The allelopathic effect of some plant extracts on the vegetative and physiological traits of *Vicia faba* L. plant

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ABSTRACT

A pots experiment was conducted at a private nursery in the Khan Bani Saad district of Diyala Governorate, located in the north of Iraq's capital, Baghdad, During the winter growing season of 2023. The aim was to evaluate the allelopathic effectiveness of leaf extracts of acacia, eucalyptus, and moringa plants on the vegetative and physiological traits of faba bean plants. The experiment was designed according to a Randomized Complete Block Design (RCBD) and the extracts were applied as foliar sprays on the faba bean plants at concentrations of 5% and 10% as well as the control treatment. There were a total of 7 treatments, each with three replicates, making the total number of experimental units, 21. The results indicated that the moringa extract at a concentration of 10% outperformed the other extracts by recording the highest averages for all studied traits, except for the dry weight of the root and the phosphorus and potassium content of faba bean leaves. For the dry weight of the root, the acacia extract at a concentration of 10% , recorded the highest average, while the 5% concentration of acacia extract recorded the highest average of Potassium percentage of faba bean leaves. Additionally, In addition, the results showed that the eucalyptus extract at a concentration of 5% recorded the highest average of phosphorus percentage of the faba bean plant leaves. The use of plant biostimulants is a new and environmentally friendly strategy for sustainable crop production, which is constrained by issues such as water scarcity, resource depletion, environmental stresses, and climate change.

Keywords: Allelochemicals, faba bean, *Acacia* extract, *Eucalyptus extract, Moringa extract*.

INTRODUCTION:

Plants produce different secondary metabolites, which may be inhibitory or stimulatory the growth of other plants, a phenomenon known as allelopathy or interaction effect. This effect is defined as any influence, whether positive or negative, directly or indirectly, of one plant on another through the production and release of allelochemicals into the environment (20). Allelochemicals can either stimulate or inhibit the germination and/or growth of plants, as well as enhance the resistance of crops to both biotic and abiotic stresses. Chemicals released by plants do not leave behind any toxic residues, making them ideal alternatives to synthetic herbicides (11). Studies have proven that natural extracts from forest trees increase the production of crops and improve their quality by increasing plant absorption of nutrients, stimulating seeds germination, enhancing plant growth and increasing its resistance to abiotic stresses (51, 49 and 31). However, the frequent cultivation of these tree on roadsides and near agricultural lands can lead to the accumulation of plant chemicals released from it, posing a risk to the productivity of surrounding or adjacent crops (34).

Acacia saligna is a perennial tree belonging to the Fabaceae family, native to Australia, and it has spread to various parts of the world including Asia, Africa, and Europe (28). Acacia tree is indeed a fast-growing, woody tree known for its invasive characteristics. It is valued for its high protein content, which typically ranges from 18.25% to 35.5% (3). It is an important source of gum, tannin, wood, and fuel, as well as fodder and medicines, indicating its low toxicity and high nutritional

value (12). Its allelopathic effect comes from its high content of phenols, flavonoids, and terpenes which plays an important role in suppressing the free radicals resulting from various biological activities in plants by increasing the activity of antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) and thus improving the various growth characteristics of the plant (25) .

Eucalyptus globulus belongs to the Myrtaceae family, and it is an evergreen tree cultivated in many cities worldwide in parks, gardens, and along roadsides, in addition to the presence of other wild species (39). A significant part of the Eucalyptus tree's value lies in its aromatic leaves containing essential oils, which is considered the most effective compound in the Eucalyptus plant. Eucalyptus leaves also contain other compounds such as phenols (26), terpenoids (8), and flavonoids (27), which have been confirmed to have roles as antimicrobials (13), insecticides (29), and as a means of weed control (18), as well as tannins and citronellol. The eucalyptus plant possesses high allelopathic activity due to its content of coumaric, gallic, gentisic, hydroxybenzoic, syringic, and vanillic acids (39, 15 and 45).

Moringa oleifera tree, known as the "miracle tree" for its diverse benefits, belongs to the Moringaceae family and grows in tropical and subtropical environments (37). It is characterized by its fast growth and good resistance to drought. Moringa is cultivated for its numerous advantages, as it contains various industrial, medicinal, and allelopathic compounds. Extracts from *Moringa oleifera* leaves are rich in essential minerals, plant growth hormones such as gibberellins, auxins, and cytokinins, as well as vitamins, flavonoids, phenols, tannins, amino acids, and ascorbic acid (42, 9 and 22). Additionally, Moringa extract alleviates abiotic stresses encountered by plants during different life stages, such as salinity, drought, heat, and heavy metals, by increasing the activity of antioxidant enzymes and enhancing the plant's content of phenolics, flavonoids, and sugars, thereby reducing the levels of reactive oxygen species. Moringa extracts also act as a biopesticide against various plant pathogens (5).

Faba bean *(Vicia faba* L.) plant belong to the winter crops of the Fabaceae family, and they are among the most important and oldest cultivated crops worldwide, being a significant source of protein (30). Mature Faba bean seeds are rich in proteins (26.1%), carbohydrates (58.3%), and dietary fibers (25.0%) (47). Faba bean also contain a range of biologically active compounds such as total phenolics and flavonoids with antioxidant activity (48). Mediterranean countries, India, China, Egypt, Afghanistan, Ethiopia, North Africa, and Northern Europe are among the major producers of this crop (41). Faba been are cultivated as a human food source, animal feed, and for nitrogen fixation in soil, reducing the need for synthetic fertilizers (50).

The aim of the current study is to investigate the allelopathic effect of aqueous extracts of acacia, eucalyptus, and moringa leaves on the vegetative and physiological characteristics of faba bean plants.

MATERIALS AND METHODS:

Plant Sample Collection and Diagnosis:

Leaves of acacia, eucalyptus, and moringa plants were collected from various areas in Diyala Governorate, which spans between longitudes (44.22- and 45.56) east and latitudes (3.33- and 35.6-) north and located in the north of Iraq's capital, Baghdad, in February 2024. The plants were classified at the College of Education for Pure Science/University of Diyala. The plant leaves were washed with ordinary water, then rinsed with distilled water, and left to air dry in the shade. The samples were periodically turned to prevent fungal contamination.

Preparation of Aqueous Leaf Extracts:

Aqueous extracts of the plants were prepared following the method described by (21). Previously dried plant leaves of each of the three plants were ground using an electric

grinder. Subsequently, the aqueous extract of each plant was prepared by adding 5 g of the dry powder into a 500 ml glass flask, then boiling water was added to the powder until the final volume reached 100 ml to get a 5% concentration, A concentration of 10% was prepared by adding 10 g of dry powder into a 500 ml glass flask and boiling water was added to the powder until the final volume reached 100 ml. The mixtures were then blended using a horizontal shaker at medium speed for half an hour. Afterward, the mixtures were left to settle for an hour and then filtered using multiple layers of mesh to separate solid residues. The filtrate of each mixture was taken and stored for later use.

Experimental Design and Treatments:

A pots experiment was conducted in a private plantation in the Deli Abbas district, located in Diyala Governorate, which is in the north of Baghdad, the capital of Iraq, in the winter of 2023. The faba bean seeds were obtained from local markets.

The experiment was conducted using RCBD (Randomized Complete Block Design) and included two concentrations of each of the three plant extracts (5%, and 10%) with control treatment. The total number of treatments was 7, as shown in Table 1, and they were replicated three times, resulting in a total of 21 experimental units.

Soil samples were collected from the study area before planting. The samples were thoroughly mixed, and a composite random sample was taken and sent to the Soil Department Laboratories at the Directorate of Agriculture in Diyala Governorate to study some of the soil's chemical and physical properties. The results of the soil analyses are presented in Table 2.

Faba bean seeds were planted at a rate of three seeds per hill. After the plants reached the two-leaf stage, thinning was carried out to maintain one plant per hill. At the three-leaf stage, DAP (Diammonium phosphate) fertilizer was applied based on the agricultural recommendations of growing faba beans in Iraq. The aqueous extracts of the three plants were then sprayed onto the faba bean plants, following specified concentrations. This was done using a hand sprayer with a 1-liter capacity. The application occurred in two stages, the first application took place when the plants reached the 3-4 leaf stage, and the second application was done two weeks later. Control plants were sprayed with distilled water only. The agricultural services, including watering and weeding, were carried out whenever it necessary.

Table 1: The experiment Treatments

No. of Treatments	The Treatments
T1	Control treatment 0% (without adding plant extracts)
T2	Aqueous extract of acacia leaves at a concentration of 5%
T3	Aqueous extract of acacia leaves at a concentration of 10%
T4	Aqueous extract of eucalyptus leaves at a concentration of 5%
T5	Aqueous extract of eucalyptus leaves at a concentration of 10%
T6	Aqueous extract of moringa leaves at a concentration of 5%
T7	Aqueous extract of moringa leaves at a concentration of 10%

Table 2: Chemo physical properties of the study soil

Soil properties	Unit	Value	
pH	----------	7.1	
EC (1:1)	dS/m	4.4	
Total nitrogen	mg/kg	83.2	
Available phosphorus	mg/kg	5.4	
Available potassium	mg/kg	114	
Organic matter	%	1	
Clay	%	31	Silty clay loam
Sand	%	12	
Silt	%	57	

Studied Characteristics:

Physiological traits were studied 2 weeks after the last spraying of plant extracts, while vegetative traits were studied at the beginning of the flowering stage.

1- Plant height (cm): The plant height was measured using a metric ruler from the soil surface to the top of the plant stem.

2- Leaf area (cm^2): The leaf area of faba bean plant leaves which selected randomly from 3 plants from each experimental unit was measured according to (44) method. First, the plant leaf was photographed using a photocopier, and then the weight of the entire photocopied paper was measured. Next, the shapes of the photocopied plant leaves were cut out of a piece of photocopy paper and weighed using a sensitive balance. Since the dimensions of the photocopy paper are known (length L 29.7 cm and width W 21.1 cm), the area of the plant leaves was calculated according to the following formula:

Plant leaf area = (Photocopied paper area × photocopied paper weight) / (Weight of the photocopied plant leaf).

3- Leaves number ($Leaf.plant^{-1}$) : All leaves of plant were counted.

4- Shoot dry weight (g): The dry weight of the shoot was measured after separating the shoot from the root, drying it in the air for 3 days, and measuring the weight using a sensitive electronic balance.

5- Root dry weight (g): The dry weight of the root was measured after washing it several times with regular water and letting it dry in the air for 5 days, then measuring the weight of the dried root using a sensitive electronic balance.

6- N % : The percentage of nitrogen was estimated using the Micro-Kjeldahl apparatus according to the method described in (24).

7- P % : The percentage of phosphorus was estimated using ammonium molybdate and ascorbic acid with a Spectrophotometer at a wavelength of 882 nanometers, according (36).

8- K% : The percentage of potassium was estimated using a Flame Photometer according to the method described by (7).

Statistical Analysis:

The experiments were conducted using a Randomized Completely Block Design (RCBD), and the results were statistically analyzed using Analysis of Variance (ANOVA) with the SPSS software. Mean comparisons were made using the Duncan's Multiple Range Test at a significance level of 5% probability (6).

RESULTS:

1- Plant Height (cm), Leaf Area (cm²) and Leaves number (Leaf.plant^{-1}):

Table 3 results indicate non-significant differences between the averages of the plant height trait due to treating the faba bean plant with acacia, eucalyptus, and moringa extracts at concentrations of 5% and 10%. It is evident from the results that the highest average of this trait resulted from treating faba bean plants with acacia extract and moringa extract at a concentration of 10%, reaching (9.980 cm), while the lowest average resulted from the control treatment , which was (6.867 cm). As for the leaf area, the results presented in Table 3 reveal significant differences among the averages of the leaf area trait when faba bean plants were sprayed with different plant extracts at concentrations of 5% and 10%. However, moringa plant extract at a concentration of 10% exhibited the highest average for this trait, measuring 15.332 cm², followed by acacia plant extract at a concentration of 5% which recorded an average of 15.123 cm^2. while the lowest average was observed in faba bean plants treated with eucalyptus extract at a concentration of 10%, measuring 10.987 cm². The analysis presented in the same Table demonstrates no significant differences between the averages of the number of leaves per plant trait resulting from treating faba bean plants with different plant extracts at concentrations of 5% and 10%. The highest average number of leaves was observed when treated with moringa plant extract at concentrations of 5% and 10%, reaching 35.000 leaf per plant and 39.667 leaf per plant respectively , while the lowest average resulted from control treatment and amounted to 25.333 leaf per plant.

Table 3: The effect of foliar spraying with extracts of acacia, eucalyptus, and moringa on plant height (cm), leaf area (cm^2) and leaves number (leaf.plant^{-1}) .

Treatments	Plant hight (cm)	Leaf area (cm^2)	Leaves number (Leaf.plant^{-1})
Control (0%)	**6.867 a**	**11.572 bc**	**25.333 a**
Acacia (5%)	**9.467 a**	**15.123 ab**	**31.667 a**
Acacia (10%)	**9.980 a**	**12.032 abc**	**33.667 a**
Eucalyptus (5%)	**8.933 a**	**13.116 abc**	**30.833 a**
Eucalyptus (10%)	**7.980 a**	**10.987 c**	**33.000 a**
Moringa (5%)	**9.923 a**	**13.369 abc**	**35.000 a**
Moringa (10%)	**9.980 a**	**15.332 a**	**39.667 a**

Values followed by the same letters are not significantly different at P=0.05.

2- Shoot and Root Dry Weights (g):

The results presented in Table 4 indicate significant differences among the averages of the dry weight of the shoot trait resulting from treatment with acacia, eucalyptus and moringa extracts at concentrations of 5% and 10%. Treating faba bean plants with moringa plant extract at a concentration of 10% recorded the highest average dry weight of shoot (2.573 g), followed by eucalyptus extract at a concentration of 5% which recorded an average of 2. 423 g. The lowest average resulted from treating faba bean plants with eucalyptus plant extract at a concentration of 10%, which was 1.543 g.

As for the dry weight of the root, the analysis presented in Table 4 reveals significant differences among the averages of this trait obtained due to treatment of faba bean plants with acacia plant extract at concentrations of 5% and 10%, recording the highest averages of (0.603 g) and (0.940 g) respectively. This was followed by eucalyptus extract at a concentration of 10%, which recorded an average of 0.526 g, while the lowest average (0.303 g) was obtained from the control treatment (0%).

Table 4: The effect of foliar spraying with extracts of acacia, eucalyptus, and moringa on shoot dry weight (g) and root dry weight (g) .

Treatments	Shoot Dry Weight (g)	Root Dry Weight (g)
Control (0%)	1.980 ab	0.303 c
Acacia (5%)	1.990 ab	0.603 b
Acacia (10%)	2.250 a	0.940 a
Eucalyptus (5%)	2. 423 a	0.446 bc
Eucalyptus (10%)	1.543 b	0.526 bc
Moringa (5%)	2.243 a	0.340 c
Moringa (10%)	2.573 a	0.360 c

Values followed by the same letters are not significantly different at P=0.05.

6- Nitrogen% , Phosphorus% and Potassium% of Faba Bean Leaves :

The results presented in Table 5 indicate significant differences among the averages of nitrogen percentages in the leaves of faba bean plants due to treatment with various plant extracts. The highest average **3**.313 %, resulted from treatment with moringa extract at a concentration of 10%, followed by acacia plant extract, which recorded an average of 3.000 % at concentrations of 5% and 10%, whereas the lowest average of 1.967% was observed in plants treated with eucalyptus extract at a concentration of 10%.

There were no significant differences observed among the phosphorus percentage averages of faba bean plants as a result of treatment with plant extracts at concentrations of 5% and 10%, as outlined in Table 5. The results indicated that the eucalyptus plant extract at a concentration of 5% yielded the highest average of 0.770 %, whereas the 10% concentration of eucalyptus extract recorded the lowest average of phosphorus percentage registering 0.066 %.

As for the potassium percentage trait of faba bean plant, the results presented in Table 5 indicate significant differences among the averages of this trait due to treatment of faba bean plants with acacia, eucalyptus and moringa extracts at concentrations of 5% and 10%. The results indicated that the acacia plant extract at a concentration of 5% recorded the highest average, 1.450%, while the lowest average resulted from treatment with eucalyptus plant extract at a 10% concentration, amounting to 0.613%.

Table 5: The effect of foliar spraying with extracts of acacia, eucalyptus, and moringa on the the faba bean leaves content of N, P and K (%).

Treatments	N%	P%	K%
Control (0%)	2.0667 c	0.110 a	1.130 ab
Acacia (5%)	3.0000 ab	0.453 a	1.450 a
Acacia (10%)	3.0000 ab	0.110 a	1.060 b
Eucalyptus (5%)	2.0333 c	0.770 a	1.250 ab
Eucalyptus (10%)	1.967 c	0.066 a	0.613 c
Moringa (5%)	2.8000 b	0.096 a	1.130 ab
Moringa (10%)	3.3133 a	0.116 a	1.200 ab

Values followed by the same letters are not significantly different at P=0.05.

Discussion:

The results of our study showed a noticeable improvement in all the studied traits as a result of treatment with acacia plant extract, as the acacia plant extract is characterized by containing a diverse range of compounds that promoting plant growth, including proteins, carbohydrates, phenols, gallic acid, coumaric acid, palmitic acid, oleic acid, and linoleic acid (4 and 14). The abundance of these chemical substances in acacia plant extract is responsible for enhancing plant growth, improving water use efficiency, increasing nutrient absorption, enhancing photosynthesis efficiency, and boosting the plant's ability to resist various harsh conditions encountered during its life stages by stimulating the antioxidant defense mechanism in the body (2, 19, 43 and 35). However, the decrease in leaf area of pea plant resulting from treatment with acacia plant extract may be attributed to the allelopathic effect of secondary metabolism compounds in acacia plant, such as phenols, terpenoids, organic acids, or alkaloids, which, at high concentrations, inhibit cell division and elongation.

Eucalyptus plants have a high allelopathic effect, which may be inhibitory or stimulatory, depending on the concentrations used (46). Eucalyptus leaf extract contains various phenolic acids such as caffeic, ferulic, benzoic, chlorogenic, hydroxybenzoic, and cinnamic acids (16). The results of our study showed an increase in most of the studied traits following treatment with eucalyptus extract. The results indicated that a 5% concentration had a better growth stimulating effect than the a 10% concentration. The results of this study consistent with the finding of (17), demonstrating significant growth enhancements in pea plants upon treatment with various concentrations of eucalyptus plant extract.

Our study results revealed that treating faba bean plants with moringa extract led to an increase in the averages of the studied traits, surpassing other plant extracts by achieving the highest averages for all traits, except for root dry weight and leaf content of phosphorus and potassium. Moringa leaf extract acts as a natural growth stimulant (40) because moringa leaves are a source of cytokinin hormones and important micro-nutrients and vitamins necessary for plant growth (33, 38 and 32). Moreover, some extracts, such as those found in *Moringa oleifera* leaves, rich in proteins, fats, carbohydrates, minerals, vitamins, and amino acids, have a stimulating effect on plant growth and productivity (1 and 23). Our findings align with those of (10) in their investigation of the impact of the moringa plant extract on the growth and development of bean plants.

CONCLUSION:

Based on the results of this study, it is concluded that the active compounds found in the extracts of moringa and acacia plants do not have harmful or toxic effects, even at high concentrations on the traits of the treated plants, while the active compounds found in the eucalyptus plant extract at low concentration showed a better growth stimulating effect than the high concentration**.**

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