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Allelopathic Effect of *Vicia faba* L. Extracts on Stimulating or Inhibiting the Seeds Germination and Seedling Growth of *Lycopersicon esculentum* Mill

ABSTRACT

This study was carried out for investigating the stimulatory and inhibitory effects of the aqueous extracts of *Vicia faba* on the seed germination and the growth of the *Lycopersicon esculentum* seedlings. The experiment was carried out at the research stations and laboratories of College of Agriculture, Tikrit University, and the Department of Examination and Certification of Seeds in Tikrit, Salah Aldeen, Iraq. In this study, different concentrations of the aqueous extracts of various parts of *Vicia faba* were tested in this study, namely (4, 8 and 12% root extracts; 4, 8 and 12% stem extracts; 4, 8 and 12 % leaf extracts) for determining their effects on the seed germination and the seedling growth of the *Lycopersicon esculentum* plant. Water was used as the control treatment. All parameters used in the experiments were randomly distributed based on the Completely Randomised Design (CRD). The results were the mean of 4 replicate experiments. The results indicated that the aqueous root extracts (4%) of *Vicia faba* showed a significant stimulatory effect on the different growth parameters like radicle length and the dry weight of the radicle of the *Lycopersicon esculentum*. The different aqueous extracts of the *Vicia faba* showed differing effects (either stimulatory or inhibitory) on the seedling growth. The mode of action was dependent on the extracts of the plant parts that were used. The results indicated that the different concentrations of aqueous extracts of various parts of *Vicia faba* showed an allelopathic effect.

INTRODUCTION:

Vegetable crops are regarded as an important component of the regular human diet. One of the most popular and common vegetable crops which is used, worldwide, is *Lycopersicon esculentum*. It is a major vegetable crop that feeds people (Hasan, 2015a).

Though Iraq has many favourable factors for growing this crop, the production is very low, i.e., 22187 Kg. ha^{-1}. This production ranks No. 14 amongst all the other Arabic countries (AOAD, 2016). The *Lycopersicon esculentum* crop is grown in a crop rotation sequence after growing many cycles of leguminous crops such as *Pisum sativum*, *Vicia faba* and *Phaseolus vulgaris* (Hasan, 2015b).

The production of this crop was less in Iraq and other Arabic countries due to the exploding population, which further decreased their ability to produce vegetable crops. Though these countries use large quantities of pesticides and fertilisers, they have to import the crops for fulfilling the demands of the people. Earlier studies have reported that the production yields of the plants like *Lycopersicon esculentum* were dependent on several factors like pest management, nature of the soil,

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crop variety used, seed germination and other problems related to the growth of seedlings. The yield is also affected by the growth of its neighbouring plants owing to the allelopathic effects.

It was noted that the allelopathic effect was dependent on the exudates and the crop residues which negatively affected the growth, yield and the germination of the *Lycopersicon esculentum* crops (Kuzyakov et al., 2007). Komguem et al. (2016) observed that the aqueous extracts of different plants like *Cupressus lusitanica*, *Tephrosia vogelii*, and *Callistemon viminalis* significantly inhibited the seed germination. On the other hand, the aqueous extracts of plants like *Tephrosia vogelii*, *Callistemon viminalis*, *Cupressus lusitanica*, *Senna spectabilis*, and *Polyscias fulva* decreased the radicle length of the *Lycopersicon esculentum*. Furthermore, when the aqueous extracts of *Vigna radiata* were sprayed during the vegetative stage, the seed germination and the seedling growth of plants like *Zea mays* var. rugosa and *Abelmoschus esculentus* was stimulated, compared to when they were sprayed during the maturing and flowering stages (Ghassan et al., 2016).

Allelopathy is defined as a phenomenon that plays a vital role in the environmental and agricultural systems since it affects the production of various crops. This effect is produced due to a biochemical interaction amongst the crops, between the weeds and crops or between microbes and plants (Rice, 1984).

The allelopathic effects are noted when different chemical compounds are released by the plants. These chemical compounds are water-soluble and solubilise in the surrounding water, or get leached, volatilised or enter the plants through the root exudates and decomposing crop residue in the soil. These water-soluble compounds show an allelopathic effect and are released by the leaves, roots, stems, seeds and flowers of some plants (Rice,1984).

Based on the above facts, the researchers hypothesised that the *Vicia faba* plant probably contained several chemical compounds that would affect the seed germination of the *Lycopersicon esculentum* crop and thereafter, its plant vigour index. They carried out a preliminary *in vitro* study for verifying these facts before conducting field investigations. Hence, this study was designed for investigating the *in vitro* allelopathic effects of the aqueous extracts of different parts of the *Vicia faba* plant on the seed germination and the vigour index of *Lycopersicon esculentum*.

2. MATERIALS AND METHODS:

The experiment was conducted from October to March, 2018, at the College of Agriculture, Tikrit University and the Department of Examination and Certification of Seeds in Tikrit city, Salah Aldeen, Iraq.

2.1 Sample preparation:

The *Vicia faba* plants were harvested during maturity in March. The different plant parts were cut into small pieces. Thereafter, 40, 80 and 120 g of the cut plant parts were added to distilled water (1 L) in a flask, which led to the final concentration of 4, 8 and 12% v/v or 40, 80 and 120 g L-1. The mixture was stirred for ten minutes and left in at room temperature for 48 h. The plant extracts were filtered with the help of 2 layers of cheesecloth. The resultant mixture was again filtered through Whatman Number 1 filter paper. The final extracts were stored at 5°C, until further use. The filtrates were taken out of the refrigerator 24 h before being used experimentally, in order to achieve room temperature.

2.3 Germination, seedling growth and vigour index:

In this study, the *Lycopersicon esculentum* seeds were used as the test crops. 25 seeds for used for every treatment. These seeds were placed in a sterile Petri plate (13.8 cm diameter), which were lined with 2 layers of filter paper. Thereafter, the aqueous *Vicia faba* extracts (4, 8, and 12%, respectively for the different plant parts) were added to every Petri plate (10 mL). Water was used as the control treatment. The experiment was carried out in the dark, with minimal exposure to light during the data collection. The Petri plates were placed in a seed germinator (AGROSAW) at a

constant temperature of temperature 25° C. After 8 days of the treatment, the seed germination was determined by counting the number of seeds which germinated in every petri dish. These results were expressed in per cent values (Saied 1984). 5 seedlings were left in every Petri plate. After the 14th day, the measurements were carried out the below-mentioned:

1. Seed germination: The seed germination was measured after 8 days, using the below formula (Saied, 1984):

Germination (%) = Number of seeds germinated /total number of seed x 100

2. Inhibition percentage: The inhibition percentage was measured using the below formula (Chung et al., 2003).

$$Inhibition(\%) = \frac{control - extract}{control} \times 100$$

3. Speed of germination: The speed of germination was determined using the formula described by Gairola et al. (2011) and Kebede and Yidinekachew (2014) as follows:

Speed of germination = *n1/d1 + n2/d2 + n3/d3 + ----------+ nm/dm*

where, *n* = number of germinated seeds; *d* = number of days; *m* = *m*th round count/days

4. Hypocotyl length: The Hypocotyl length was measured from the base of the stem to the top of the seedling (using a graph paper).
5. Radicle length: The researchers determined the radicle length from the base of the stem to the root tip (using a graph paper).
6. Dry weight of hypocotyl: The dry weight of the hypocotyl was determined after the plant material was dried in the oven at medium heat (70° C) until a constant weight was noted.
7. Dry weight of radicle: The researchers determined the dry weight of the radicle using the same technique used for determining the dry weight of the hypocotyl.
8. The vigour index: The researchers calculated the vigour index of the plant using the formula described earlier by Abdul-Baki and Anderson (1973) as follows:

Vigour index = Germination (%) × total seedling length

2.4 Statistical analysis:

The researchers carried out the bioassay experiments using the Complete Randomised Design (CRD) which included 1 factor. The experiments were replicated 5 times. In this study, the researchers tested different concentrations of the aqueous extracts of various parts of *Vicia faba* (i.e., 4, 8 and 12% root extracts; 4, 8 and 12% stem extracts; 4, 8 and 12 leaf extracts) for determining their effects on the seed germination and the seedling growth of the *Lycopersicon esculentum* plant. Water was used as the control treatment. Thereafter, the analysis of variance (ANOVA) was applied using the SPSS program. The mean values were analysed using Duncan's test at probability levels of 0.05.

3. RESULTS AND DISCUSSION:

3.1. Effect of extract on percentage of germination (inhibition or stimulation):

With regards to the major treatments, the *Vicia faba* aqueous extracts reduced the seed germination of the *Lycopersicon esculentum* compared to the negative control sample (Fig. 1). The results indicated that the aqueous leaf extracts (12%) showed the lowest stimulatory effect on the seed germination when compared to the negative control. However, 12% root and leaf extract samples showed highest inhibitory effects. The control treatment significantly increased the seed germination of the *Lycopersicon esculentum*, i.e., 96.00%.

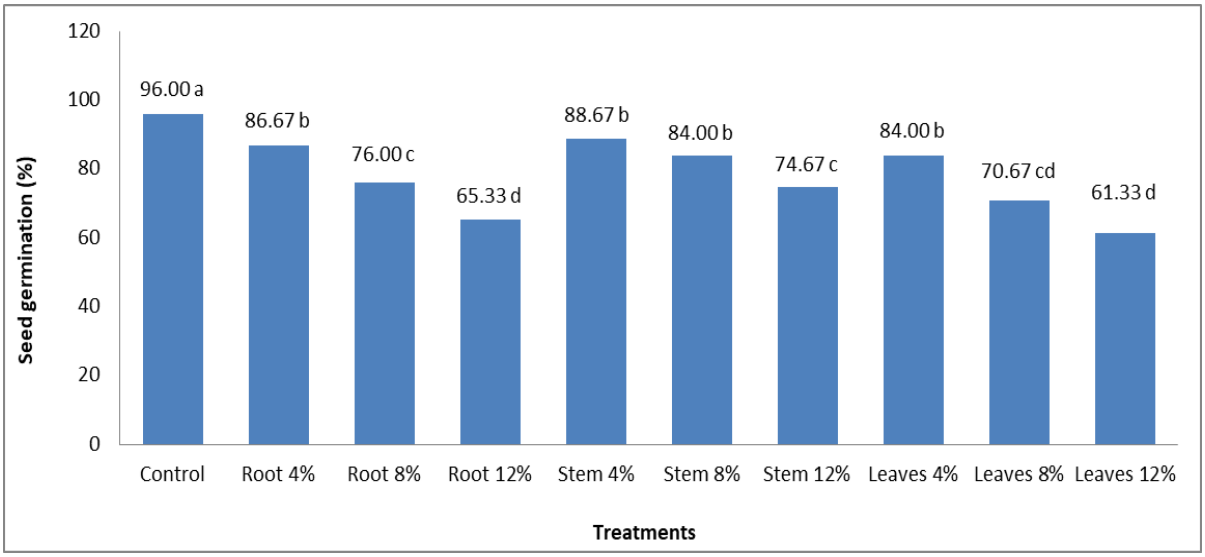

Fig. 1. Effect of *Vicia faba* extract on seed germination of *Lycopersicon esculentum* (%)

(Different alphabets show significant difference using Duncan's Multiple Range test (P≤ 0.05))

The results of the Duncan multiple comparison test for the average seed germination indicated that the leaf extracts (12%) showed the highest inhibitory effect on the seed germination of the *Lycopersicon esculentum*, i.e., 36.11% inhibition (Fig. 2).

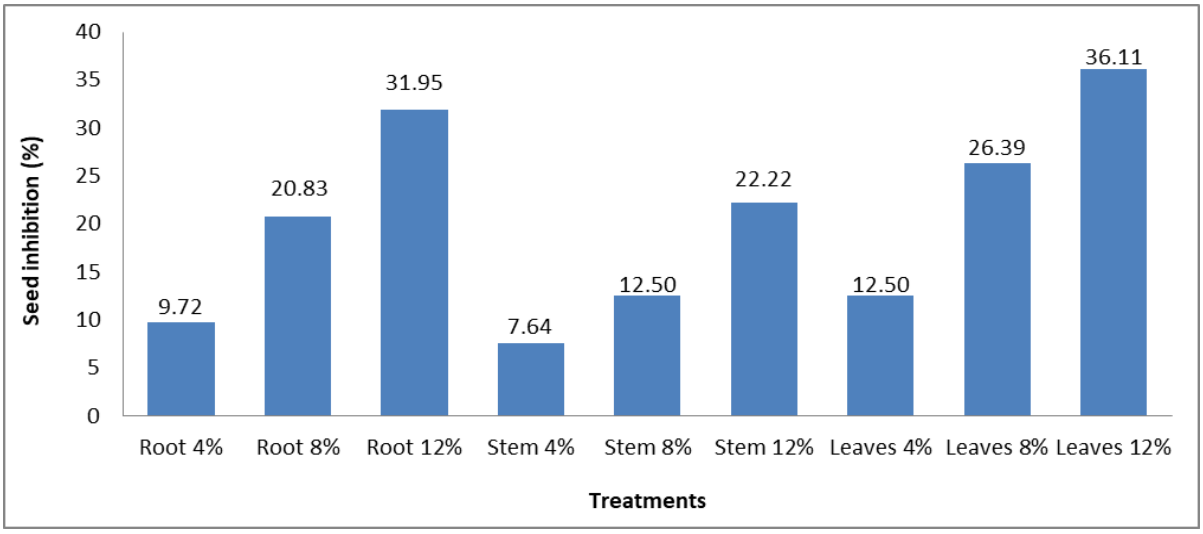

Fig. 2. Effect of *Vicia faba* extract on seed inhibition of *Lycopersicon esculentum* (%)

The results indicated that the leaf extracts showed higher phytotoxicity compared to the root and stem extracts. This was attributed to the photosynthesis process and accumulation of many probable phytochemicals in some plant parts. Furthermore, this effect was based on the concentration, plant types, type of plant material that was used and plant parts. These results were similar to those reported earlier (Sukumarn et al., 2011; Talukder et al., 2015; Gowsiya and Santosh, 2016). Thus, it was concluded that the aqueous extracts showed stimulatory or inhibitory effects on the seed germination of the crops that were tested.

3.2 Effect of extracts on speed of germination:

The *Vicia faba* extracts decreased the speed of germination in the *Lycopersicon esculentum* (Fig. 3), hence the researchers concluded that the extracts probably consisted of many inhibitory phytochemicals. On the other hand, the control treatments showed variable results, which was attributed to the fact that they did not contain any allelopathic compounds.

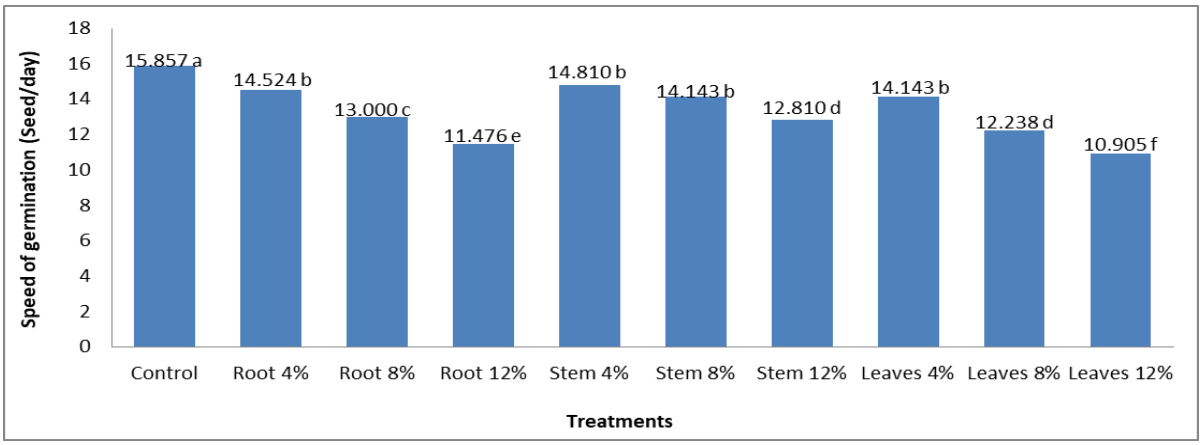

Fig. 3. Effect of *Vicia faba* extract on speed of germination of *Lycopersicon esculentum* (seed day^{-1})

(Different alphabets show significant difference using Duncan's Multiple Range test (P≤ 0.05))

3.3 Effect of extracts on hypocotyl length:

The results indicated that the plant extracts (different concentrations) suppressed the plant growth and showed a negative effect on the test crops. The different plant extracts showed differing results, compared to the control, especially when their concentrations were increased. The different *Vicia faba* extracts at varying concentrations significantly decreased the hypocotyl length of the test crops (Fig. 4). It was noted that the leaf extracts (i.e., 8 and 12%) significantly decreased the total hypocotyl length of the test crops (i.e., 4.067 and 3.133 cm, respectively), compared to the control sample (7.467 cm). This could be attributed to that fact that the leaf extracts contained some phytotoxic compounds which decreased the hypocotyl length of the plants.

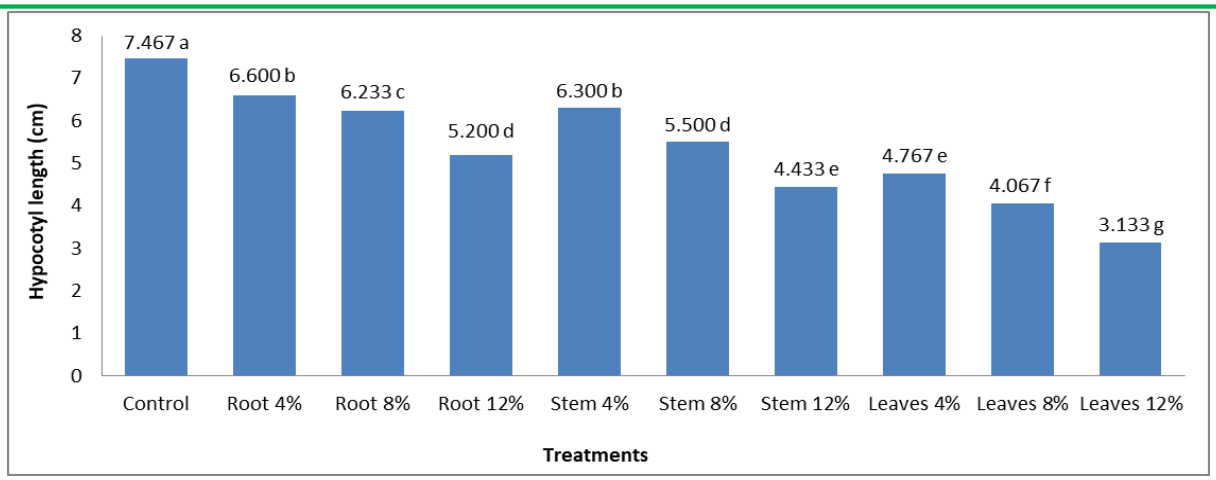

Fig. 4. Effect of *Vicia faba* extract on hypocotyl length of *Lycopersicon esculentum* (cm).

(Different alphabets show significant difference using Duncan's Multiple Range test (P≤ 0.05))

3.4 Effect of extracts on radicle length:

The researchers determined the effect of the plant extracts on the radicle length of the *Lycopersicon esculentum*. It was noted that the aqueous root extracts (4%) increased the radicle length, i.e., 6.733 cm compared to the control (Fig. 5). This indicated the presence of some stimulatory compounds in these extracts. Furthermore, the other extracts and the control treatment showed variable results. The aqueous leaf extracts (12%) decreased the radicle length of the *Lycopersicon esculentum* (1.933 cm). No significant difference was noted in the radicle length of *Lycopersicon esculentum*, when they were treated using the root extracts (8%), stem extracts (4%) or control.

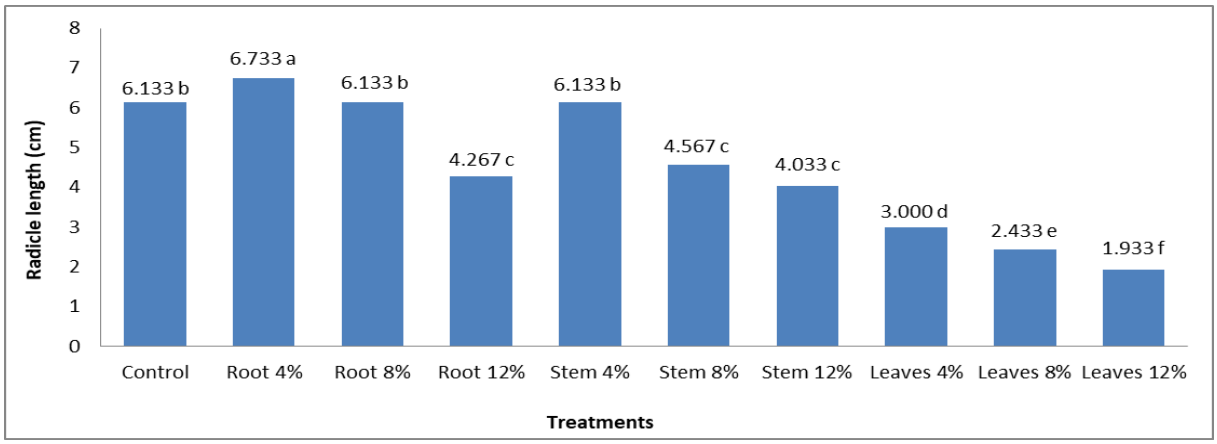

Fig. 5. Effect of *Vicia faba* extract on radicle length of *Lycopersicon esculentum* (cm).

(Different alphabets show significant difference using Duncan's Multiple Range test (P≤ 0.05))

The aqueous leaf extracts negatively affected the *Lycopersicon esculentum* crop. However, the root and stem extracts showed different effects. Similar results were presented by Sarkar et al. (2012), who noted that though the allelochemicals were present in the complete plant, the concentration of these compounds differed amongst the various plant parts. In this study, it was noted that the aqueous leaf extracts of *Vicia faba* showed a higher inhibitory effect on the radicle length of the *Lycopersicon esculentum* seedlings compared to the root and stem aqueous extracts. Similar results were reported by Maharjan et al. (2007), who stated that the aqueous leaf extracts of *P. hysterophorus* exhibited the maximal allelopathic effect on the seed germination compared to the other vegetative plant parts. Some other studies (Tefera 2002; Clarence et al., 2013) observed that the leaf extracts of *Parthenium* plants showed a higher inhibitory allelopathic effect compared to the extracts of other vegetative plant parts. Srivastava et al. (1985) stated that the aqueous inflorescence

and leaf extracts showed a higher inhibitory effect on the seed germination and seedling growth in the wheat, peas and barley plants. However, Guzman (1988) noted that the difference in the intensity of the inhibitory effects of different plant parts was attributed to the release of various phytotoxic compounds like sesquiterpenes, phenolics and lactones, from the roots and vegetative plant parts, along with the achene due to exudation.

3.5 Effect of extracts on dry weight of hypocotyl:

Results presented in Fig. 6 showed that the plant extracts significantly decreased the dry weight of the hypocotyl of the *Lycopersicon esculentum*. On the other hand, the control, root and stem extracts (4%) gave highest values in the hypocotyl dry weights of the *Lycopersicon esculentum*, i.e., 0.03833, 0.03600 and 0.03767 mg, respectively.

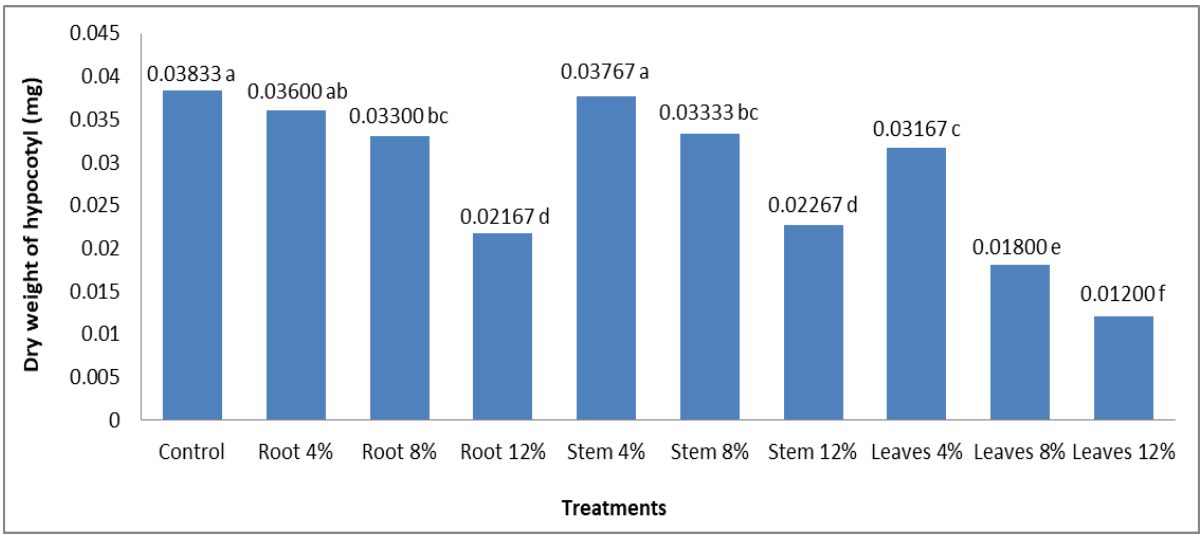

Fig. 6. Effect of *Vicia faba* extract on dry weight of hypocotyl of *Lycopersicon esculentum* (mg).

(Different alphabets show significant difference using Duncan's Multiple Range test (P≤ 0.05))

Similar results were reported earlier by Maharjan et al. (2007) and Clarence et al. (2013), who noted that the germination of the test crops was significantly decreased when increasing concentrations of aqueous leaf extracts of *P. hysterophorus* L plant were used in their study. They noted that the *P. hysterophorus* extracts inhibited the seedling growth and decreased the fresh or dry matter production. Similar results were also noted in many earlier studies (Guzman 1988; Mersie and Singh 1988; Swaminathan et al., 1990; Evans, 1997; Tamado et al., 2002), which noted similar effects on crops like sorghum and maize, pumpkin, multi-purpose trees, and *Lycopersicon esculentum*.
An et al. (2005) observed that different species showed a marked difference with regards to their susceptibility to the allelochemicals.

3.6 Effect of extracts on dry weight of radicle:

Results presented in Fig. 7 showed that the aqueous extracts of the *Vicia faba* plant display stimulatory and inhibitory effects on the dry weight of the radicles of *Lycopersicon esculentum*. The root extract (4%) stimulated the dry weight of *Lycopersicon esculentum* radicles, i.e., 0.04267 mg, but there was no significant different with control treatment and root extract (8%), whereas the other aqueous plant extracts inhibited the dry weights of the *Lycopersicon esculentum* radicles.

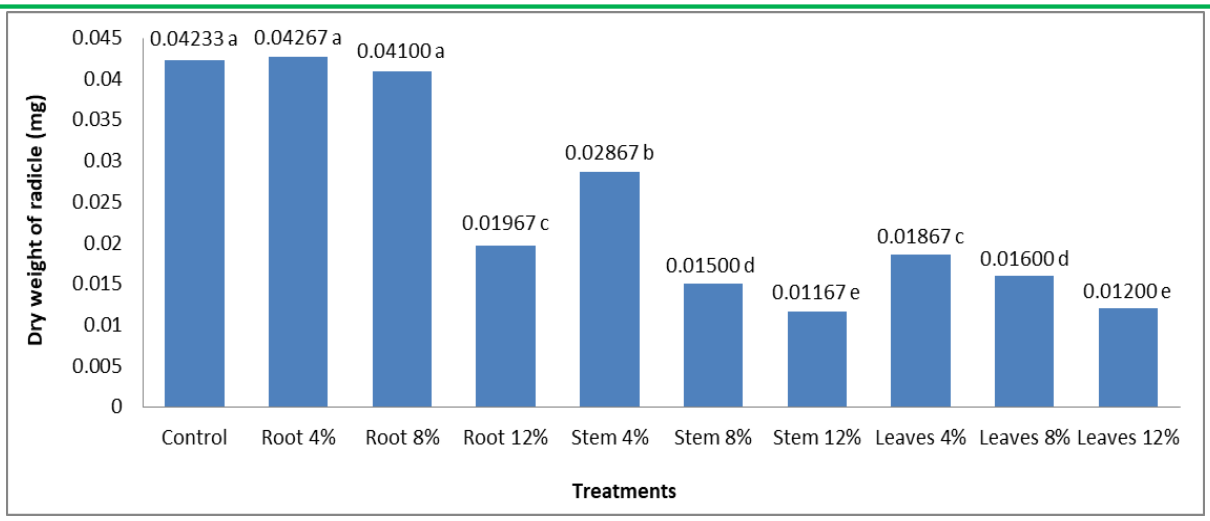

Fig. 7. Effect of *Vicia faba* extract on dry weight of radicle of *Lycopersicon esculentum* (mg).

(Different alphabets show significant difference using Duncan's Multiple Range test (P≤ 0.05))

These results confirmed the inhibitory and the positive effects of the allelochemicals present in the various *Vicia faba* extracts at different concentrations. This study highlighted the fact that the extracts of the *Vicia faba* plants were water-soluble and consisted of many stimulatory and inhibitory compounds.

3.7 Effect of extracts on vigour index:

Fig. 8 showed that the root and stem extracts (4%) along with the control treatment gave highest values which were 11.560, 11.025 and 13.056, respectively, the vigour index in comparison to the other treatments.

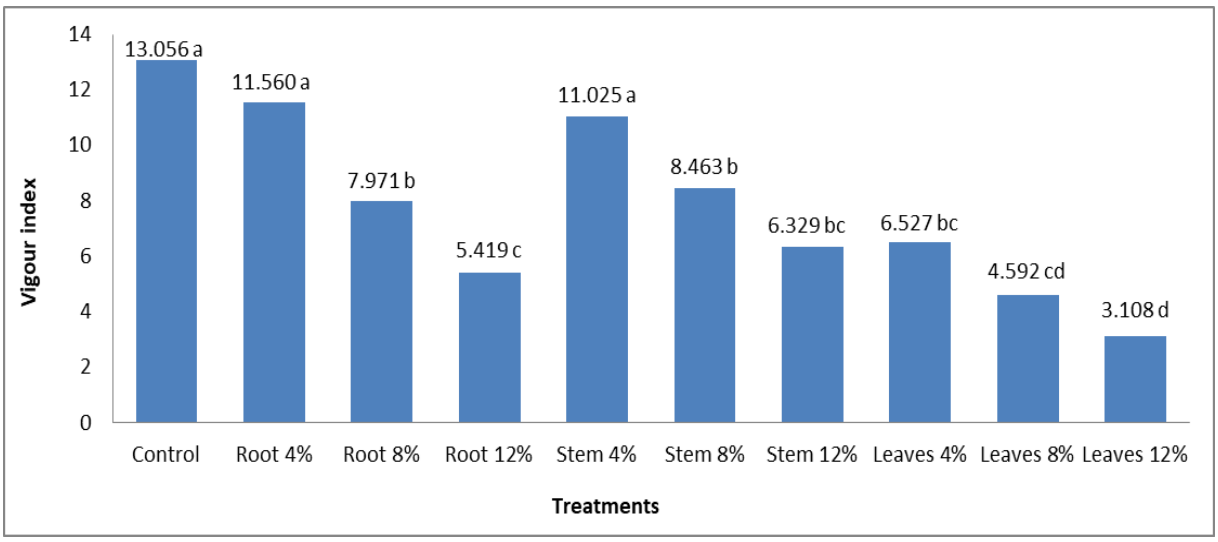

Fig. 8. Effect of *Vicia faba* extract on vigour index of *Lycopersicon esculentum*

(Different alphabets show significant difference using Duncan's Multiple Range test (P≤ 0.05))

The inhibitory effect of the plant was dependent on the concentration of the extracts. This inhibitory effect increased with an increase in the aqueous extract concentration. Similar reports were reported earlier (Chou and Yao, 1983; Kavitha et al., 2012), wherein the researchers noted the inhibitory effects of the aqueous leaf extracts of the *Vitex negundo* plant on the *Lactuca sativa*, *Brassica chinensis*, *Degitaria deacumbens* and *Mimosa pudica* crops. In this study, the researchers concluded that the phytotoxic effects are caused by many chemical compounds, which are

concentrated in the leaves. The various crops show a different reaction to these chemical compounds. The allelochemicals in the plants exhibit an inhibitory effect (Chaturvedi and Jha, 1992). In their study, Swaminathan et al. (1989) observed that the potential chemical compounds like phenolic acids could display an inhibitory effect on the test species.

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تأثير الأليلوباثي لمستخلص الباقلاء في تحفيز أو تثبيط أنبات البذور ونمو شتلات الطماطة

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[2] قسم الفحص وإصدار الشهادات للبذور في تكريت ، صلاح الدين ، العراق

المستخلص

أجريت التجربة في محطة الأبحاث ومختبر كلية الزراعة ، جامعة تكريت ، ودائرة فحص و تصديق البذور في تكريت، صلاح الدين، العراق، بهدف معرفة التأثيرات المثبطة والمحفزة للمستخلص المائي للباقلاء على إنبات البذور ونمو شتلات الطماطة. أستخدمت معاملات مختلفة من المستخلصات المائية للباقلاء وهي: مستخلص الجذور والساق والأوراق بنسب 4% و 8% و 12% لكل منها، بالإضافة الى الماء كمعاملة مقارنة. تم توزيع التجربة بشكل عشوائي باستخدام التصميم العشوائي الكامل (CRD) وبأربعة مكررات. أظهرت النتائج أن المستخلص المائي لجذر الباقلاء بتركيز 4٪ كان له تأثير معنوي أيجابي على بعض الصفات مثل طول الجذير و الوزن الجاف للجذير في الطماطة. أظهرت الدراسة أن المستخلصات المائية للباقلاء كانت لها تأثيرات مختلفة (مثبطة ومحفزة) على أنبات بذور ونمو شتلات الطماطة وفقا لجزء النباتي المستعمل. وكذلك تشير النتائج إلى أن المستخلص المائي للباقلاء للأجزاء النبات المختلفة والتراكيز المختلفة كان لها تأثير أليلوباثي.

الكلمات المفتاحية: أليلوباثي، المستخلصات المائية، قوة الأنبات، الباقلاء، الطماطة