ASSESSMENT OF PHYTOHRMONES OF *TAMARIX APHYLLA* (L.) H.Karst., 1882 INFECTED WITH *PRAPODIA SINAICA* (Frauenfeld, 1859) (GELECHIIDEA: LEPIDOPYERA) IN SOUTH OF SINAI

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ABSTRACT

Tamarix aphylla is a medicinal plant that grows well in arid areas and is widely regarded as one of the most significant plants for insect invasion. *Tamarix aphylla* trees cover a huge portion of the sand dunes in the Wadi Asel region in southern Sinai. *Parapodia sinaica* attacks *Tamarix aphyllla* and forms galls that hinder the growth and development of the plant. The aim of this study is to investigate the changes in phytohormone levels in *Tamarix aphylla* plants caused by insect infestation, especially by *Parapodia sinaica* insects that cause the formation of galls. In this study, indole-3-acetic acid (IAA), gibberellic acid (GA3) and Zeatin (Zn) were used in induced and non-induced plants during two distinct seasons, the dry and wet seasons between March and August 2021. The amount of each hormone was determined by high-performance liquid chromatography (HPLC). The level of (IAA) increased more in healthy plants in the wet season than in the dry season and decreased in infected plants. Zeatin (Zn) showed the same trend as in hormone (IAA). However, hormone (GA3) showed the same trend in both healthy and infected plant. The role and effects of entophytic fungus on galls caused by insects should be further investigated.

Keywords: *Tamarix aphylla*, Phytohormone, Gall-inducing insect, *Parapodia sinaica*.

INTRODUCTION

Tamarix aphylla belongs to the Tamaricaceae family and is native to Asia, northern Africa, and southeastern Europe (Jasiem *et al*., 2019). It is commonly known as tamarisk and is used by locals for therapeutic purposes (Bahramsoltani *et al*., 2020). Desertification, salinity, toxicity, drought, heat, nitrogen deficiency, flooding, sandstorms, and frost are all environmental stressors for *Tamarix aphylla*

Vol. (52); Iss. (10); No. (4); Oct. 2023
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

growing in the wild (Kuzminsky *et al*., 2014). Tamarix species have also been shown to be highly resilient to a variety of biotic stressors (Zhang *et al*., 2002). This makes the study of their species diversity and inherent tolerance to certain stressors quite interesting (Bencherif *et al., 2020).*

T. aphylla thrives on saline sandy soils at the edges of salt marshes (El Hadidi and Hosni, 1996). Therefore, it is one of the most important plants providing food and shelter to phytophagous insects (Minaei and Mound, 2020). Galling insects are a type of herbivores (entophytic herbivores) that necessarily form galls on the host plant to complete their life cycle (Crneiro *et al*., 2009). Certain insect groups can promote the formation of a new structure on plant organs, called a gall, by disrupting the non-galligerous growth of the affected plant organ.

Parapodia sinaica insect (Lepidoptera: Gelechiidae), induces a spindle gall on the stem of *Tamarix*. (Fusu, 2017p Bidzilya *et al.,* 2019 ; Kamel *et al*., 2021 ; Baugnée *et al*., 2022). For the first time, *P. sinaica* (the host of *T. tamaricis* sp.) is reported to develop galls on *Tamarix canariensis* Willd (Fusu *et al*., 2017).

Plant hormones are chemical compounds produced by plant tissues in response to external stimuli (Khan *et al*., 2020). Herbivores can produce plant hormones or hormone mimics to manipulate the host defense responses (Gätjens-Boniche, 2019). Plant hormones play a role in all phases of plant-herbivore interactions, from the initial detection of insect contact and pressure until the very end of the process. When an insect produces a gall, several chemicals, including indol acetic acid (IAA), gibberellin (GA_3), and zeatin (Zn), are involved in the development of both hypertrophy and hyperplasia (Body *et al*., 2019). The current study aimed at evaluating phytohormone changes caused by insect infestation in *T. aphylla* during wet and dry seasons.

MATERIALS AND METHODS

Sampling site: Healthy and infected plants were collected in the middle of the dry desert. Freshly collected branches were immediately stored in ice boxes in zip-lock plastic bags and tightly sealed. Fig.(1) showed the location of the samples were collections were down in the town of Ras Sudr in South Sinai Governorate. This area is about 50 km from the Ahmed Hamdi tunnel. Fresh samples of healthy and infected strains of *Tamarix aphylla* were collected in March (rainy season) and August (dry season) 2021 at the Wadi-Asal site bounded by latitude 29□ 32 '47.57 "N, longitude 032□ 48 '37.6"and Altitude 174.

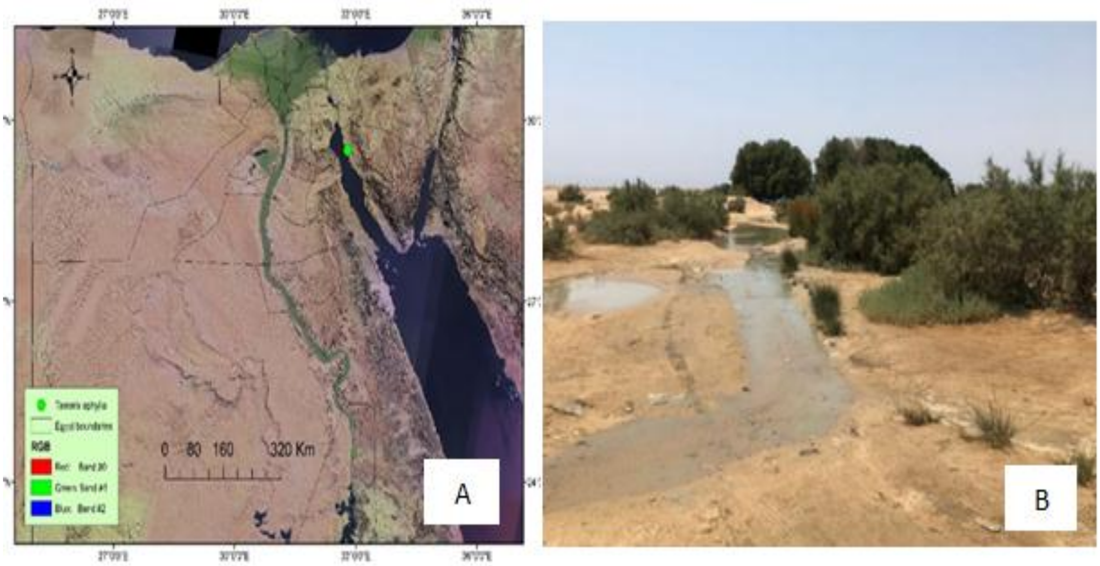

Figure (1): A: Location map showing sampling sites for *T. aphylla*.in the Wadi-Asel in South of Sinai(Map source: google Maps). B: *T. aphylla* community. This picture was taken from the collection sites during the year 2021 rainy season

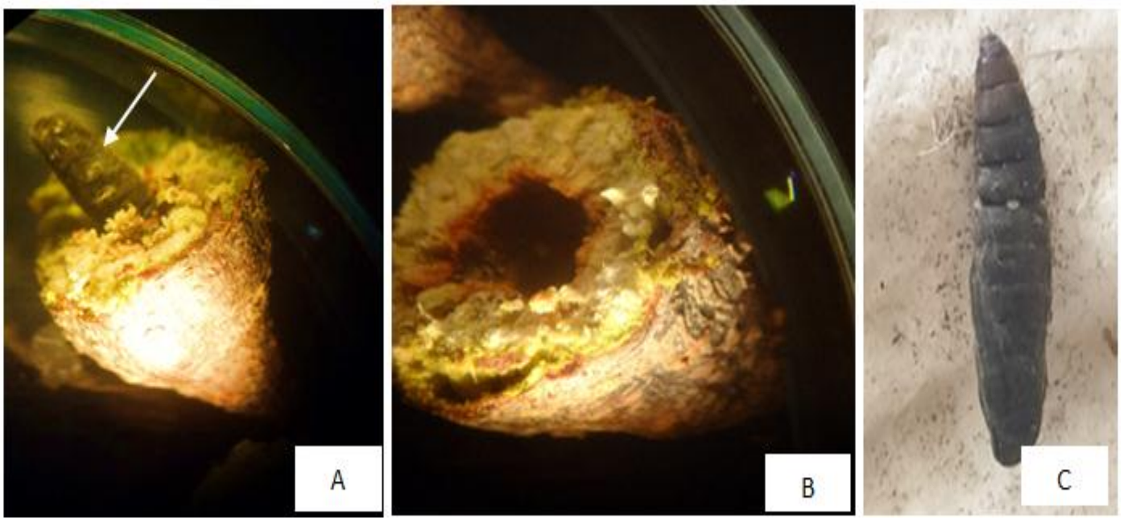

Figure (2): A: cross section of gall induced with *Parapodia sinaica* white arrow illustrate pupa. B: Empty chamber after removal of pupa of *Parapodia sinaica ,and* C: pupa (10 mm) of *Parapodia sinaica.*

The life cycle of this insect is characterized by a long duration, which can last more than a year (larvae (5) mm diameter), which transform into pupae. Fig (2) C) (10) mm) diameter) in early March and then into adult moths (12 mm diameter) in May (kamel *et al*.,2021), In Fig (2) (A)illusterated the stage of Pupa wthe the gall and Fig(2) B) showing the only one empty chamber of gall after removal the pupe. The larva prepares an exit aperture and turns a tube that extends slightly from the opening before pupation. The pupa lies down in this tube with its head pointing toward the exit. This period was chosen because the galls matured during the flowering period of the plants. The larvae of Lepidoptera species possess two pairs of gustatory sensilla (medial and lateral sensilla styloconica) located in the maxillae galea; these sensilla play a crucial role in larval food selection (Yang *et al.*, 2020 ,Schoonhoven and van Loon, 2002,).

Sample preparation : Galled and non-galled branching three replictes samples were collected separately in plastic Zip-Lock bags from the same site in the same year during March and August 2021. Indol acetic acid (IAA), Gibberellin (GA_3), and Zeatin (Zn), were extracted from freshly collected branches according to the method described by (Trapp *et al*. (2014) extraction of phytohormones: composition of the extraction solution and type of plant samples (fresh or dry material). Initially, tubes containing 100 ± 1 mg of plant material were either stored at −80°C or dried overnight in a freeze dryer at −42°C. Extraction was performed by adding 1.0 mL of ethyl acetate, dichloromethane, isopropanol, MeOH, or MeOH:water (8:2) to each tube containing dry or fresh plant material. Samples were shaken in a Starlab shaker for 30 min and centrifuged for 5 min at 16,000 g and 4°C. The supernatant was transferred to a new 1.5-microcentrifuge tube and dried in Speed-Vac. After drying, 100 µl MeOH was added to each sample, homogenized under vortex, and centrifuged for 10 minutes at 16,000 g and 4 °C. The quantity of each hormone was determined using high-performance liquid chromatography (HPLC; E-Chrom Tech instrument, LC 1620, USA). Samples were assayed against Indol acetic acid (IAA), Gibberellin (GA_3), and Zeatin (Zn) as internal standards.

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

The content of the three tested hormones (IAA, GA_3, and Zn) were estimated in both the infected galled stems and the non-galled ones during wet and dry seasons **Table (1)**: show the relative retention times and area percentage of plant hormones detected in extracted of galled and non-galled plants tissues of *Tamarix aphylla*.

Table (1): content of phytohormone (IAA,GA3 and Zn)of gall and non-gall tissues of T.aphylla during wet and dry seasons data are means± SD of three replications

Sample	IAA	GA_3	Zn
Non-galled plant in wet season	3.32±0.01[b]	222.3±1[b]	3.25±0.005[d]
Galled plant in wet season	3.31±0.01[b]	248.67±0.67[a]	5.03±0.01[a]
Non-galled plant in dry season	3.18±0.01[c]	129.36±0.66[c]	4.47±0.005[c]
Galled plant in dry season	3.61±0.003[a]	103.09±0.95[d]	4.97±0.005[b]
LSD	0.007	0.68	0.006

Values in the same column with the same letter is non-significant .

As shown in Table(1), Indol acetic acid (IAA) level was higher in healthy plant than infected one during wet season, However, this increase was non-significant. The opposite was observed during the dry season, as the hormone increased in the infected plant than in the healthy plant.

Gibberellin (GA_3): The results showed a significant increase of GA_3 during the wet season in the infected plant than in the healthy one, and the opposite in the dry season where the hormone decreased in the infected plant compared to the healthy plant.

And Zeatin (Zn): is adenine-type of cytokinins the level of Zn, The level of the hormone in the time of dry is higher than the rates of all other samples.

Vol. (52); Iss. (10); No. (4); Oct. 2023
ISSN 1110-0826
ONLINE ISSN 2636 - 3178

DISCUSSION

One goal of phytohormonal ecology is to study the interactions between biotic and abiotic stresses at hierarchical levels of biological organization. From an ecological perspective, exposure to one stress can alter the plant's probability of being exposed to another stress (Thaler and Bostock, 2004). The role of phytohormones in mediating plant defense responses against insect herbivores has greatly improved in recent years (Erb *et al*., 2012)

There is compelling evidence that gall-induced tissues contain higher concentrations of indole-3-acetic acid (IAA) and/or gibberellins (GAs) compared to non-galled tissues (Takei *et al*., 2015; Xiang et al., 2020). Galls serve as plant sink organs capable of absorbing nutrients and phytohormones from other plant organs (Hirano et al., 2020).However, the precise role of phytohormones in gall formation remains unknown (Wang *et al*., 2022).

Although it has been hypothesized that gall-forming insects can alter the balance of phytohormones to keep plant tissues in the sink status, the manipulation's methods are largely taxa-independent (Oliveira *et al*., 2016). Higher than average (IAA) levels concentrations in biliary tissues indicate the induction of (IAA) biosynthetic pathways triggered by attacking herbivores that enhance defense responses against insect species (Ederli *et al*., 2020)

Increased GA3 concentration in gall tissue during the rainy season compared to controls suggests a critical role of GA3 in gall development and immune responses (Hou *et al.*, 2013). The decrease in GA3 concentration in the infected plant during the dry season is an attempt by the host tree to slow the growth of the gall, and this host tree response is compensated by the size of the aphid population, resulting in less down-regulation within the gall (Wang *et al.*, 2016).

CONCLUSION AND RECOMMENDATION

Healthy plants had higher amounts of Gibberellin (GA3), Indol acetic acid (IAA), and Zeatin (Zn) throughout the wet season, while infected plants had higher levels of GA3 during the wet season and lower levels during the dry season. Three different phytohormones were produced and accumulated in greater amounts as a result of the growth of galls on the stems of T. aphylla. Some of these hormones function as covert insect defenses. In order to create nutrient-rich tissue for herbivores to eat, other phytohormones are raised. Finally, it's still unclear what role insect-induced galls play and what impact they have. It is yet unclear how phytohormones engage in these processes. I advise more investigation into this complex cycle.

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تقييم الهرمونات النباتية فى نبات تامركس افيلا المصاب بحشرة البارابوديا سينيكا(جلاشيديا ليبوديبترا) فى منطقة جنوب سيناء –مصر

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المستخلص

تامركس أفيلا هو نبات طبي ينمو بشكل جيد في المناطق القاحلة وتغطي أشجاره جزءًا كبيرًا من الكثبان الرملية بمنطقة وادي عسل بجنوب سيناء. يعتبر أحد أهم النباتات المعرضة لغزو الحشرات ومنها حشرة بارابوديا سينيكا التي تهاجم النبات وتشكل عفصات تعيق نمو النبات وتطوره. تهدف هذه الدراسة إلى تقييم التغيرات التى تحدث في مستويات الهرمونات النباتية في نباتات تامركس أفيلا الناتجة عن الإصابة الحشرية، وخاصة حشرة بارابوديا سينيكا التي تسبب تكوين العفصات. في هذه الدراسة تم تقييم ثلاث هرمونات وهى: حمض الإندول-٣-الخليك (**IAA**) وحمض الجبريليك والزيتين في النباتات المستحثة وغير المستحثة خلال موسمين: الموسم الجاف والموسم الرطب في شهري مارس وأغسطس ٢٠٢١ على التوالي. تم تحديد كمية كل هرمون بواسطة الاستشراب السائل عالي الأداء. ارتفع مستوى حمض الإندول-٣-الخليك في النباتات السليمة في موسم الأمطار عنه في موسم الجفاف وانخفض في النباتات المصابة. وأظهر الزيتين نفس الاتجاه؛ أما هرمون حمض الجبريليك فقد أظهر نفس الاتجاه في كل من النباتات السليمة والمصابة. مما سبق يتبين انه ينبغي إجراء مزيد من الدراسة حول دور وتأثيرات الفطريات المستنبتة على العفصات التى تسببها الحشرات.