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Effects of indole acetic acid (IAA) hormone and bitter apple extract on growth development and nutrient content of mung bean plant *In vitro*

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Abstract
Background and objectives: Mung bean is an important bean crop in Arab region and other Asian Countries. It is used as different kinds of food sources. The objective of the experiment was to investigate the effect of bitter apple (Handal) extract and plant hormone [indole-3 acetic acid (IAA)] on the growth, development and nutrient content of mung bean (*Vigna radiata)* plant *In vitro.*
Materials and Methods: Handal fruits were ground with motor and pestle by adding 30 ml distilled water. Then extract was filtered and kept it in the refrigerator. Four replicates were made from fruit sample. 50ppm Indole 3-Acitic Acid was chosen as auxin.
Results: Germination percentage was found higher in the IAA (50ppm) treated plant followed by water control, fruit extract and leaf extract. The lowest germination rate was found in the leaf extract treated plant. However, the shoot and leaf growth (length) were higher in the leaf extract treated plant than other like fruit extract, IAA 50ppm and water control treatments. Root length was higher in the IAA treated plant than that of other treatments. Moreover, nutrient content like K and NO3 was found higher in the leaf treated plant compared to other treatment followed by fruit extract, IAA 50ppm and water control treatment. =
Conclusion: The results conclude that IAA (auxin like hormone) was the best for germination and root growth and leaf and fruit extract were the best for shoot and leaf growth of mung bean plant.

Keywords: Bitter apple extract, IAA, root and shoot growth, mung bean

Introduction

Mung bean (*Vigna radiata*), alternatively known as the bean, green gram, lentil is a plant species in the legume family [1]. Native to the Indian subcontinent, the mung bean is mainly cultivated at present in India, China, Arab countries and Southeast Asia. It is also cultivated in hot, dry regions in Southern Europe and the Southern United States [2]. It is used as an ingredient in both savory and sweet dishes. Mung beans can also be used in a similar fashion as whole beans for the purpose of making sweet soups [3]. Bitter apple or wild gourd, is a desert vinyl plant native to the Mediterranean Basin and Asia, especially in Turkey [4]. It resembles a common watermelon vine, but bears small, hard fruits with a bitter pulp.

Clinical studies have shown medicinal benefits of bitter apple in patients with diabetes, diabetic neuropathy, and hyperlipidemia. In a randomized clinical trial (RCT), HbA1c and fasting blood glucose levels were decreased in patients using 300 mg of *C. colocynthis* dry fruit powder daily for 2 months [5]. In another trial, intake of 300 mg of powdered seed can lower the triglyceride and cholesterol concentration significantly in nondiabetic hyperlipidemic patients Topical *C. colocynthis* also showed significant efficacy in treatment of patients with painful diabetic neuropathy [5].

It was reported [6] that the higher concentration of KNO3 gave the maximum flowers number. Dry weight increased due to spray with the nutrient. All treatments significantly enhanced cucumber productivity especially at the higher concentration of KNO_3.

Nitrogen, phosphorous, potassium and calcium content increased as the concentration increased. Potassium nitrate at both concentrations was the best in keeping the Total Soluble Solid (TSS) at higher levels.

Hormone increases plant growth and fruit quality and develop the fruit properly. Gibberellic acid (GA3), IAA, IBA, BAP can be effective for plant and fruit growth and development [7, 8]. Indole-3-acetic acid (IAA) is the most common, naturally-occurring, plant hormone of the auxin class. It is the best known of the auxins, and has been the subject of extensive studies by plant physiologists [9].

Indole-3-acetic acid (IAA) is the most common, naturally-occurring, plant hormone of the auxin class. It IAA enters the cell nucleus and binds to a protein complex composed of a Ubiquitin-activating enzyme (E1), a Ubiquitin-conjugating enzyme (E2), and a Ubiquitin ligase (E3), resulting in Ubiquitination of Aux/IAA proteins with increased speed [10].

IAA has many different effects, as all auxins do, such as inducing cell elongation and cell division with all subsequent results for plant growth and development. On a larger scale, IAA serves as signaling molecule necessary for development of plant organs and coordination of growth. No literature found on the handal extract and few literatures are on IAA effects on mung bean. That is why this project was designed to get new information regarding this. The objectives of the research were undertaken

1. To investigate the effects of bitter apple extract and IAA on the growth of mung bean
2. To identify the k and nitrate content after affecting the plant

Materials and Methods

Plant material

Mung bean plant was used to observe the growth effect.

Treatment preparation

Handal plant and fruit were collected from the desert area in Hail. Leaves were collected from the plants. Leaves were ground with motor and pestle by adding 30 ml distilled water. Then extract was filtered and kept it in the refrigerator. Four replicates were done from leaf sample. Four fruit sample were chosen from the collected fruit. Fruits were ground with motor and pestle by adding 30 ml distilled water. Then extract was filtered and kept it in the refrigerator. Four replicates were made from fruit sample. 20% extraction was used in the case of both leaf and fruit of handal.

Plant Hormone concentration

Indole 3-Acitic Acid was chosen as auxin. 50ppm concentration was prepared by adding distilled water following the equation of 50ppm=50mg/1000ml (1ppm = 1mg/1000ml).

Germination and Treatment setting

Mung bean seeds were collected from the Farm of Farmer in Hail. 20 seeds were germinated with the cotton and water in one petri dish. For leaves extract four petri-dishes were set as four replicates. Four fruit extract also four pertidishes were set and for plant hormone (IAA) four petridishes were set. Water control was set in four petri dishes.

Data collection

Germination percentage was determined by calculating the germinated seed/total seed x 100.

Root length, shoot length and leaf number were measured.

Nutrient content determination

Mung bean plants were ground with motor and pestle using distilled water 15 ml and extract was collected. Nutrient content (NO3 and K) was determined by using Horiba NO3 and K meters (USA). Three drops of juice sample (from the plant extract) put on the disc sensor of the meter using small dropper and data were displayed and recorded.

Statistical analysis

Least Significant different test (LSDT) was used in this experiment.

Results and Discussion

It has been shown in Figure 2 that germination percentage was the highest in the plant hormone (IAA50ppm) treated seed. However, the lowest percentage was found in the 20% leaf extract. It might be due to the more effectiveness of the breaking of the dormancy by the auxin, IAA. It was reported [11] reported that to accelerate breaking of seed dormancy, hormones have been applied in several studies. Plant growth regulators such as GA, IAA, IBA and Kintein have been recommended to break dormancy and enhance germination.

From Figure 3, it has been shown that root growth represented by length was the highest in the plant hormone (IAA50ppm) treated plant. However, the lowest percentage was found in the 20% leaf extract. It was higher in the fruit extract than water and leaf extract. It might be due to the enhancement of the root growth by the auxin, IAA. It was reported [12] that auxin like IAA, IBA stimulates the root growh. It was found higher shoot length in the leaf extract than in fruit extract followed by IAA and water control (Fig. 4).

It might be due to the more nutrient content in the leaf and fruit extract. Nutrient content enhanced the growth of shoot and leaves. In figure 5, it has been seen that the leaf growth was higher in the leaf extract treated plant than in fruit extract followed by IAA and water control. It might be due to the more nutrient content in the leaf and fruit extract treated plants. Nutrient content enhanced the growth of leaves earlier in the leaf extract and water control than IAA and fruit extract. In Table 1, it has been found that K+ and nitrate (NO3) were higher in the leaf and fruit extract than in IAA and water control treated plants. That is why our results showed the higher shoot and leaf growth in the leaf and fruit extract of bitter apple. Fig. 5 shows the root growth at different treatments. Fig. 6 shows the procedure of the nutrient measurement.

Fig 1: Photograph shows the leaf and fruit grinding process

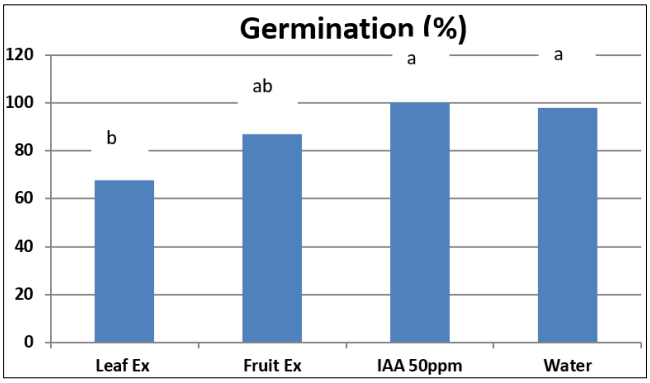

Fig 2: Effect of treatments on germination percent. Mean (n=5). Ex. Extract. 20% Leaf Ex. and 20% Fruit Ex used. IAA= Indole acetic acid. Same letters (b, b) showed no difference at 5% level of significant by Least significant difference (LSD) test.

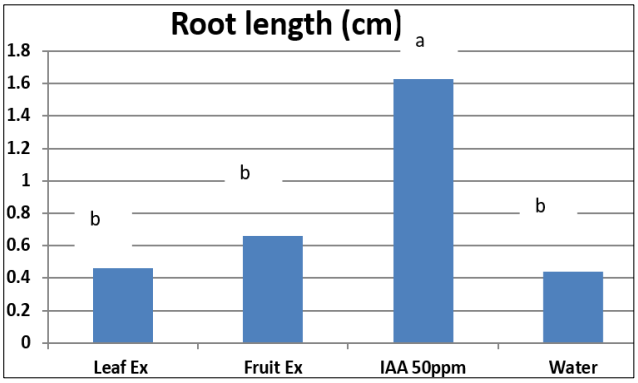

Fig 3: Effect of treatments on root growth (length). Mean (n=5). Ex. Extract. 20% Leaf Ex. and 20% Fruit Ex used. IAA= Indole acetic acid. Same letters (b, b) showed no difference at 5% level of significant by Least significant difference (LSD) test.

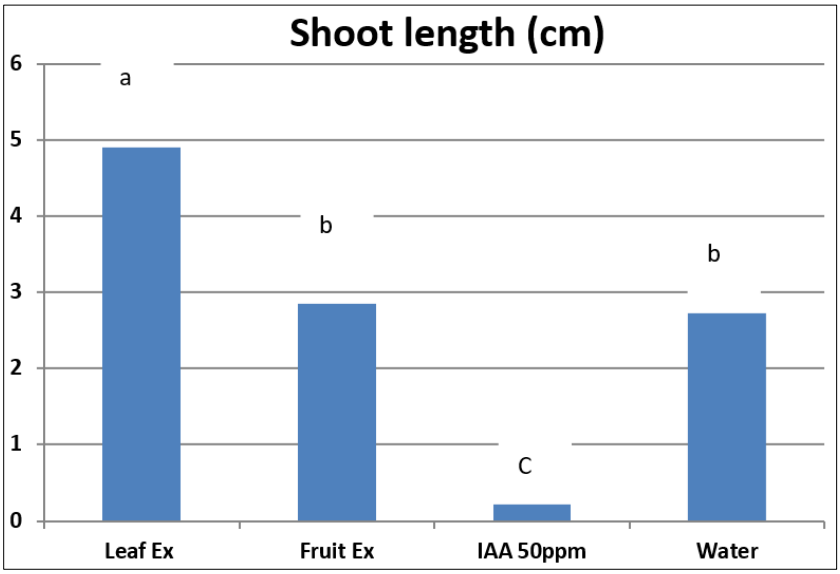

Fig 4: Effect of treatments on the shoot length. Mean (n=5). Ex. Extract. 20% Leaf Ex. and 20% Fruit Ex used. IAA= Indole acetic acid. Same letters (b, b) showed no difference at 5% level of significant by Least significant difference (LSD) test.

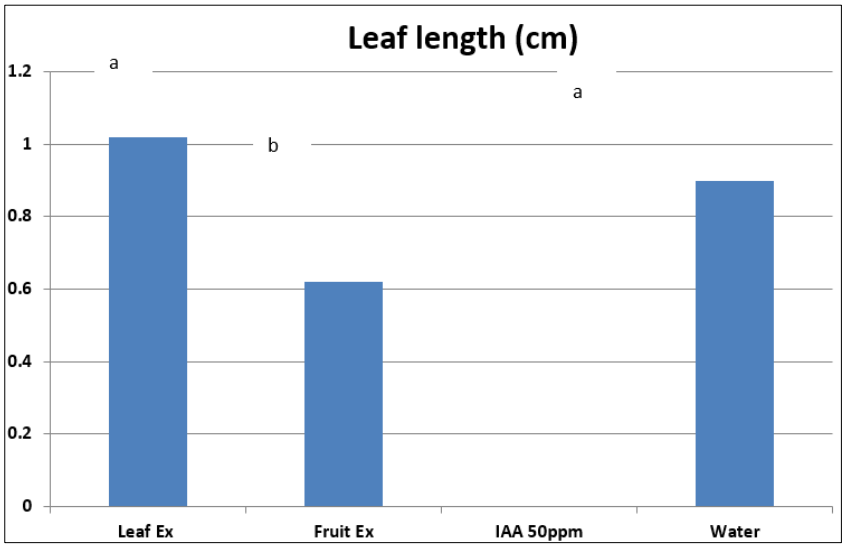

Fig 5: Effect of treatments on the leaf length. Mean (n=5). Ex. Extract. 20% Leaf Ex. and 20% Fruit Ex used. IAA= Indole acetic acid. Same letters (a, a) showed no difference at 5% level of significant by Least significant difference (LSD) test.

Fig 6: Effect of leaf, fruit extract and hormone on bean plant growth

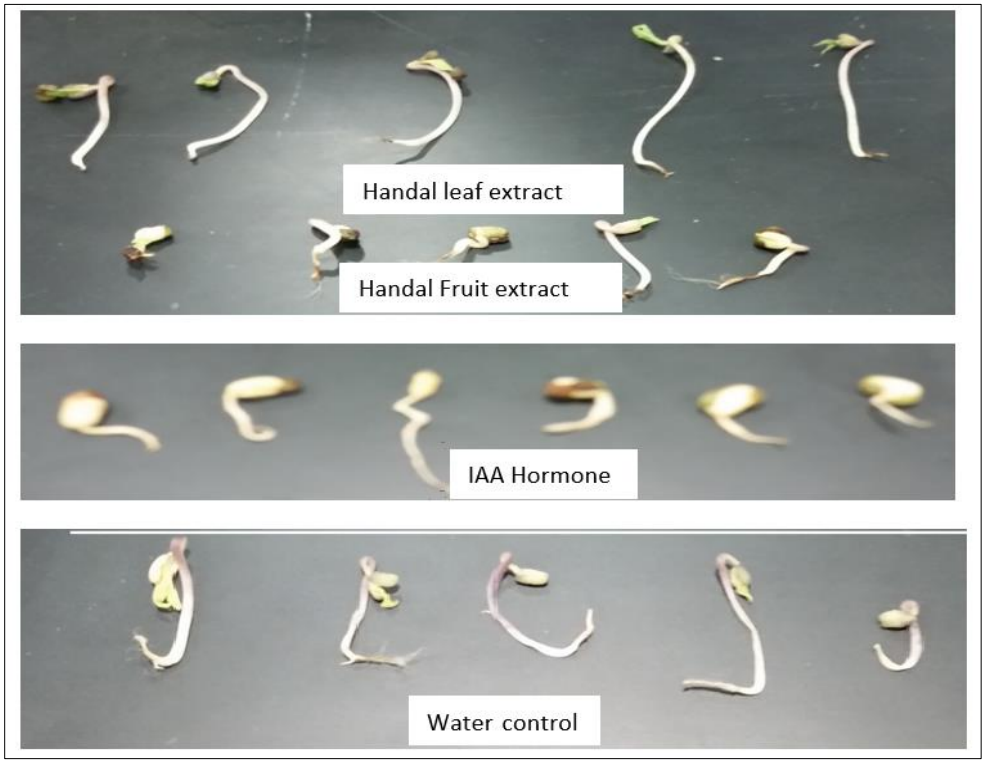

Fig 6a: Effect of leaf, fruit extract and hormone on bean plant root, shoot and leaf

Fig 6b: Potassium and Nitrate determination by HORIBA NO3 and K Meters

Table 1: Potassium and nitrate determination from mung bean plant at different treatments.

Treatment	Potassium (K+) [ppm]	Nitrate (NO3) [ppm]
Leaf Extract (20%)	300±0.5	410±0.5
Fruit Extract (20%)	250±0.3	350±0.6
IAA 50ppm	100±0.4	180±0.3
Water control	240±0.5	340±0.4

Conclusion

From our results it is concluded that bitter apple leaf and fruit extract exhibits better growth for shoot and leaf than IAA 100ppm. Moreover, IAA 100 ppm shows better effect for root growth and germination than bitter apple extract.

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