

## Improving seed palm germination using biomaterials Fenugreek seed extract (*Trigonella foenum-graecum* L.)

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The Barhee cultivar of date palm (*Phoenix dactylifera*) holds significance due to its nutritional value. This study explores the impact of Fenugreek (*Trigonella foenum graecum* L.) seed extract on the growth of Barhee seedlings. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of Fenugreek seed extract at various concentrations (0.0%, 0.5%, 1%, 1.5%, 2%) revealed pronounced effects on the vegetative growth and phytochemical composition of Barhee seedlings. Our findings demonstrate a positive correlation between the Fenugreek seed extract treatments and the growth parameters of Barhee seedlings, particularly noteworthy when concentrations were below 2%. Notably, chlorophyll content increased by 58%, while soluble carbohydrates and proteins saw improvements of 55% and 48%, respectively. The antioxidant activity, measured through catalase (CAT) and superoxide dismutase (SOD), also exhibited enhancements. Phenolic compounds registered a substantial increase of 47%. Moreover, Fenugreek seed extract contributed to membrane integrity by reducing levels of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and malondialdehyde (MDA), indicating a positive impact on oxidative stress. These results collectively underscore the beneficial effects of Fenugreek seed extract on the growth and physiological aspects of Barhee date palm seedlings. In conclusion, this study highlights the potential of Fenugreek as a growth-enhancing agent for date palm cultivation, specifically the Barhee variety. The positive outcomes observed in various parameters suggest that incorporating Fenugreek seed extract at appropriate concentrations could be a valuable strategy for optimizing the productivity and quality of date palm crops.

**Keywords:** Date palm, fenugreek seed extract, allopathic compound, growth parameter, phytochemicals, Vegetative growth, Phytochemical composition, Growth parameters, Soluble carbohydrates, Catalase (CAT), Oxidative stress.

### INTRODUCTION

*Phoenix dactylifera* L. barhee cv. is one of the date palm plant it belongs to a family Aracaceae, it is characterized by its great economic value and is spread in large parts of the world especially the middle east. It is a tree with a single stem bearing large pinnate leaves located at the top of the stem, it is a dioeciously, monocot plant that includes several species and varieties, cv. barhee is one of the most desirable varieties because of the high nutritional value in its fruits (Johnson, 2011). An important feature of the plant is that it has ability to synthesize organic compounds and molecules, their carbon structure is called a secondary metabolites, these compounds are important for the life of the cells as well as play an interactive role with the neighboring organisms and have a role either defensive or supportive of growth and these compounds have low molecular weight and vary among plant species (Xu et al., 2023). Some secondary compounds act as

support for the growth of other plants adjacent to it because these compounds have a behavior allelo chemicals, as it stimulates or inhibits other plant growth, it also plays an active role in the plants defense mechanism (Ridanour and Call away., 2011). There are many studies that have proven that compounds allopath contribute to the ability to support plant growth and increase its productivity (Hiero and Cilaway, 2003).

It can be defined allopathic compounds as chemical reactions that occur between the plants and other organisms, and this characteristic can be support other plant to modulate growth or increase productivity, also we can define allopath, an ecological phenomenon can effect direct or in direct in some plant (Hamida, 2019), and there are studies that have proven the positivity of these compounds in attributing the growth of other organisms. Generally this compound which plant can products it can called secondary metabolites, produced by plants in special reactions, and they are final products that



have a role in the physiology of plants and other organisms. Most of these products are characterized by the presence of one or more rings, most of which have aromatic rings, or they have a closed benzene ring linked directly to the aromatic ring, which is characterized by the presence of a hydroxyl ring as well as containing CH<sub>3</sub> OR COOH (Zhiqiang, 2021). One of the most important nutrients are nitrogenous compounds have effect on the productivity of various plant (Wang *et al.*, 2020), also phenolic compounds constituents among these allelochemicals can be accumulated in many parts of tissues of plant such as seeds (Omezzine *et al.*, 2014). Allelochemicals can be extract into environment from plants such as roots or aerial parts, these compounds can effect of other plant community.

*Trigonella foenum-graecum* L. plant herbaceous, an annual dicotyledonous 30cm long branched trifoliolate ovate alternate, white flower, legume fruit Chemically have many component carbohydrate 45-60%, mucilaginous fiber galactomannan, proteins (lysine, tryptophan 20-30%), lipid 5-10%, phenols (coumarin, scopolamine) and flavonoids (lutein quercetin and apigenin, and alkaloids trigonelline, steroidal saponin, volatile, minerals and vitamins. oils This plant rich with secondary metabolites used in pharmaceutical in worldwide, also used as food for human and animal feed purposes Seeds of Fenugreek characterized by phytochemical compound that have antioxidant properties (Reta *et al.* 2019). Because of a great importance of the date palm cv. barhee, and because of slow growth process of this plant, this study came to clarify some methods that contribute to accelerating growth using the allopathic effect of Fenugreek seeds.

## MATERIALS AND METHODS

The seeds of fenugreek plant and seeds of date palm (cv. barhee) were obtained from an agricultural nursery (a commercial supplier). The seeds of fenugreek plant (500g) are ground to a powder and the extract prepared by methanol alcohol, and evaporate in vacuum at 45 °C, a residue was dissolved in 100ml distilled water this residue as regarded 100% seeds extract (w/v). Uniformly selected 60 seeds of date palm cv. barhee were surface-sterilized for 2min in 2% (v/v) sodium hypochlorite then rinsed thoroughly with tap water then double distilled water seeds replicates were placed in 9cm petri dishes (after sowing with tap water 12h) containing one disk of Whatman No.2 filter and covered with other filter paper with irrigated by 5ml of D.W. After week, 2mm radicle emerged from the seeds were considered germinated seeds, then the seeds were transferred to pots filled by mixed soil sand with clay (1:2 w/w), then irrigated with many concentration of fenugreek seeds extract (0.0, 0.5, 1, 1.5, 2%) then incubated in growth chamber at 22±5°C by 12 photoperiod and 85% humidity for four weeks. Dry weight of a fine powder seeds of fenugreek plant used for GC mass

analysis to study the types of compounds of Fenugreek seeds qualitatively and quantitatively.

**GC-Analysis (Gas Chromatography-Mass Spectroscopy analysis):** Methanol use as a solvent to extract seeds of Fenugreek, seeds were dried and ground by using an electric grinder, 10gm of the powder was dissolved in 100ml of methanol, extracts was filtered, then incubated at 40°C.

GC-MS analysis was conducted by Thermo Scientific Trace GC Ultra/ISQ Single Quadrupole MS – TG-5MS, the column was used silica capillary column 30mm 0.25 mm 0.25µm film thickness, then complete the extract of Fenugreek plant seeds according to the method of (Adams, 1989).

**Total Soluble Carbohydrate:** Total soluble carbohydrate was estimated by according to Watanabe *et al.* (2000), 1 g. of powdered from Fresh leaves tissue was used. 1ml of alcoholic extract transferred with 3ml from anthrone reagent, the absorbance was measured at 620nm by UV-spectrophotometer and total soluble carbohydrates were estimated by the glucose standard curve.

**Estimation of soluble protein:** Estimation of soluble protein was conducted according to Bavei *et al.* (2011), from powdered leaves tissue with liquid nitrogen and PMSF (phenylmethanesulfonyl fluoride) and TCA (trichloroacetic acid), absorbance was measured at 595nm, protein concentration values by standard curve of albumin.

**Estimation of total phenolic compound:** According to method of (Milan, 2011) phenolic compound was estimated UV-Spectrophotometer and Folin-Ciocalteu reagent 1ml from leaves tissue extract and Na<sub>2</sub>CO<sub>3</sub> was added to mix, the absorbance was measured at 550 nm and standard curve was estimated by gallic acid

**H<sub>2</sub>O<sub>2</sub> and Malondialdehyde (MDA) content:** According to (Fu and Huang, 2001) 0.5gm. fresh tissue was homogenizing in 0.1% TCA (tri-chloroacetic acid) at pH 7 from phosphate buffer and potassium iodide 0.2ml, the absorbance at 390nm, all materials reaction mixture are the blank without potassium iodide, then the absorbance of H<sub>2</sub>O<sub>2</sub> was subtracted from the mixture.

Estimation of MDA according to (Fu and Huang, 2001) 0.5gm fresh leaves in 4ml TCA (0.1% w/v) with 0.5% thiobarbituric acid, the absorbance was measured at 600nm and 532 TBA using as standard

**Estimation of Total Chlorophyll:** Total chlorophyll was estimated according to method of Asar-Boamah *et al.* (1987) 1gm of leaf tissue grounded with 80% acetone and spectrophotometrically using 80% acetone and their concentration were calculated.

**Antioxidant activity (Super Oxide dismutase SOD) and (Catalase CAT) enzymes:**

**Enzymes Extraction and estimation:** 0.5gm fresh tissue grounded with liquid nitrogen and extract by 2ml of (100mM) from phosphate buffer at pH=7.5 then complete the extract by method of (Ying *et al.*, 2018).



Estimate of (SOD) 0.2 ml of the enzyme extract with methionine and potassium phosphate, complete the steps according to the method of Gong *et al.* (2005), and estimate of CAT enzyme was conducted by method of Aebi *et al.* (2004) using 100µl from enzyme extract with mixture of potassium buffer solution at pH=7 and H<sub>2</sub>O<sub>2</sub>, absorbance of SOD was measured at 560nm and CAT at 240nm.

**Statically analysis:** All data were conducted in 5 treatments with 10 replicates, all the results were calculated as a mean ± standards deviation, SPSS (version 25) was used for statistical analyses. the significant Value at P>0.05.

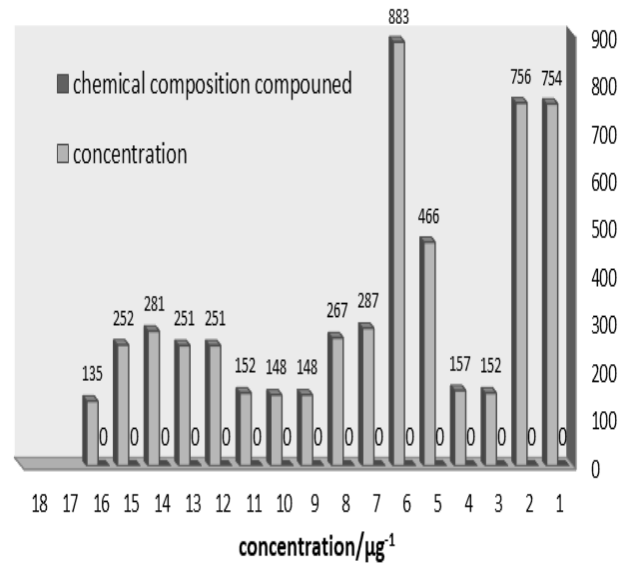
## RESULTS AND DISCUSSION

The study was performed to evaluate the effects of Fenugreek seed extract to improving germination of date palm barhee cv. according to the allele pathy. The Fenugreek seed extract was analyzed by the GC/MS device, several compounds appeared (Butylamine, Butanamine, Propanam-Succinimide, Veratramide, 6-Methylaminomethyl, Paromomycine, 4-Hydroxybenzoicacid, Estragole, Anthole, Cystine, Tetraacetyl-xylonicnitrite, 3-7diacetamido-7H-S-Trizolo-Strizole, Tricyclo-undeeccan lamine, Benzamethanol, Tetraacetyle-d-xylonicnitile). The most concentrated compound was Methylaminomethyl was 883µg<sup>-1</sup> and the least concentrated was Benz methanol 135µg<sup>-1</sup>. Figure 1. So by observing the effect of treatments of Fenugreek seed extract and their effect on vegetative growth of the date palm plant and its effect on the shoot and roots, the positive effect appeared with clear significance at the level p< 0.05 starting from concentration 0.5% to 1.5% while a decline in growth of date palm appeared at concentration 2% from Fenugreek seed extract.

In general, plants contain many chemical compounds or allopathic compounds, which are characterized by biological effectiveness, These compounds may be primary metabolites or secondary metabolites. It appeared through an analysis of Fenugreek seed extract that they are almost organic compounds that contain nitrogen, As it is known, nitrogen is considered very important element participate in building plant cell basic organic compounds that contribute to the growth.

There are studies that showed the effect of phytochemical compounds on some vegetative characteristics as *vicia faba* and *maize* this study focused on the effect of phenolic compounds isolated from Fenugreek seed extract, By this study it was shown that the amino organic compounds were the most concentrated in the Fenugreek seed extract, which indicates the clear effect of nitrogen in improving the growth of the date palm plant Figure 2. Nitrogen is considered have importunes that help in the growth, development of plant parts through its entry into the construction of many compounds necessary for plant growth as it is involved in the construction of photosynthetic pigments and the formation of energy

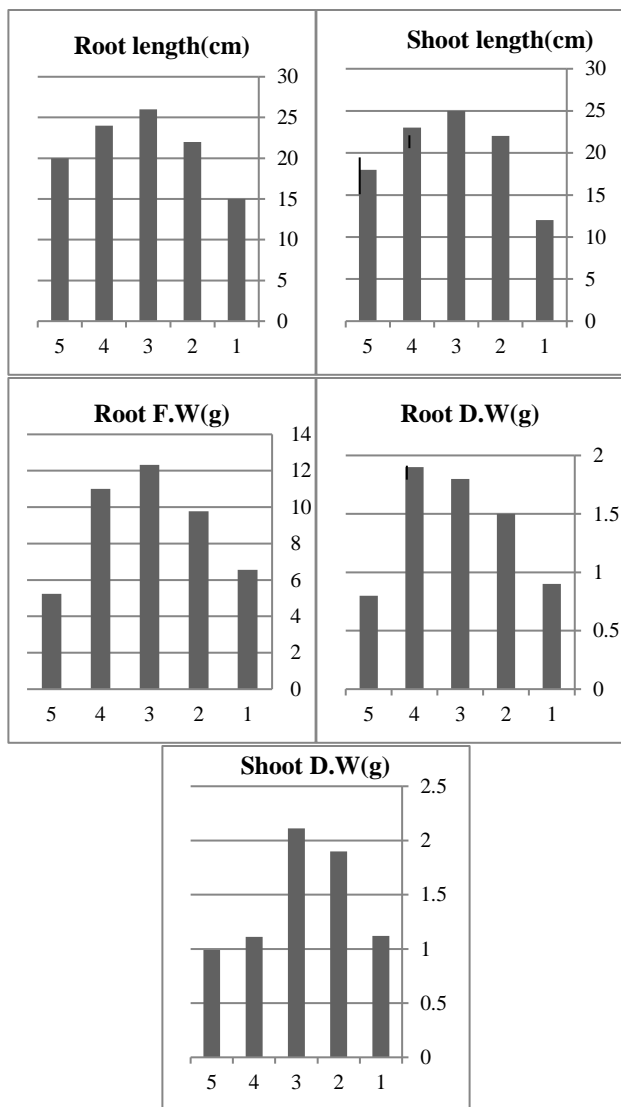
compounds and the construction of cell membranes mitochondria and chloroplasts (Chen *et al.*, 2021), this is consistent with many researchers who have proven the effect of nitrogenous compounds on plant growth such as (Delon *et al.*, 2012) on svana plant and (Arvin, 2019) on Savory plant. The improvement in the growth indicators of the date palm plant may be due to the fact that the nitrogenous compounds in the Fenugreek seed extract have helped in building amino acids nucleic acids, as well as being one of the basic components in building the chlorophyll molecule, also the availability of nitrogen helped in building cytokines, which stimulate cell division, so it was found through the study that the improvement of the growth positively associated with the improvement of fresh and dry weight Fig 2, Table 1. this modulate also helped in the construction of protein and nucleic acids DNA, RNA and the building of energy molecules NADPH needed to convert the acetyl Co-Enzyme molecule to gibberellin and in the formation of tryptophan acid which is considered the main compound for building indol acetic acid, which is a catalyst for cell elongation, these nitrogenous compounds also help in building the oxine hormone which stimulates the process of cell division (Shunkai *et al.*, 2020) the treatment of seeds with a concentration of 2% from Fenugreek seed extract is the reason for the decline in growth indicators, this concentration may be sever and the cell cannot absorb it.



**Figure 1.** Analysis of seed extract of fenugreek plant (qualitative and quantitate analysis) using GC/MS analysis(µg-1). [1- Butylamine 2- Butanamine 3- Propanamine 4- Succinimide 5- Veratramide 6-Methylaminomethyl 7- Paromomycine 8-4-hydroxybenzoic acid 9- Estragole 10- Anthole 11- Cysteine 12- Cystine 13- Tetraacetyl-Xylonic nitrite 14-3,7 diacetamido-7H-S-Trizolo-S-trizole 15-



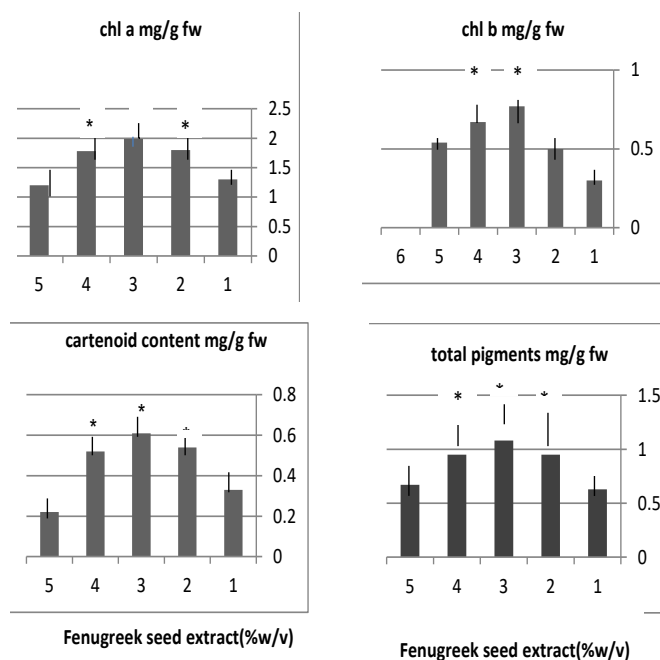
**Tricyclo-undecan-1-amine 16- Benz methanol  
17- Tetraacetyle-d-xylene nitrile].**



**Figure 2.** Showed many concentrations of Fenugreek seed extract on A: shoot length. B: Root length C: shoot fresh weight D:Root fresh weight: shoot Dry weight E: Root Dry weight of date palm seedling. The mean value of treatments statistically different significantly with the control of date palm cv. barhee at  $p < 0.05$  (\*).

It was observed that photosynthetic pigments were affected significantly by the level of treatments with seed extract of fenugreek and starting from concentration 0.5%, 1%, 1.5% w/v and it was noted that concentration 2% showed a decrease in the content of photosynthetic pigments from chl a, chl b, carotenoids and total pigments were be 2.29, 0.88, 0.62 mg/gm. and 58% respectively (Fig. 3).

Chlorophyll is one of the essential pigments for the photosynthesis process, it is found in plants that perform photosynthesis and carotenoids, photosynthetic pigments can be relied upon as one of the important indicators of the physiological changes that accrue in the plant when exposed to a specific condition, because its effect is directly in the manufacture of carbohydrates and the fixation of  $CO_2$ , by this study a clear improvement in photosynthetic pigments are carotenoids when treated with different concentrations of Fenugreek plant extract in a concentration less than 2% w/v, the reason may be attributed to the improvement in the level of enzymes that help in the process of building chlorophyll molecules in terms of providing the carbon structure needed to build chlorophyll and carotenoids molecules (Fevria *et al.*, 2023). Also the phenolic substances in the Fenugreek seed extract helped to remove the reactive oxygen molecules (ROS) that arise from the activity of vital process, thus providing the necessary conditions for an increase in the building of photosynthetic molecules.



**Figure 2.** Showed the different concentrations of Fenugreek seed extract on photosynthetic pigments. mean value for treatments is statistically different from the control of date palm cv barhee at  $p < 0.05$  (\*).

In the stages of plant growth, some materials must be available as basic requirements for building cells from these materials, which are, proteins and carbohydrates, these materials are broken down during the growth stages to provide the basic materials for building plant tissue cells. Through this study it became clear that the soluble



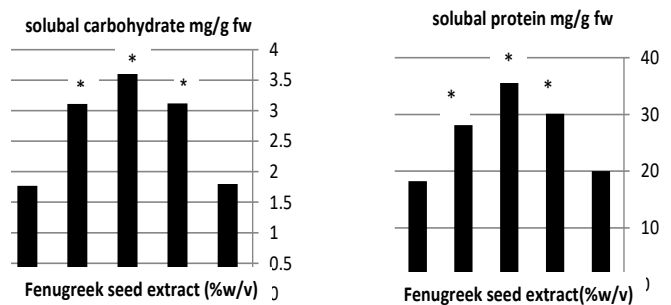


carbohydrates and soluble proteins accumulated in date palm seedling when treated with Fenugreek extract starting from concentration 0.5%, 1% and 1.5% but no significant changes appeared in treatment 2% and it was noted that concentration 1% the best accumulation increased of both soluble carbohydrates 55% and soluble proteins 48% Figure 4. The changes that occurred in the plant content of carbohydrates or proteins has an important role in the study of physiological changes which occur in the plant because of its direct relationship to the process of photosynthesis or respiration and transpiration (Palma *et al.* 2002; Jouyban, 2012; Ahmed *et al.* 2016), the improvement in soluble proteins and soluble carbohydrates must be associated with the activity of enzymes amylase and protease and this is consistent with what he mentioned of Mdany and Khalil (2017), a clear improvement was found in the activity of amylase & protease when the bean plant and maize plant were treated with Fenugreek seed extract in several concentrations. Other study was conducted by Shatha (2021) on Fenugreek plant when treated by 2mg/kg boron also showed improvement in soluble protein and soluble carbohydrates, I mentioned that enhancing the level of photosynthesis when this treated with this concentration of boron led to the availability of CO<sub>2</sub> led to enhancing the metabolism of nitrogen and proteins, and this agreement with mentioned of Bestias *et al.* (2004) in a study on maize plant.

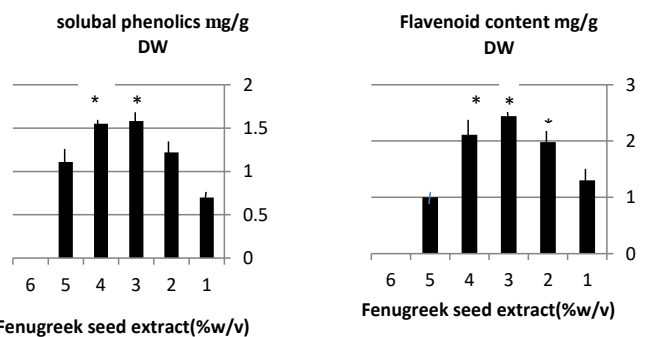
Also, the increase in the content of soluble carbohydrates was found associated with the increase in the content of date palm of photosynthetic pigments, and this indicates the effectiveness of the photosynthesis process this is consistent with what was mentioned by in maize plant was treated with some concentration of salicylic acid, and other studies were conducted by Saleh & Abu El-Soud (2015) on wheat plant have showed that there is an improvement in the photosynthetic pigments when the plant is treated with cumarin, which results in an improvement and increase in the activity of amylase and protease, other studies were conducted by Orabi *et al.* (2013) showed that the carbohydrates increased in the bean plant when treated with salicylic acid, this indicates that treating the plant with some substances the plants content of soluble proteins and soluble carbohydrates. Also, when studying the content of secondary compounds phenols and flavonoids, it was found that they had changed significantly at the level  $p < 0.05$  when the concentration of the treatments increased to more than 0.5%  $\mu\text{g/g.dw}$  was noted that the total soluble phenols increased by 47% and 45% for flavonoid for treatment 1% and continued to increase in treatment 1.5% and then decrease concentration to more than 1.5% maybe sever treatments on date seedling figure 5. Phenolic compounds are considered important indicators of physiological changes when the plant is exposed to any factor, and it is one of the compounds that can act as antioxidants in these.

These concentration indicates an increase in the vital activities in the plant (Ayoub *et al.*, 2016). Also phenolic

compounds in the plant play a role in inhibiting enzymes, nutrient absorption, photosynthesis and protein synthesis, Among the main compounds in phenolic compounds are flavonoids that respond quickly to physiological changes in the plant (Sharma *et al.*, 2012; Fayez and Bazaid, 2014). The changes in the content of total soluble phenols and flavonoids that occurred in the date cv. barhee plant with exposed to treatments concentrations of Fenugreek seed extract led to increase w significantly in photosynthesis process, and the outputs of this process are carbon and nitrogen products transported to the product of secondary metabolites (Fritz *et al.*, 2006) and these result agreement with Madany and Khalil (2017) on Vicia and Maiz plant.



**Figure 3. Showed effect of different treatments of Fenugreek seed extract on soluble carbohydrate and soluble protein. seed extract mean value of treatments is statistically different from the control of date palm at  $p < 0.05$  (\*).**



**Figure 4. Showed effect of different treatments of Fenugreek on soluble phenolic and flavonoid. seed extract mean value of treatments is statistically different from the control of date palm at  $p < 0.05$  (\*).**

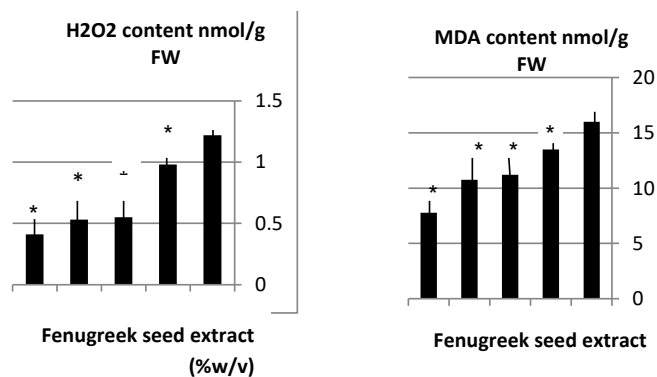
To study the integrity of the cellular membranes of date seedling during treatment with different treatments of Fenugreek seed extract significant reduction in H<sub>2</sub>O<sub>2</sub> and MDA content was observed at all concentrations of treatments starting from treatment 0.5% of Fenugreek seed extract with significantly at the level  $p < 0.05$ , figure 6 for oxidation is one of the processes that occur in all living



organisms and membrane oxidation alone is an indicator that determines the extent of changes that occur in the cell membranes when the plant is exposed to any beneficial or harmful external factor, and it does not only affect cell function, but also can oxidative through the production of derived radicals or lipids, lipid peroxide is the first product that forms in membranes during vital processes and formation of ROS and which deserves the decomposition of unsaturated fatty acids (Ghosh *et al.*, 2015).

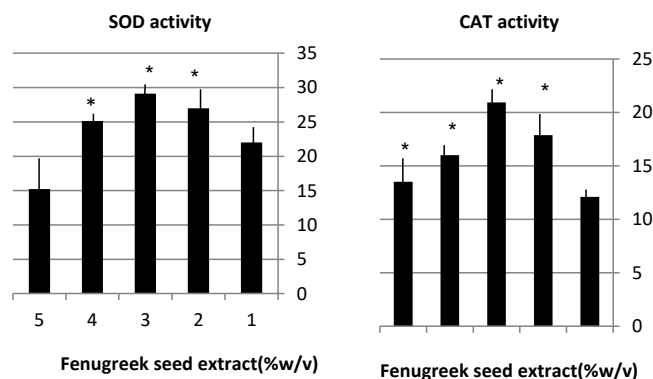
Among the most important compounds that are formed as a result of oxidation of membranes by  $H_2O_2$  is a ranged compounds that are responsible for causing damage to membranes, such as fluidity of membranes, ion transport, and inhibition of the effectiveness of enzymes and proteins in membranes (Sharma *et al.*, 2012). It was noted that clear significant changes occurred at the level  $p < 0.05$  in the content of  $H_2O_2$  and MDA it was noted through the study that the concentrations of the treatments with Fenugreek seed extract had a clear effect on the reduction of each of  $H_2O_2$  and MDA and with the increase in the concentrations of the treatments it has reduced  $H_2O_2$  by 40% and 34% for MDA with increase concentration of treatment, which indicates a clear significant reduction with the increase in the concentrations of the treatments, this reduction that occurred in the level compounds that act as antioxidants that work in removing toxicity of  $H_2O_2$  and removal of MDA accordingly, which is the result of lipid oxidation, and this led to enhancing the integrity of cellular membranes this effectiveness maybe due to the reduction of  $H_2O_2$  due to the phenols found in seeds of Fenugreek, that enhance antioxidant compounds that act to remove free radicals including  $H_2O_2$  compound (Michalak, 2006). The integrity of the membranes means enhancing the process of photosynthesis and thus enhancing growth of date seedling, also Ali (2023) who proved through a study on the tomato plant, that it has a direct relationship to the photosynthesis process, and other researchers conducted by León-López *et al.*, (2020) on chickpea plant, this agreement with Madany & Khalil (2017) study on Vicia and Maize plant and there are studies that have proven the effectiveness of phenolic compound in removing free radicles such as (Singh & Kaur, 2014).

And shedding light on the activity of regulatory enzymatic antioxidant SOD, CAT by the effect of treating date seedling with different concentrations of Fenugreek seed extract, it was noted an increase in activity of SOD, CAT in significantly at  $p < 0.05$  with an increase in the concentrations of treatments, figure.7 with indicates that the activity of enzymes starts from the beginning the plant was treated with concentration 0.5% it peaked at treatment 1% and 1.5%, which indicates the response of date seedling to this concentration of treatments, it is known that the metabolic activities in the plant induce reactive oxygen species ROS, as products accompanying these processes a mechanism that removes these ROS from which enzymatic antioxidant SOD, and CAT



**Figure 5. Showed Effect of different treatments of Fenugreek on  $H_2O_2$ , MDA content, mean value of treatments are statistically different from the control of date palm cv. barhee plant at  $p < 0.05$ (\*).**

As we noted previously, a clear positive effects between the level of treatments and the improvement in the process of photosynthesis, proteins and carbohydrates, this improvement that occurred in these indicates the effectiveness of the enzymes amylase, protease that stimulate the building of these compounds, in general led to improve in activity of SOD and CAT to remove the ROS, this agreed with (Aline *et al.*, 2023), also fined with many studies the other treatment can stimulate activity of antioxidant such as treatment with salicylic acid (Sabry *et al.*, 2023), So treatment with boron can enhanced activity of antioxidant system in Fenugreek plant with study on Fenugreek plant conducted by Shatha. (2021)



**Figure 6. Showed effect of different treatments of seed extract of Fenugreek plant on SOD, CAT enzyme activity mean value of treatments is statistically different from the control of date palm at  $p < 0.05$ (\*)**

**Productivity of date palm cv. Barhee:** The results obtained through this study, an increase in productivity of date palm cv. barhee when treated with concentrations of Fenugreek



extract (1%, 1.5 %) We notice this through an increase in soluble carbohydrates and proteins and an increase in the activity of enzymatic antioxidants that help the plant in promoting growth and increasing productivity.

**Conclusion:** It can be concluded that the Fenugreek seed extract with different concentration proved all growth indicators of date palm cv. barhee seedling, it was found through the study that the best concentration 1% and 1.5% and this clear improvement and enhancement in the growth of date palm seedlings is due to the chemical compounds found in Fenugreek seeds especially the amino acid compounds, which were more concentrated, in addition to the phenolic and flavonoid compounds. The alcoholic analysis of Fenugreek seed extract showed many chemical compounds that took an allopathic effect on date palm seedlings It was observed through the study that the date palm seedlings showed a clear improvement in the vegetative characteristics associated with a significant improvement in the content of chlorophyll, carotenoids and phenolic substances, and it was related with an increase of Enzymatic antioxidant activity, which led to the integrity of cellular membranes and decrease in H<sub>2</sub>O<sub>2</sub> and MDA, we conclude from that the Fenugreek seed extract has an effective and good role in promoting the growth of date palm seedlings and acceleration of date for growth of seedlings.

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**Author contribution:** The authors of this paper participated in preparing, and planning the paper. Dr. Nasser prepared the information and editing the paper, and shatha carried out the chemical analysis, plant cultivation, and data preparation.

**Conflict of interest:** Confirmation by the authors there is no conflict with this article.

**Availability of data and material:** This article submitted manuscript our is our work.

**Ethical statement:** Article don't use any study with human or animal.

**Code availability:** 1127

**Consent to participate:** All authors participated in this research.

**Consent for publication:** All authors submitted agreement to publish this research.

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