamarix nilotica (Tamaricaceae) is known in Egypt as Nile Tamarisk. It is diversely used in traditional herbal medicine (Abdelgawad, 2017), in addition to its recent economic application as a biofuel (Younis, 2020). *Tamarix nilotica* grows in saline sandy soils located on the edges of salt marshes and Nile banks (El Hadidi & Hosni, 1996), thus rendering it one of the most important plants for providing food and shelter to phytophagous insects (Minaei & Mound, 2020). *Tamarix nilotica* stems are infected by thrips, which is a class of well-known gall-forming insects (order, Thysanoptera).

Several thrips species are injurious pests to vegetable, ornamental, and commercial crops worldwide, either via direct damage through feeding on the plants or via their action as vectors of viruses for economically important plants (Riley et al., 2011). Thrips are involved in several types of host associations with plants (Diaz-Montano et al., 2011; Mound, 2020). *Tamarix*-associated thrips have been recently described by Minaei & Mound (2020). However, knowledge on the development of *Tamarix nilotica* stem galls and their subsequent effects on this organ is lacking. Therefore, the present work was undertaken to assess the structural, physiological, and biochemical changes that occur in the stem galls of *Tamarix*, as well as the endophytic fungi that are associated with such galls.

Materials and Methods

Healthy and galled branches of *T. nilotica* were collected in April 2019 from the plants growing around the Cairo–Suez road, Egypt, delimited by latitude 29°58’04.9 N. and longitude 32°08’25.4 E., to analyze the alterations induced by gall-forming insects. This period was selected as the plants were in the flowering stage when gall maturation occurred. The freshly collected branches were immediately placed in liquid nitrogen and brought to the laboratory for storage at −80°C until they were used in biochemical analyses. Other branches were collected in ice boxes in plastic Zip-Lock bags and sealed tightly, for the isolation of endophytic fungi. Some stems were kept in 70% alcohol, for anatomical studies, all collected at the same site and characteristically with the same age. The gall tissue was dissected using clean microdissection needles and observed for the presence of a gall maker. A single insect chamber was observed under the surface of the gall. The gall inducers were identified as thrips, which belong to order Thysanoptera (Thrips Wiki, 2020).

Determination of hormone levels

ABA, GA_3, SA, and zeatin (Zn) were extracted from freshly collected branches according to the method described by Trapp et al. (2014). Endogenous 24-epibrassinolide was determined

using the method reported by Ding et al. (2013). The quantity of each hormone was determined using high-performance liquid chromatography (HPLC; E-Chrom Tech instrument, LC 1620, USA). Samples were assayed against ABA, GA_3, SA, Zn, and 24-epibrassinolide, as internal standards.

Structural changes

To examine the anatomical and cellular changes that occurred in the galled stems compared with the healthy ones, stems were gradually dehydrated in an ethanol series, slowly infiltrated, and embedded in paraffin; subsequently, transverse sections of galled and ungalled samples were prepared on a manual rotary microtome using a razor blade. The 15–20-µm-thick sections were stained using safranine and light green. Characterization of the sections was carried out using a clear camera (digital premiere MA88-900, Italy) coupled to a compound light microscope (Optica, Italy) and submitted to the Image J Analyzer.

Chlorophyll and fluorescence spectroscopy

The levels of chlorophyll a, chlorophyll b, and total carotenoids in leaves were measured spectrophotometrically according to Metzner et al. (1965), and their content was calculated according to the formula of Lichtenthaler (1987). Fluorescence emission spectra were measured as described by Lang et al. (1991) using a spectrofluorometer (Perkin Elmer LS 50B, USA). Red and far-red fluorescence were recorded between 650 and 800nm. The leaf extracts of plants that received the different treatments were excited at λ= 435nm.

Analysis of shoot primary metabolites

Total soluble sugars and polysaccharides were determined using the method of Clegg (1956). Sucrose content was estimated according to Hubbard & Mason Pharr (1992). Total free amino acids were determined using the method of Muting & Kaiser (1963). Proline content was determined according to the method developed by Bates et al. (1973). Total soluble protein was estimated based on the method of Bradford (1976). Na^+, K^+, Ca^{2+}, and Mg^{2+} were measured according to Chapman (1961) using an atomic absorption spectrophotometer (Perkin Elmer 3100, USA).

Analysis of shoot secondary metabolites

The determination of total phenolics was carried out using the method of Makkar et al. (1993). Tannins were measured based on the method of Nwinuka et al. (2005). In turn, the method of Chang et al. (2002) was used for total flavonoid determination. Finally, total alkaloids were determined according to Obadoni and Ochuko (2002).

Lipid peroxidation assay

The malondialdehyde (MDA) content was measured using the method of Heath and Packer (1968).

Determination of ascorbic acid

Ascorbic acid content was measured using the method of Mukherjee & Choudhuri (1983).

Determination of hydrogen peroxide (H_2O_2)

H_2O_2 was estimated based on Velikova et al. (2000).

DPPH radical scavenging assay

The method of Hatano et al. (1988) was used to determine DPPH radical scavenging. The antiradical activity was finally expressed as IC_{50} (mg g^{-1} FW).

Determination of antioxidant enzymes

Antioxidant enzymes were extracted from healthy and infected *Tamarix* shoots using phosphate buffer (pH 7) (1:4 w/v), and the crude extracts were used for enzymatic assays. Superoxide dismutase (SOD, EC 1.15.1.1) was measured according to Kong et al. (1999). Polyphenol peroxidase (PX, EC 1.11.1.7) activity was determined based on the method of Shannon et al. (1966). Polyphenol oxidase (PPO, EC 1.10.3.1) activity was measured using the method of Trejo-Gonzalez and Soto-Valdez (1991). Catalase (CAT, EC 1.11.1.6) activity was assayed according to the method of Aebi (1984). Ascorbate oxidase (AO, EC 1.10.3.3) and peroxidase (APX, EC 1.11.1.11) activities were measured using the methods of Diallinas et al. (1997) and Chen & Asada (1989), respectively. Phenyl ammonia lyase (PAL, EC 4.3.1.5) activity was assayed based on the method described by McCallum & Walker (1990).

Fungal endophyte determination

Fungal isolation

Within 24h of collection, fungal endophytes were cultured from galls and from healthy *Tamarix* stems. The galled and healthy stems were washed under running tap water, a 1-cm long section of standardized sections of healthy stems proximal to the gall was obtained. Surface sterilization was

performed on all samples according to the protocol of Deckert et al. (2001). Under sterile conditions, galled and healthy segments were plated on potato dextrose agar (PDA) plates with chloramphenicol. Two pieces from galls and healthy stems were placed in each petri dish containing PDA. The dishes were incubated in the dark at 28 °C for 4 weeks, and checked every 2 or 3 days until the emergence of microorganisms. When isolates appeared, they were isolated, purified, and maintained on PDA slants, for identification. The genus *Aspergillus* was identified based on cultural characteristics and microscopic observation, according to Raper & Fennell (1965). The identification of other endophytic fungi isolates was carried out at the Mycological Center (AUMC), Faculty of Science, Assuit University, Assuit, Egypt.

Statistical analysis

Statistical analyses were carried out using the SPSS v20.0 software package (SPSS Inc., Chicago, USA), and the comparison of the average values of galled and healthy stems was based on two-tailed unpaired *t*-tests at $P \leq 0.05$.

Results and Discussion

In the current study, the content of the tested hormones was increased in the infected galled stems compared with the healthy ones (Table 1). An elevated GA_3 concentration in the gall tissues relative to the controls (Table 1) suggests a critical role for GA_3 in gall development and immune responses (Hou et al., 2013). In turn, increased SA levels in the gall tissues indicate the induction of the SA biosynthetic pathways that are triggered by attacking herbivores and enhance the defense responses against insect species (Ederli et al., 2020). ABA and Zn were slightly increased in the galled stems (Table 1); increased ABA and cytokinin levels were also observed in galls induced by different insects on several plant species (Tokuda et al., 2013; Body et al., 2019), which may be attributed to plant defense responses to gall-inducing insect feeding (Appel et al., 2014). Furthermore, the increase in Zn levels in *T. nilotica* galled tissues might affect herbivory-induced defenses indirectly by controlling the resources associated with strong sink strength, which can increase the synthesis of defense compounds (Arnold et al., 2004). The elevated BR levels detected in the gall tissues agreed with previous findings reported by Yokota et al. (1982), who reported BR accumulation in insect-induced chestnut galls. BR signaling positively contributes to the production of antioxidant metabolites (Aljbory & Chen, 2018), in addition to its crucial role in regulating the JA and SA pathways and native immune defenses in response to insect infestation (Miyaji et al., 2014). Similarly, the role of BRs in the defense response against herbivores has also been described in previous studies (Divi et al., 2016; Gruszka, 2019).

The hormonal alterations induced by the herbivore attacks eventually manifested as morphological and anatomical changes in the infected stems. The stems of infected *T. nilotica* shrubs showed many fusiform-shaped galls that were mostly located on young branches. Anatomical differences were detected between galled and healthy stems: the healthy stems were rounded in transection, solid in pith (Fig. 1a), with a periderm containing 3–9 layers of phellem with a thickness of 30.3µm, 1–2 layers of phellogen with a thickness of 2µm, and 3–10 layers of phelloderm with a thickness of 25.5µm (Fig. 1b). The vascular cylinder of healthy stems consisted of many collateral vascular bundles arranged in a circle penetrated by medullary rays (Fig. 1a). Each vascular bundle consisted of many secondary phloem elements with traces of a primary one, 1–2 layers of vascular cambium, and many secondary xylem elements with patches of the primary one, with each bundle being covered by a fiber cab (Fig. 1b). In contrast, the mature galls of infected stems had a large diameter (3–6mm) (Fig. 1c). Moreover, a large portion of the vascular cylinder had disappeared, leaving a large C-shaped cavity in which the mature insect nested (Fig. 1c, d). Galled stems exhibited an increase in the thickness of the periderm, an increment in the number of phellem layers to 8–12, and few crashed old phellem layers with a thickness of 48 µm (Fig. 1e, f). Remnants of xylem elements of many vascular bundles were observed attached to the narrow parenchymatous pith, and few phloem elements were detected, as only 4–5 complete vascular bundles remained that connected the pith with the periderm; moreover, the fiber caps had disappeared (Fig. 1e). Furthermore, the number of phellogen layers increased to 1–3, with a thickness of 7 µm, whereas the phelloderm tissue directions and layers were unrecognized and became wider (110-µm thickness) (Fig. 1f). Such anatomical alterations have been reported to promote the trophic activity of the inducer (Isaias et al., 2014). Structural changes in galls induced by other insects have also been reported by Guedes et al. (2018) and Muthukumar et al. (2020).

TABLE 1. Content of phytohormones, photosynthetic pigments, primary metabolites and elemental analysis of Tamarix nilotica healthy and infected branches. Data are means ± SD of three replications

Phytohormones (ng g^{-1} FW)	Healthy branches	Infected branches
Abscisic acid (ABA)	56.17 ± 1.17	60.32 ± 0.30*
Zeatin (Zn)	76.41 ± 2.01	108.12 ± 3.02*
Gibberellic acid (GA_3)	22.77 ± 0.70	38.46 ± 1.10*
Salicylic acid (SA)	7.98 ± 0.08	9.09 ± 0.50*
24-epibrassinolide (BR)	1.46 ± 0.03	3.54 ± 0.16*
Photosynthetic pigments (µg g^{-1} DW)		
Chl. a	187.85 ± 2.15	133.27 ± 7.25*
Chl. b	51.35 ± 2.74	28.31 ± 3.69*
Chl. a+b	239.21 ± 0.59	161.58 ± 3.56*
Chl. a/b	3.66 ± 0.23	4.78 ± 0.88*
Carotenoid	48.34 ± 1.53	37.33 ± 1.8*
Chl. (a+b)/c	4.95 ± 0.16	4.29 ± 0.12ns
Primary metabolites (mg g^{-1} DW)		
Total soluble sugar	34.53 ± 2.5	31.80 ± 3.2*
Sucrose	62.08 ± 7.30	53.58 ± 7.20*
Polysaccharide	100.80 ± 7.50	127.25 ± 9.25*
Total free amino acids	0.606 ± 0.043	0.560 ± 0.041*
Total soluble protein	11.41 ± 0.62	7.063 ± 0.53*
Elemental analysis (mg g^{-1} DW)		
Na^+	2.93 ± 0.03	2.87 ± 0.02 ns
K^+	1.32 ± 0.02	0.77 ± 0.005*
Ca^{2+}	2.08 ± 0.04	1.08 ± 0.03*
Mg^{2+}	0.49 ± 0.05	0.27 ± 0.01*

*, data are significantly different at P≤ 0.05, ns, non-significant.

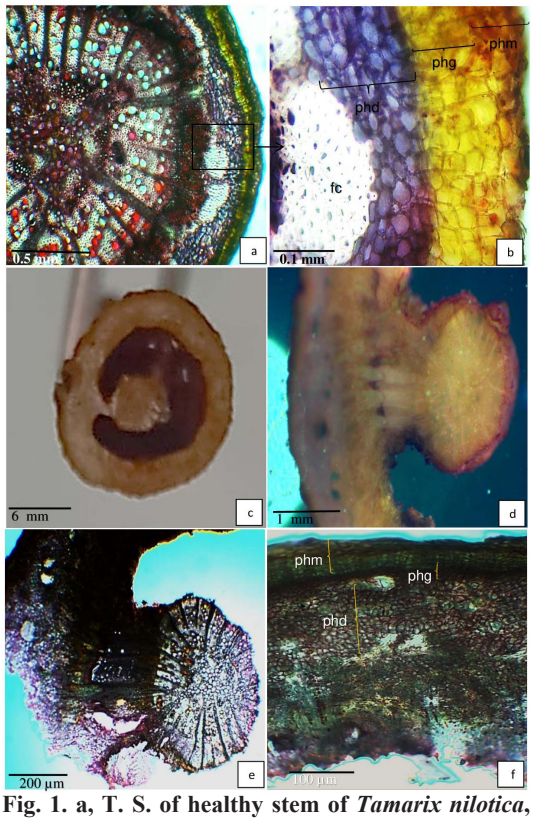

Fig. 1. a, T. S. of healthy stem of *Tamarix nilotica*, b: Magnified T. S. showing periderm, c: T. S. of *T. nilotica* fusiform-shaped galls, d: T. S. of infected *T. nilotica* stem, e: Magnified T. S. of infected stem showing remains of vascular bundle, f: Magnified T. S. of infected stem showing periderm layers [ph= Phloem, xy= Xylem, phm= Phellem, phg= Phellogen, phd= Phelloderm, mr= medullary ray]

The feeding activity of thrips in stem galls of *Tamarix* phloem tissues most likely changes the balance between source and sink tissues (Oliveira et al., 2017) and reduces the photosynthetic capacity in the tissues that surround the gall (Costa Rezende et al., 2018). A low content of photosynthetic pigments in infected *Tamarix* leaves (Table 1) is a common physiological disorder that occurs after insect attack (Costa Rezende et al., 2018). Such decline in chlorophyll content in gall developmental sites was associated with increased emission of chlorophyll a fluorescence (Fig. 2), which implies a reduction in the conversion of the excited light energy into chemical energy as an injurious result of the insect attack (Murchie & Lawson, 2013). Some studies have also reported a significant inhibitory effect of insect feeding on PSII activity and photosynthetic pigments (Kot et al., 2018; Martini et al., 2020).

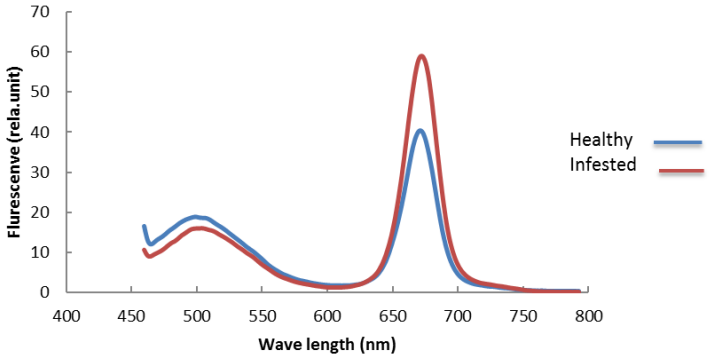

Fig. 2. Room temperature red fluorescence emission spectra of freshly shoots from non-galled and galled *Tamarix* plants [Ex λ=435nm]

Galling insects have been found to assess their required nutritional resources from the host plants; therefore, the reduction in total soluble sugars, sucrose, total free amino acids, and total soluble protein observed in the galls of *Tamarix* plants (Table 1) were most probably caused by the drainage of the assimilates by the thrips. A similar reduction in nutrient levels in the galls formed on other plant species was reported in previous studies (Kot et al., 2018; Body et al., 2019). Furthermore, it has been demonstrated that soluble sugars are utilized in feeding the secondary metabolism (War et al., 2020), which strongly agrees with the elevated levels of phenolics, tannins, alkaloids, and flavonoids detected in the galled stems of *T. nilotica* in the present study (Table 2). Other published works similarly reported the induction of phenolic compounds in plant parts infected by different insects (Kot et al., 2018; Coelho Kuster et al., 2020). Arnold et al. (2012) proposed that plant phenolic accumulation might be an adaptive strategy to sequester the excess carbon that accumulates in the gall. The reduced level of Na^{+}, K^{+}, Ca^{2+}, and Mg^{2+} detected in *Tamarix* shoots (Table 1) may be explained by a reduction in their absorption by the plant and/or their utilization by the insects for nutrition (Semhi et al., 2019). It is worth noting that the observed decrease in Mg^{2+} levels was accompanied by a low pigment content in infected *Tamarix* branches; Mg^{2+} is an essential element in chlorophyll biosynthesis. A decrease in mineral uptake in response to insect infestation has been consistently demonstrated in other plants (Khattab, 2007; Xu et al., 2018).

Thrips infestation has been shown to induce ROS production, resulting in oxidative stress, which was consistent with the elevated content of H_2O_2 and MDA detected in the galled *Tamarix* stems (Table 2). An increase in H_2O_2 level in the galls is indicative of the oxidative stress imposed by the galling insects, as reported by War et al. (2012) and Isaias et al. (2015). The increased proline content observed in infected *Tamarix* galls (Table 2) most likely had an adaptive function, because proline has been reported as a universal osmolyte that accumulates in response to several stresses, serves as an energy source, and plays a protective role as a hydroxyl radical scavenger (Mansour & Salama, 2020). Proline accumulation has also been observed in several infested plants (Khattab, 2007; Bufon et al., 2020).

Table 2 shows that the galled tissues exhibited the highest radical scavenging capacity (a lower IC_{50} reflects a higher antioxidant activity in the DPPH assays) compared with the healthy ones. These results indicate the participation of non-enzymatic antioxidants (proline and phenolic compounds, Table 2) in the scavenging of ROS in the gall extracts, and interpreted their overproduction in the galls compared with enzymatic antioxidant activities, which were significantly reduced (Table 2). The reduction in ASA content and in the activities of its related enzymes, AO and APX, in the galled stems (Table 2) is suggestive of role absence in the defense mechanism against herbivorous thrips in *T. nilotica* galls. In addition, the reduced activities of SOD, CAT, PX, and PPO antioxidant enzymes observed in the galled stems of *T. nilotica* (Table 2) indicate that non-enzymatic antioxidants, rather than antioxidant enzymes, contributes to ROS detoxification. It is proposed that gall-inducing species may suppress the enzymatic defensive response in host plants to meet their own needs. Interestingly, PAL activity was enhanced in galled *T. nilotica* tissues compared with ungalled ones (Table 2), which explains the increased level of SA detected in *T. nilotica* galls, as PAL is involved in SA biosynthesis (Chen et al., 2009). Moreover,

PAL triggers the secondary metabolism cascades (Hernández-Soto et al., 2015), which is consistent with the biosynthesis of lignin observed in the galled stems (Fig. 1f).

TABLE 2. Content of antioxidant parameters of *Tamarix nilotica* healthy and infected stems

Antioxidant parameters	Healthy stems	Infected stems
Malondialdehyde (MDA) (µg g^{-1} FW)	12.13 ± 0.13	15.44 ± 0.95*
H_2O_2 (m mole g^{-1} FW)	28.89 ± 0.56	36.11 ± 1.11*
Proline	0.151 ± 0.08	0.265 ± 0.15*
Phenolic contents (mg g^{-1} DW)	16.5 ± 0.008	29.4 ± 0.018*
Tannins (mg g^{-1} DW)	2.87 ± 0.037	3.80 ± 1.80 *
Total alkaloids (mg g^{-1} DW)	2.93 ± 0.020	3.47 ± 0.41*
Total flavonoids (mg g^{-1} DW)	3.72 ± 0.97	4.21 ± 0.57*
Ascorbic acid (µg g^{-1} FW)	130.77 ± 1.80	73.82 ± 1.38*
IC_{50} (µg g^{-1} FW)	44.54 ± 0.41	29.58 ± 0.41*
SOD (U g^{-1} protein)	1597.17 ± 5.66	949.48 ± 2.63*
PPO (a g^{-1} FW min^{-1})	0.17 ± 0.01	0.081 ± 0.001 *
PX (a g^{-1} FW min^{-1})	1.06 ± 0.11	0.89 ± 0.001*
CAT (µM of H_2O_2 g^{-1} FW)	4.06 ± 0.34	2.75 ± 0.27 *
AO (a g^{-1} FW min^{-1})	2.16 ± 0.014	2.01 ± 0.014*
APX (a g^{-1} FW min^{-1})	4.00 ± 0.04	3.61 ± 0.036 *
PAL (a g^{-1} FW min^{-1})	2.68 ± 0.08	3.18 ± 0.04 *

Data are means ± SD of three replications.

* means that data are significantly different at P≤ 0.05.

The investigation of the presence of endophytic fungi by culturing (Fig. 3), followed by their isolation and identification led to the detection of six species in the galls, namely, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Rhizoctonia solani*, *Penicillium chrysogenum*, and *Penicillium digitatum*; however, no fungal endophytes were detected in the healthy branches. The occurrence of such fungal infections is most likely associated with gall conditions exclusively. Similarly, Seifert et al. (2004) reported the association of various *Penicillium* species with galls of *Cynipidae* species on *Eucalyptus* and *Rosa* leaves. Moreover, the molds of the genera *Penicillium* are facultative gall associates, as they have the ability to grow in habitats with limited water availability (Heneberg et al., 2016). The current work documented for the first time the association between such fungal species and *Tamarix nilotica* galls. However, further research is needed to elucidate their effects and roles on herbivorous insects and their host plants.

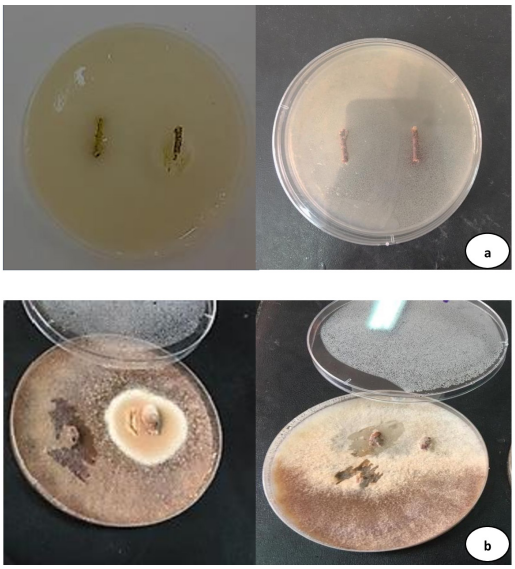

Fig. 3. Fungal endophytes isolated from a: Healthy- and b: Galled-stems of *Tamarix nilotica*

Conclusion

The development of thrips galls on *T. nilotica* stems had a negative effect on the host plant, as reflected in the disruption of photosynthesis, modification of the levels of metabolic substances in the surrounding tissues, and reduction in the enzymatic antioxidant potential of the host plant. Further cytological research is needed to characterize the inducible defense mechanisms and the host targets, which may contribute to the development of cost-efficient thrips-resistant crops. Finally, the roles and effects of endophytic fungi in insect-induced galls remain an open question.

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التعديلات الهيكلية والفسيولوجية والبيوكيميائية على جذع المرارة من التامريكس نيلوتيكا

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تامريكس نيلوتيكا هو نبات طبي بري مهم. وهو يسكن بيئة شبه قاحلة ، حيث غالبا ما يكون أحد أهم النباتات المناسبة لتغشي الحشرات. تصيب الثربس نبات تامريكس نيلوتيكا وتسبب المرارة التي تؤثر على نمو النبات. ولذلك تم الاضطلاع بالعمل الحالي لدراسة الجوانب التشريحية والبيوكيميائية والفيزيولوجية الناجمة عن الإصابة بالحشرات قد تبين زيادة الزيتين، الجبريلين، حمض الساليسيليك، البرسينوستيرويد وحمض الابسيسيك في السيقان المصابة نسبة إلى السيقان السليمة. أظهرت المرارة زيادة سمك طبقات البريدرم والفلوديرم في حين اختفى جزء كبير من الاسطوانة الوعائية تارك تجويف كبير على شكل C حيث تسكن الحشرة الناضجة. وقد انخفضت محتويات الكلوروفيل التي ارتبطت مع انخفاض في انبعاث الفلوروسنس fluorescence في المرارة. في حين أن السكريات القابلة للذوبان الإجمالية، والسكروز، والأحماض الأمينية الحرة الإجمالية، والبروتين القابل للذوبان الكلي ومحتويات المعادن، فضلا عن أنشطة SOD، CAT، PX، PO، APX، وAO انخفضت بشكل كبير في السيقان المصابة، تم زيادة الكربوهيدرات، البرولين، المالوندايلدهايد والهيدروجين بيروكسيد مقارنة مع السليمة منها. أظهرت المرارة زيادة النشاط المضاد للشوارد والتي كانت متزامنة مع تراكم الفينولات، التنين، القلويدات، الفلافونويدات وتعزيز PAL. ستة فطريات شّنائية تسكن مرارة التاماريكس. تطور الثربس على التامريكس نيلوتيكا يؤثر بشكل كبير على استقلاب الهيكلية والخلوية للمضيف وكذلك تسبب الإجهاد التاكسدي. لا تزال أدوار وتأثيرات الفطريات الشّنائية على المرارة المستحثة بالحشرات بحاجة إلى مزيد من الاستكشاف.