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The Stimulating Effect of Nummularine Aqueous Extracts on Growth and Germination of Faba Bean (*Vicia faba* L.)

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ABSTRACT

This study targets to examine the stimulating effect of Nummularine aqueous extracts from the stem bark of *Ziziphus nummularia* on the growth and germination of faba bean (*Vicia faba* L.) at several concentrations (50, 100, and 150) µg/ml compared to control treatment employing the direct irrigation method. Results have revealed that the aqueous extracts of Nummularine had an evident stimulating effect in their various concentrations toward the growth and germination of the faba bean (*Vicia faba* L.). Over and above, the concentration of 150 µg/ml showed the strongest stimulating effect compared to the control treatment and to other concentrations where the length of the shoot system reached 23.9 cm, whilst the seed germination rate was 98%. The control treatment at the concentration of 0 mg/ml of the aqueous extract showed the weakest stimulating effect where the length of the shoot system recorded 4.6 cm, whilst the seed germination percentage was at 83%.

1. INTRODUCTION

Nummularine ($C_{23}H_{32}N_4O_4$) is a cyclopeptide alkaloid and antifungal extracted from the stem bark of the medicinal plant *Ziziphus nummularia*, a species of the genus of *Ziziphus* under the order of Rosales that belongs to the family of Rhamnaceae. *Ziziphus* is indigenous to the Thar Desert in western India, southeast Pakistan, south Iran, Afghanistan, Lebanon, and Zimbabwe. It is commonly known as Jujube. *Ziziphus nummularia* is a thicket-forming shrub that grows to a height of at least 6 meters (20 feet). The leaves are spherical and have pubescence on the adaxial surface. The plant is seen in dry regions, hills, plains, and farmlands. *Ziziphus nummularia* is a thorny, heavily branched bush. The twigs and branches are silky and pale violet in color. Long and extending far into the substrate are the lateral roots. The 2.5 cm (1 in) long, simple, ovate leaves are placed alternately;

the upper surface of the blade is glossy and dark green, and the underside is downy and white. Two brown spines, one of which is a small, downward-curving hook, frequently replace the stipules and the other is a robust, straight 1 cm (0.4 in.) long spine. The individual pale yellow blooms are bisexual with five segments and frequently have no petals and are followed by tiny reddish or blackish drupes that are less than 1 cm (0.4 in.) in diameter and contain a hard stone with typically two seeds inside (Lin et al., 2021).

Ziziphus nummularia has been widely used to stop erosion, lessen wind damage, and stabilize deposited debris, creating a microhabitat that encourages the growth of other plants like grasses. The shrub might be planted to create a windbreak since its root system produces numerous suckers, sometimes in conjunction with *Crotalaria burhia*. The wood can be used to build structures, create farm equipment, and manufacture charcoal and useful fuel. In Asian regions, the leaves are harvested, dried, and stored, yielding roughly 1000 kg (2200 lb.) of dry foliage per acre. The leaves can serve as feed for livestock. The fruits are palatable, tangy, and sweet. They can be used to

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make candy, eaten raw, pickled, dried, or pickled. The fruit is utilized in poison baits to draw in rats and gerbils, which are also drawn to it (Sharma *et al.*, 2013).



Figure (1): Leaves and Fruits of *Z. nummularia* (Jujube) (Pandey, 2010)

The family of flowering plants known as the Fabaceae or Leguminosae sometimes referred to as the legume, pea, or bean family, is a sizable and crucial one for agriculture. It includes trees, shrubs, and herbaceous plants, both perennial and annual, which are distinguished by their leguminous fruit and compound, stipulate leaves. With over 765 genera and nearly 20,000 recognized species, the family is widely dispersed and is the third-largest land plant family in terms of species behind the Orchidaceae and Asteraceae (Saikia *et al.*, 2020). Faba bean (*Vicia faba* L.) is an annual plant belonging to the family of Fabaceae. The stem is straight and four-sided, and the leaves are feathery compounds. The inflorescence is short, the fruit is a large, thick pod, cylindrical and flat, and the seeds are globose to ovate.

Vetch is also known as the wide bean, fava bean, or faba bean. It is widely grown as a crop for human consumption and as a cover crop. Field bean, tic bean, and tick bean are varieties with smaller, tougher seeds that are fed to horses or other animals. The approved designation for the horse bean variety is *Vicia faba* var. *equina* Pers. Southern European, Northern European, East Asian, Latin American, and North African cuisines all frequently use this legume (Venkataswamy *et al.*, 2018). Based on Karkanis *et al.*, (2018), the faba bean is crucial as a crop for both pulses and vegetables for their benefits to human nutrition as a dietary source of fiber and protein, dry and fresh seeds or pods are advised. Adding faba beans to crop rotation systems also enhances soil from an agronomical standpoint because they increase

soil organic matter and can fix atmospheric N_2 in levels up to 200 kg N ha^{-1} . As a result, its integration into rotational systems significantly enhances the sustainability of agricultural systems.

Mesmar *et al.*, (2022), have highlighted that numerous analyses were carried out on the antibacterial activity of *Ziziphus nummularia* fruit, leaf, and bark extracts against the gram-positive strain *Staphylococcus aureus* and the gram-negative strain *Escherichia coli* applying the agar well diffusion method. Findings showed the strongest antibacterial activity against both of the tested strains, followed by extracts made from hexane and chloroform. Interestingly, the plant's fruit component significantly reduced the growth of several gram-positive strains tested. Other investigations have also shown that the *Ziziphus nummularia* fractions in chloroform and ethyl acetate were efficient against both gram-negative and gram-positive bacteria and some fungal species, but the fractions in methanol and water showed no activity against any of the examined pathogens. According to Sharma *et al.*, (2013), the alkaloids, flavonoids, glycosides, and saponins found in the plant's parts extracts are what give *Ziziphus nummularia* its antibacterial and antifungal properties.

MATERIALS AND METHODS

This study was carried out at the laboratory of the Microbiology Department, Faculty of Science, Alasmarya Islamic University, Zliten, Libya based on an experiment that extended throughout November of 2022. The main purpose of the study was to test the stimulating effect of different concentrations of the antifungal Nummularine (50, 100, and 150) $\mu\text{g/ml}$ extracted from the stem bark of *Ziziphus nummularia* on germination, shoot length, and shoot and root weight of faba bean (*Vicia faba* L.). The experiment was designed based on 4 treatments and each treatment was randomly distributed and 4 times repeated as illustrated in the following scheme:

150 $\mu\text{g/ml}$	C_1	100 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$
100 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	C_2	150 $\mu\text{g/ml}$
50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$	150 $\mu\text{g/ml}$	C_3
C_4	150 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$

Figure (2): Random Distribution of Faba Bean (*Vicia faba* L.) Replicates

Local varieties of faba bean (*Vicia faba* L.) seeds were obtained from the popular market of Zliten city. The soil used for plantation was collected, transferred to the laboratory, air dried, sieved with a 2 mm sieve, and placed in plastic pots, and each pot was labeled with the date, treatment number, and replication number.

Four faba bean seeds were planted in each pot at a depth of 3 cm. After 2 days of the plantation, plants were treated with different concentrations of Nummularine (50, 100, and 150) µg/ml, and then the plants were harvested after 30 days of germination. Length, wet weight, and dry weight measurements of both the shoot and root system were carefully measured and recorded.

Germination and seedling emergences were daily observed and the emergence of the first seedling was recorded after 3 days of the plantation. The germination percentage was determined after 10 days of plantation utilizing the following formula:

Germination rate (%) = (number of germinated seeds) / (total number of planted seeds) X 100

RESULTS & DISCUSSION

Based on Table (1), findings have shown that the aqueous Nummularine aqueous extracts had an evident stimulating effect in their various concentrations toward the germination of the faba bean (*Vicia faba* L.) as the germination rate showed a direct proportion with Nummularine aqueous extract concentration. The stimulating effect of the aqueous extracts of Nummularine could be due to its antifungal properties against various fungal genera (Salhi *et al.*, 2017).

Table (1): The effect of Nummularine on the Germination Rate of (*Vicia faba* L.)

Treatment	Germination rate (%)
Control (0 µg/ml)	83%
50 µg/ml	88%
100 µg/ml	93%
150 µg/ml	98%

Table (2) has also confirmed the stimulating impact of Nummularine regarding the length of the shoot system of (*Vicia faba* L.) which showed a direct proportion with Nummularine aqueous extract

concentration. It was observed that the highest length of faba bean was at the concentration of 150 µg/ml whilst the shortest length was seen with the control treatment (0 µg/ml).

Table (2): Shows the Shoot System Length of (*Vicia faba* L.)

Treatment	Shoot system length (cm)
Control (0 µg/ml)	23.9
50 µg/ml	18.3
100 µg/ml	11.7
150 µg/ml	4.6

Results may be interpreted due to the effect of Nummularine on the morphological and anatomical characteristics of the faba bean, which led to an increase in the number of cells and the length of the internodes.

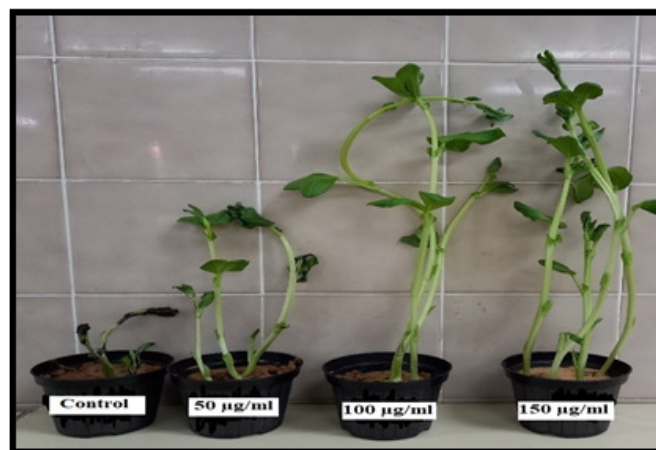


Figure (3): The Stimulating Effect of Nummularine on (*Vicia faba* L.)

Wet and dry weight measurements of both the shoot and root system revealed a positive effect, which was associated with the concentration intensity and its stimulating effect on the absorption of water and the necessary elements in plant tissues and the increase in the water content and thus the size of the vegetative and root systems (Kramer, 1945).

CONCLUSION

To conclude, this study confirms the stimulating effect of the aqueous extracts of Nummularine extracted from the stem bark of *Ziziphus nummularia* on the growth and germination of faba bean (*Vicia faba* L.). Whereas, germination percentage, length, and wet and dry weight measurements of both the shoot and root system

showed a direct proportion towards Nummularine concentration. The 150 µg/ml concentration showed the highest stimulation effect compared to the control treatment and to other concentrations where the shoot system length scored 23.9 cm, whilst the seed germination rate was 98%. On contrary, the control treatment showed the lowest stimulating effect where the length of the shoot system was at 4.6 cm, whilst the seed germination percentage was at 83%.

Table (3): Wet and Dry Weight of Shoot and Root Systems of (*Vicia faba* L.)

Treatment	Shoot System		Root System	
	Wet weight (gm)	Dry weight (gm)	Wet weight (gm)	Dry weight (gm)
Control (0 µg/ml)	1.8	0.8	1.5	0.3
50 µg/ml	2.0	1.9	3.4	0.7
100 µg/ml	17.5	7.6	5.0	0.9
150 µg/ml	22.5	13.9	5.7	1.6

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Recommendations

The author of this work would highly recommend other researchers conduct similar studies to confirm the results.

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