The effect of *Schanginia aegyptica* and *Urtica dioica* powder on the growth of *Trigonella foenum* seedlings in laboratory sterilized soil.

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Abstract

The study included isolating and diagnosing the fungi accompanying fenugreek seeds *Trigonella foenum* , most of the species belonged to the genus *Aspergillus*. The study showed that the use of *Schanginia aegyptica* and *Urtica dioica* powder alone for the fungi treatments led to a significant increase in the percentage of germination by 100% and in the length and weight of the seedlings compared to the fungi treatments alone and that adding fungi to the soil treatment had led to reduce the percentage of germination, the most common of which are the two fungi, *Aspergillus flavus* and *Aspergillus terreus*, at a rate of 71% for each, and the most affected fungi by adding nettle and sage plant powder is the fungus *Pencillium*, at a rate of 100%.

Keywords: Schanginia aegyptica, Urtica dioica, Trigonella foenum, Aspergillus, Penicillium.

1. Introduction

Fenugreek *Trigonella foenum-gracum* is one of the plants belonging to the family Leguminosae and it is one of the most important medicinal plants as it is cultivated in different regions of Iraq for several purposes, including as food for humans and fodder for livestock as well as for its uses in medicines and medical drugs. The fenugreek contains several important active substances in terms of the most important of which are Isoleucin, galactomannans, sapogenis, and sterodial [1].

Fenugreek seeds are infected with several types of fungi, which may be inside or outside the seeds [2]. isolated the fugi *Alternaria alternate, Aspergillus niger, Aspergillus flavus, Fusarium oxysporium, Fusarium solani, Fusarium moniliforme and Rhizopus* from fenugreek seeds. The damage caused by fungi from an economic point of view is to search for natural compounds that have an effect on microorganisms, which can be obtained from some plants.

Schangania aegyptica plant belongs to the family Chenopdiaceae, and it is one of the wild plants that grow in Iraq in home gardens and fields. It is characterized by containing antibiotic substances through root secretions [3], and these substances increase the fertility of the land and have an effect on crop plants, causing a reduction in germination as it contains a seedling growth inhibitor [4]. The seedling is also distinguished by its phenolic compounds [5]. Through previous studies, it was found that the aqueous extract of *Schanginia aegyptica* had an effect on the germination of Roselle plant [6, 7]. found the effect of hot aqueous extracts for each of the vegetative and root system of a group of plants found in Iraq, including *Schanginia aegyptica* on the growth inhibitor of the fungus *Rhizoctonia solani*, which causes the rotting of seeds and the death of some plants.

Urtica dioica belongs to the Urticaceae family of medicinal plants used in Traditional medicine for centuries. *Urtica dioica* contains several active substances, including carotenoids, fatty and amino acids, carbohydrates, phenols and alkaloids [9], The alcoholic extract of *Urtica dioica* showed antifungal activity (*Alternaria alternate, Fusarium oxysporium, Fusarium solani, Rhizoctonia solani and Candida albican* Ghaedi et al. [10] indicated that The ethanolic extract of *Urtica dioica* flowers had an effect on the growth of the fungi *Candida albican* and *Aspergillus niger*.

The current study aimed to indicate the effect of plant powders for each of *Schanginia aegyptica and Urtica dioica* because of their importance and to find economic alternatives in the field of controlling the fungi accompanying fenugreek seeds, which are *Aspergillus flavus, Aspergillus terrues, Aspergillus niger* and *Pencillium.*

2. Materials and Methods

Seed sources

Fenugreek seed samples were obtained from the local markets office in the city of Samarra. The samples were kept in tightly closed nylon bags at a temperature *of 4°C until use.*

Collecting, drying and preserving plants

Schanginia aegyptica and *Urtica dioica* leaves were

doi.org/10.31838/hiv22.02.50 Received: 16.01.22, Revised: 10.04.22, Accepted: 06.08.22

collected at the flowering stage from Samarra University, the leaves were washed to dry in laboratory conditions, taking into account the stirring to prevent the occurrence of rotting. Then the dry leaves were crushed with the electric grinder to obtain a fine powder, and then kept in opaque glass bottles at a temperature of 4°C until use.

Preparation of the PDA culture medium

The medium of potato extract Agar was prepared by dissolving 39 g of it in 1000 ml of distilled water, then placing the solution in an electric autoclave for 15 minutes, under pressure of 1 atmosphere and temperature of 121 °C. 250 mg/L to inhibit bacterial growth [11].

Isolation and diagnosis of fungi from fenugreek seeds

Fungi were isolated from sterile seeds according to International Seed Testing Association (ISTA) [12]. The seeds were superficially sterilized with 5% sodium hypochlorite solution for 5 minutes, then washed with sterile distilled water three times, then dried and planted in culture dishes containing culture media (PDA). For the purpose of developing fungi, the seeds were planted so 10 seeds were placed in each dish, and the dishes were observed at a temperature of 25 °C for 7 days, and after the incubation period ended, the fungi were diagnosed according to the taxonomic keys.

Adding *Schanginia aegyptica* and *Urtica dioica* powder with or without fungi to the growth of fenugreek seedlings in laboratory sterilized soil.

Preparation the fungal vaccine

The seeds of local millet, *Panicum miliaceum*, were used for the purpose of preparing fungal vaccines and loading the seeds with the fungi to be used. The seeds were washed well several times to remove dirt and impurities and left submerged in water for the next day. The excess water was removed using a regular sieve. The washed seeds were spread on a gauze cloth and left in the laboratory atmosphere for the next day and the fan left running to generate an electric current that speeds up the drying process. 50 g of them were placed in each 250 ml beaker, and then sterilized with a sterilizer at a temperature of 121 ° C and a pressure of 1 atmosphere for an hour, and the sterilization was repeated on the next day at the same temperature and pressure and the same time. The beakers containing the seeds sterilized with fungi were placed in the incubator at a temperature of 25 °C for 10 days, taking into account the shaking of the beakers every 3 days in order to distribute the fungus to all the seeds [13, 14].

Prepare sterile soil

Mixed soil was used (1 moss + 1 sandy soil + 1 clay soil) volume / volume and it were sterilized by sterilizer at a temperature of 121 °C and pressure of 1 atmosphere for an hour. It was re-sterilized the next day for an hour and after cooling it was placed in sterile nylon bags and left aside until use.

Examination the effect of the interaction between the powder of *Schanginia aegyptica* and *Urtica dioica* leaves with or without the isolated fungi on the growth of fenugreek seedlings in laboratory sterilized soil.

The inoculum of each fungus was added to the sterilized soil at a rate of 1% w/w of millet seeds bearing the fungus. As 6 gm of the fungal pollen carried on millet seeds was added to 600 gm of the mixed soil and placed in a sterile cellophane bag, shake well to homogeneity of the pollen with the soil. The soil contaminated with the fungus was divided into three replicates, so that 100 gm of soil contaminated with the fungus was placed in each pot with a diameter of 6 cm.In each pot, 5 fenugreek seeds were sprinkled with powder of *Schanginia aegyptica and Urtica dioica* separately after moistening the seeds with sterile distilled water. The pots were placed inside the planting room after sterilization at a temperature of 22 °C and the intensity of illumination of 2000 candles / foot. The number of germinated seeds was calculated after 10 days of planting and after four weeks of planting the plants were carefully removed to ensure that the roots were not cut and washed with distilled water well. The treatments for the experiment were distributed among the following groups:

1. Sterilized soil mixed only
2. Soil + *Urtica dioica* powder
3. Soil + *Schanginia aegyptica* powder
4. Soil + *Schanginia aegyptica* and *Urtica dioica* powder
5. Soil + millet seeds loaded with *Aspergillus flavus*
6. Soil + millet seeds loaded with *Aspergillus flavus* + fenugreek seeds fogging with *Schanginia aegyptica* powder
7. Soil + millet seeds loaded with *Aspergillus flavus* + fenugreek seeds Fogging with *Urtica dioica* powder
8. Soil + millet seeds loaded with *Aspergillus terreus*
9. Soil + millet seeds loaded with *Aspergillus terreus* + fenugreek seeds fogging with *Schanginia aegyptica* powder.
10. - Soil + millet seeds loaded with *Aspergillus terreus* + fenugreek seeds fogging with *Schanginia aegyptica* powder.
11. Soil + millet seeds loaded with the fungus *Asprgillus niger*
12. Soil + millet seeds loaded with *Asprgillus niger* + fenugreek seeds fogging with *Urtica dioica* powder
13. Soil + millet seeds loaded with *Asprgillus niger* + fenugreek seeds fogging with *Schanginia aegyptica* powder
14. Soil + millet seeds loaded with the fungus *Pencillium*
15. Soil + millet seeds loaded with the fungus *Pencillium* + fenugreek seeds fogging with *Urtica dioica* powder
16. Soil + millet seeds loaded with the fungus *Pencillium* + fenugreek seeds fogging with *Schanginia aegyptica* powder.

3. Statistical analysis

The results were statistically analyzed using the SPSS program, as Duncan's test was used at a probability level of 0.005.

4. Results and Discussion

Isolation and diagnosis

The results of isolation and diagnosis showed the

presence of a number of fungi accompanying fenugreek seeds, which are *Aspergillus flavus Aspergillus terreus*, *Aspergillus niger* and *Pencillium*. The result agrees with what Divya et al. [15] which they found several fungus species when they isolation the fungi from fenugreek, including *Alternaria alternate*, *Aspergillus niger, Aspergillus fusarium*, *Aspergillus* , *Fusarium moniliforme* , *Verticillium dahlia* , they explained that fenugreek harbors a large number of internal fungi.

Examination the effect of the interaction between the powder of *Schanginia aegyptica* and *Urtica dioica* leaves with or without the isolated fungi on the growth of fenugreek seedlings in laboratory sterilized soil.

The results in Table (1) showed significant differences between soil treatment only (control), soil + *Schanginia aegyptica* powder, soil + *Urtica dioica* powder, soil + the powder of *Schanginia aegyptica* and *Urtica dioica* in the percentage of fenugreek seeds germination after 10 days of planting, total length, fresh and dry weight. The results showed that adding *Schanginia aegyptica* and *Urtica dioica* powder only separately and adding both powders together resulted in an increase in the percentage of germination, which reached 100% for *Urtica dioica* and for both powders together, and 98% for *Schanginia aegyptica.*

As for the addition of the fungi *Aspergillus flavus* and *Aspergillus terreus,* the percentage decreased, which amounted to 71% for each of them, compared to the control, which amounted to 83%. It was found that the two of them had the most effect in reducing the percentage of germination, while the effect of the two fungi *Aspergillus niger* and *Pencillium* was not significant in the percentage of germination amounted to 91% and 92%.

Table (1) Effect of powdered *Urtica dioica* and *Schangania aegytica* plants with or without fungi on the growth of fenugreek seedlings.

Fresh weight Mean ± SD	Stem length Mean ± SD	Root length Mean ± SD	Total length Mean ± SD	Germination% Mean ± SD	Treatment
0.033±0.005C	6.3±0.15D	5.4±0.05B	11.7±0.20C	83±5.77B	Sterilized soil mixed only
0.043±0.005B	8.1±0.15B	5.8±0.05B	14±0.15B	100±0.00A	Soil + *Urtica dioica* powder
0.046±0.005B	8±0.11B	5.6±0.10B	13.6±0.20B	98±2.89A	Soil+*Schanginia aegyptica* powder
0.036±0.046C	8.7±0.05B	8.6±0.10A	17.3±0.15A	100±0.00A	Soil + *Schanginia aegyptica* and *Urtica dioica* powder
0.010±0.000E	4.5±0.05F	3.4±0.15D	8±0.20E	71±1.15C	Soil + millet seeds loaded with *Aspergillus flavus*
0.023±0.005D	7.1±0.05C	5.6±0.15B	12.8±0.10B	93±1.00A	Soil + millet seeds loaded with *Aspergillus flavus* + fenugreek seeds fogging with *Schanginia aegyptica* powder
0.023±0.005D	6.3±0.10D	5.2±0.11B	11.5±0.20C	85±0.58B	Soil + millet seeds loaded with *Aspergillus flavus* + fenugreek seeds Fogging with *Urtica dioica* powder
0.020±0.005D	7.4±0.05C	3.4±0.10D	10.8±0.11D	71±1.53C	Soil + millet seeds loaded with *Aspergillus terreus*
0.026±0.010D	8.8±0.10B	5.2±0.05B	14.2±0.47B	98±2.89A	Soil + millet seeds loaded with *Aspergillus terreus* + fenugreek seeds fogging with *Schanginia aegyptica* powder.
0.046±0.005B	7±0.11C	5.3±0.10B	12.7±0.55B	71±0.58C	Soil + millet seeds loaded with *Aspergillus terreus* + fenugreek seeds fogging with *Schanginia aegyptica* powder.
0.036±0.005C	5.4±0.10E	4.7±0.10C	10.1±0.17D	91±1.00A	Soil + millet seeds loaded with the fungus *Asprgillus niger*
0.030±0.010C	6.6±0.10D	5.5±0.43B	12.2±0.25B	92±0.58A	Soil + millet seeds loaded with *Asprgillus niger* + fenugreek seeds fogging with *Urtica dioica* powder
0.026±0.005D	7.2±0.10B	5.5±0.10B	12.7±0.17B	91±1.15A	Soil + millet seeds loaded with *Asprgillus niger* + fenugreek seeds fogging with *Schanginia aegyptica* powder
0.036±0.005C	6.5±0.10D	3.5±0.10D	10±0.10D	92±1.00A	Soil + millet seeds loaded with the fungus *Pencillium*
0.086±0.005A	9.1±0.10A	3.8±0.10D	12.9±0.20B	100±0.00A	Soil + millet seeds loaded with the fungus *Pencillium* + fenugreek seeds fogging with *Urtica dioica* powder
0.046±0.005B	7.5±0.43C	5.2±0.10B	12.7±0.36B	100±0.00A	Soil + millet seeds loaded with the fungus *Pencillium* + fenugreek seeds fogging with *Schanginia aegyptica* powder

When adding *Urtica*

mentioned fungi to the soil significantly affected the total length of fenugreek seedlings, which amounted to 8 cm, 10.8 cm, 10.1 cm and 10 cm, respectively, compared to the control, which amounted to 11.7 cm. The addition of *Urtica dioica* powder to the fungi under study achieved a significant increase in the total length accompanied by an increase in root length and stem length, which reached 12.8 cm, 14.2 cm, 12.2 cm and 12.9 cm, respectively, compared to the control, which amounted to 11.7 cm.

Whereas, *Schanginia aegyptica* powder had a significant increase in the total length of fenugreek seedlings, which amounted to 11.5 cm, 12.7 cm, 12.7 cm and 12.7 cm compared to the control, which amounted to 11.7 cm.

The results showed that *Schanginia aegyptica* and *Urtica dioica* powder had a significant effect on the fresh weight of fenugreek seedlings, which amounted to 0.0043 g and 0.046 g, compared to the control, which amounted to 0.033 g. The addition of fungi significantly affected the fresh weight of fenugreek seedlings, as the fungus

Aspergillus flavus achieved the lowest fresh weight percentage of 0.010 g compared to the control 0.033 g. Addition of *Urtica dioica* powder to the contaminated fungi did not have a significant effect on increasing the fresh weight, except for the treatment of soil + Pencillium + *Urtica dioica* powder, which amounted to 0.086 gm, compared to the control, which amounted to 0.033 gm. *dioica* powder to the fungi treatments mentioned in the percentage of germination reached 93%, 98%, 92% and 100%, and the largest increase was caused by adding ***Urtica dioica*** powder against the fungus *Aspergillus terreus* and *Pencillium,* which amounted to 98% and 100%, respectively.

As for the *Schanginia aegyptica* powder it was found by adding it with the fungi polluting the soil and mentioned previously that the fungus powder had a non-significant increase in the germination rate of 85%, 71%, 91% and 100% compared to the control, which amounted to 83% and was the lowest percentage achieved by the fungus powder of *Aspergillus terrus* where it reached 71%, while the increase was for the fungus *Pencillium*, which amounted to 100%. The results in Table (1) showed an increase in the total length of fenugreek seedlings, as the addition of *Schanginia aegyptica* and *Urtica dioica* powder separately and both together achieved a significant increase in the total length of 14 cm, 13.6 cm and 17.3 cm, respectively, compared to the control, which amounted to 11.7 cm

The results also showed that adding the

The current study shows that the fungi have a role in inhibiting the germination of fenugreek seeds, especially the fungus *Aspergillus flavus*, this result is agrees with the study of Khokhar et al. [16], as the filtrate of the fungus had an effect on the germination of fenugreek seeds by 22.2% and at a concentration of 100%, while *Aspergillus niger* at a rate of 25.5% and at a concentration of 100 compared to the control which was 71.3% and this is due to the aflatoxin toxins produced by this fungus, which can inhibit seed germination [17].

It agrees with what was found by Al Shaam et al. [8] that the hot aqueous extract of *Schanginia aegyptica* had a role in inhibiting the fungus *Rhizoctonia solani,* which causes rotting seeds and death of some plants, as noted by Abbas et al. [6] that spraying the *Schanginia aegyptica* extract at a concentration of 10% for clove seedlings significantly increased the growth indicators such as the high of the plant, the number of leaves, and others. Al Shaam et al. [8] explained in his study that the extract of *Schanginia aegyptica* had a significant effect on increasing the vegetative and fruitful growth of eggplant seedlings; this is due to the fact that the *Schanginia aegyptica* plant contains many active substances, including flavonoids, alkaloids, soaps, tannins and glycosides [18]. The *Schanginia aegyptica* extract also contains a high percentage of elements, including iron, manganese and zinc, as iron participates in the process of respiration and photosynthesis and enters into the composition of green plastids, as one of the vital functions of zinc is its entry into the synthesis and formation of a number of enzymes and participates in the formation of starch while helping in the elongation of the plant stem and stimulating the work of the growth regulator (auxin). It is believed that zinc is necessary in the formation of tryptophan, which in turn affects the activity of the growth regulator.

The study agrees with the findings of Hadizadeh et al. [19] that the alcoholic extract of *Urtica dioica* showed anti-fungal activity, including *Alternaria alternata*, *Fusarium oxysporium*, *Fusarium solani*, *Rhizoctonia solani* and *Candida albican*. Also it was found that the roots and leaves of *Urtica dioica* plant at concentration of, 5 ,10 ,20 and 40% have effect against the fungus *Alternaria solani* isolated from tomato [20].

Ghaedi et al. [10] indicated that the aqueous extract of *Urtica dioica* had an effect on inhibiting the growth of the fungus *Candida albican* and *Aspergillus niger*. The results explain that *Urtica dioica* contains aldehydes, ketones and sulfur compounds. The leaves are rich in carotenoids, vitamins, proteins, carbohydrates and organic acids, as well as containing phenolic compounds from the most important Rutin compound [21].

We conclude from the study the presence of a number of fungi accompanying fenugreek seeds, the most important of which is the fungus *Aspergillus flavus*, which affected the growth of fenugreek seedlings. It was also found that *Urtica dioica* and *Schanginia aegyptica* plant powders have inhibitory properties for the growth of fungi, as *Urtica dioica* powder outperformed *Schanginia aegyptica* powder and thus can be used in the field of combating fungi associated with fenugreek seeds. Therefore, we recommend conducting extensive studies on the effect of plant powders on other fungal isolates and conducting field experiments to identify the possibility of plant powders and their vital ability in combating plant diseases and on the seeds of different plants, as well as preparing plant extracts from both plants and comparing them with the effect of plant powders.

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