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EFFECT OF GIBBERELLIN AND SAPONINS EXTRACT OF *SANSCEVIERIA* LEAVES ON ANISE SEEDS GERMINATION

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Abstract

The present study was carried out to evaluate the response of the anise plant seeds to the plant growth regulator gibberellin (GA_3) and the effect of the saponins extract of *Sansevieria hyacinthoides* leaves on the seeds germination and seedling growth. Various plant extracts were used to improve crop yield and quality, being safe for the environment. Firstly, the toxic effect of the leaf extract was estimated using the phytotoxicity assay on radish seeds. This assay showed insignificant decrease in root length at 1000 µg/ml, whereas an increase in root growth was observed at concentrations 10 and 100 µg/ml. Similarly, stimulatory effect on the percentage of seeds germination was observed reached (100%) at 10µg/ml. Low concentrations (0.0, 1.25, 2.5, 5.0, 10.0 µg/ml) of saponin extract were applied to test the germination and growth of anise plant. Leaves extract increased the germination of anise seeds and enhanced the growth of root length significantly at 10µg/ml in comparison with control treatment (0µg/ml). The root lengths were 1.3 and 2.3cm after 4 and 7days respectively and the percent of germination reached 100% after only 7 days. The lower concentrations did not have any effect on seeds germination rate with little effect on root length. It is concluded that the saponins extracted from *Sansevieria* leaves are safe and has no toxic effect and possess clear activity similar to the effect of the plant growth regulator gibberellin on increasing seed germination rate and promoting seedling growth.

Key words : GA_3, saponins, seeds germination, seedling growth, anise.

Introduction

Anise (*Pimpinella anisum* L.) is an annual aromatic herb of the Apiaceae family, widely planted for fruit and essential oil. Mediterranean region consider the origin of this plant and it is implanted under field conditions in a vast number of countries, especially in Southern Europe and South East Asia, Turkish, Iran but also in the United States, China, India and Chile. Since anise favors warm climatic conditions throughout the growing season, it is particularly cultivated in subtropical regions. Anise is an important raw natural material mostly used in medicine, pharmaceutics, perfumery and cosmetic industries beside their general use as spice (Acimovic *et al.*, 2014). The fruit are extensively utilized to treat pains of stomach and relief of gastrointestinal spasms (antispasmodic), used as carminative and expectorant, tonic, antidepressant, antipyretic, antiseptic, antifungal, sedative, and the consumption of aniseed in lactating women increases milk (Kara, 2015; Sonmez, 2018).

Seeds are the main way for propagate of plants. Seeds germination and seedling growth are the precise stages in plant life cycle (Jamian *et al.*, 2014). Anise is generally grown by seeds. The time from sowing to seed ripening is 4-5 months. Germination normally takes 17-25 days. Initial growth is slow: 35-40 days from germination to stem emergence (Ullah, 2012).

The demands for increased yield and improved quality can be met through improvement of the crop growth environment through irrigation, fertilization, and the use of plant protection products. Chemical growth regulators are also one of the tools used to improve both crop growth and quality. Commercially registered growth regulators are all phytohormones, one of them are gibberellins that activated embryonic vegetative growth weakening the endosperm layer that involved. During the last few decades, natural products are also distributed for their growth-regulating effects due to their chemical properties,

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availability, and simple use without side effects, in contrast to the various chemicals that are employed to increase germination and growth of vegetables that may delay and reduce seedling emergence of many vegetable crops at high rate, be expensive along with hazardous toxic effects of these chemical compounds (Andresen and Cedergreen, 2010; Talukder *et al.,* 2015).

Saponins are one of the important interesting groups of plant secondary metabolites, which are connected to the plant's natural defense system. They have a high molecular weight and a high polarity. When dissolved in aqueous solution they form soap like foaming on shaking. They are capable to interact with cell membranes and are capable to lower the surface tension of an aqueous solution (Dewick, 2002), and these surface active properties distinguished them from other glycosides. They are commonly known by their sweeter to bitter taste, acid hydrolyzed and hemolytic properties due to saponins interaction with membrane sterols of the erythrocyte causing an increase in permeability, bursting erythrocyte membrane (Hostettmann and Marston, 2005). Saponins are classified into the steroidal tetracyclic type (C-27) and pentacyclic triterpenoid type (C-30). Soap-like behavior of saponins in aqueous solutions is attributed to their molecules containing a combination of glycon (polar) and non-polar aglycon portion including steroid or triterpene (Bernards *et al.,* 2011). The carbohydrate part of saponins consists of one or more sugar moieties linked to a hydrophobic aglycone (sapogenin) (Saxena *et al.,* 2013). The kind of the aglycone, number, nature, the sequence of sugar residues in a carbohydrate chain, and the position of sugar attachment to the aglycone can differ greatly resulting in a so varied group of compounds and it was improved the important of this vary on their activities such as cytotoxicity (Podolak *et al.,* 2010). It was well known that they show various biological activities such as antimicrobial, mollusicidal, insecticidal and anti-human immunodeficiency (Khan, 2009; Chaieb, 2010; Bhardwaj *et al.,* 2014; Dewatisari, 2015). In addition, it was reported the role of saponins as regulator of plant growth (Andresen and Cedergreen, 2010; Saha *et al.,* 2010).

Saponins are openly distributed in higher plants. They have been recorded in more than 100 families of plants, in each of wild and cultivated plants. Triterpenoid saponins occur mainly in dicotyledonous plants but also in some monocots, whereas steroid saponins are found mainly in monocots, such as the Agavaceae, Lilliaceae, Asparagaceae, Droscoraceae, Trilliaceae and Smilacaceae and in certain dicotyledons, such as Fabaceae and Solanaceae (Sparg *et al.,* 2004). Steroid saponins are present in tomato seed, yucca, *Sansevieria*, *Dracaena*, capsicum peppers, asparagus, aubergine, alliums, yam, fenugreek, oats and ginseng. Triterpenoid saponins are obtained from Quillaja saponaria, a native tree of the Andes region of South America (Yücekutlu and Bildac, 2008).

Sansevieria, a member of the Agavaceae (agave family), contains more than sixty species native to Africa, the islands near the African coast, Arabia, Ceylon, Burma and India. For a long time ago, it has been utilized by many cultures and transported to many parts of the world. It was highly valued by primitive people for its strong, soft fibers. *Sansevieria* is better known to modern cultures as an ornamental plant, grown outside in the tropics and semitropics, and in a variety of containers for interior use throughout the world. Some of the common names given to the ornamental types include snake plant and mother-in-law's tongue (Henley, 1982). The whole plant is traditionally used as a cardiotonic, purgative, expectorant, tonic and for rheumatism, quick relief of a common cough and cold and in ear pain, The roots are used as a febrifuge in snake bite and hemorrhoids (Ikewuchi *et al.,* 2010). Additionally, the plant showed various pharmacological activities such as antitumor, antimicrobial, antidiabetic, leaves exhibited anticancer and antioxidant property, the whole plant also possesses antioxidant, analgesic (Obydulla, 2016). Preliminary phytochemical screening of leaves extract showed the presence of many secondary metabolites such as alkaloids, flavonoids, saponins. Although several studies reported the effect of saponins as an inhibitor of seed germination or have allelopathic potential on crop species and weeds (Gorski *et al.,* 1991; Hoagland *et al.,* 1996), no preoiase research on the effect of steroidal saponns on seeds germination and seedling growth are documented. Thus, the objective of the present study was to evaluate the potential of saponins extracted from the leaves of *Sansevieria* to stimulate seeds germination of anise.

Material and Methods

Plant Material

Sansevieria hyacinthoides or Tongue plant was obtained from agricultural nurserly in Baghdad, was classified by Dr. Sukainah Abbas, department of Biology, University of Baghdad.

Preparation of Extract

The collected *Sansevieria hyacinthoides* leaves were washed with tap water dried in oven at 40^0C and later pulverized using blender into a fine texture. Steroidal saponins extraction was carried out by following the method of (Wagner and Bladt, 2009). The ground material (18 g) was mixed with (180 ml) (70%) ethanol, put in water bath for (8 hours) and then the plant material was filtered after cooling, the filtrate was then concentrated. n-butanol at 1:3 ratio (150 ml) was added to the ethanolic extract, and then the mixture put in separated funnel, n-butanol phase was separated into two phases: the lower phase was left to dry at room temperature in petri-dish. Stock solution was prepared (45mg/ml). A series of concentration (0, 10, 100 and 1000) µg/ml were set and used in the experiments of this study.

Phytotoxicity Radish Seeds Assay

The stock solution of extract was prepared as mentioned previously. Radish seeds were washed with tap water. Ten seeds were placed in each petri-dish and treated with (5ml) saponins extract at the concentrations (0, 10, 100 and 1000) µg/ml. All petri-dishes were kept at room temperature. The root length and % of seeds germination were noted. All treatments were performed in triplicate.

Germination Test and Measurement of Shoot and Root Growth

Seeds of *Pimpinella anisum* were obtained from local herbalist in Baghdad. The viability of the seeds was assayed using distal water at room temperature. Two experiments were used for investigation of seeds germination. 1.0 mg/l GA_3 and 2.0 mg/l GA_3 solutions in distilled water were prepared. Also, different concentrations (0, 1.25, 2.5, 5, 10) µg/ml of saponin extracts were examined for this purpose, three replicates of 10 seeds for each of the above treatments were placed in petri dishes with two layers of filter paper saturated with 5ml of each concentrations (at the concentration 0, distil water was added). The dishes were placed at room temperature. Seeds were considered germinated after emergence of root and germination count was recorded up to 4 days. % of seeds germination was counted using the following equation:

$$\text{Germination}(\%) = \frac{\text{Total number of seed germinated}}{\text{Total number of seed cultured}} \times 100$$

For shoot and root growth measurement, the root length of the seedlings were measured after 4 and 7 days and the shoot length were noted after 7 days.

Experimental Design Statistical Analysis

Effect of studied treatments on different characters was statistically analyzed. Low significant differences (LSD) analysis was carried out at 0.05 of probability using statistical analysis system (SAS., 2012).

Results and Discussion

Influence of Gibberellin on Percentage and Rate of Seed Germination

Results indicated the positive effect of gibberellin on seed germination. The percentage of germination results for the anise seeds treated with different concentrations of GA_3 and without GA_3 (control) are shown in Fig. 1. Results showed that the treated with 1.0 and 2.0 mg/l GA_3 have significantly improved percentage of germination and shortened time to start germination in comparison with control treatment. The final percentage of germination in gibberellic acid treatments reached % 100 and the final time of germination decreased markedly from 14 days in control to 8 days and 7 days in 1.0 and 2.0 mg/l GA_3 treated seeds respectively. This result may be attributed to the role of gibberellin on promoting germination and counteracting the inhibitory effects of abscisic acid (Vera *et al.*, 2010). The GA_3 increases cell size and cell growth facilitating water absorption, and encourages analysis of endospermic materials in the seed. GA_3 stimulates the production of hydrolytic enzymes by aleuronic cells such as α- and β-amylase that converts starch to glucose, which can be absorbed by the embryo (Al-Hawezy, 2013).

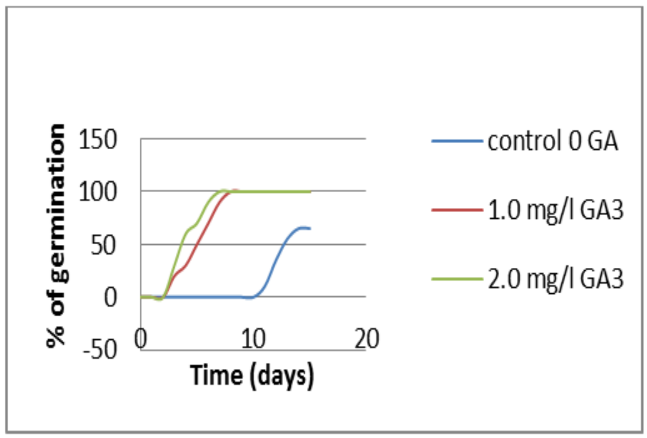

Fig. 1: Effect of GA_3 treatments on germination percentage and rate of anise seeds.

Phytotoxic Assay

For evaluation the phytotoxic activity of plant extract, assay of radish seed germination is very substantial. The activity involved the root length and the germination percentage measurements. The water was used as negative control. The phytotoxic potential of all tested samples of saponin extract are viewed in table 1 and Figure 2. Results revealed that germination (%) of radish seeds and root length decreased insignificantly at the higher concentration of 1000 µg/ml, The decrease was (86.6%, 1.603 cm) respectively in comparison with control treatment (83.3%, 4.6 cm) and increased significantly with decrease in saponins concentration that reached (100%, 2.325cm) at the lowest concentration (10µg/ml).

This assay for the cytotoxic activity are carried out because saponins are reported to have various toxic properties and was isolated as an allelochemicals which show either stimulatory or inhibitory activity for other plants species growth (Saha *et al.,* 2010).

Influence of Saponins on Seed Germination and Growth Properties

The present study assessed the effect of saponins extracted from *Sansevieria* leaves on *P. anisum* germination and seedlings growth that were investigated at different saponins concentration after 4 and 7 days of treatment. The germination of seeds under control condition was 16% and 60% after 4 and 7 days respectively. Gradual increase in germination was observed with increasing saponin concentration reached 100% after only 7 days at 10µg/ml. Similarly, treated with 10 µg/ml showed the highest seedlings growth rate and increased the shoot and root length to (2.190, 2.3 cm respectively) that significantly differ with control treatment. All results are shown in Table 2 and Figure 3. Stimulant effect of the low concentration of saponins may be attributed to their membrane tropic activity and their function as bio regulators of plant substance metabolism (Kemertelidze *et al.,* 2009). On the other hands, the magnitude of induction at all concentration was proportionate to the concentration. Previous study have shown that spraying plant tea seed powder, rich in triterpenoid saponins, have a stimulatory effect on plant growth and yield with regarding the applied dose. This effect has been attributed to the containment of these active compounds on substances with hormone-like properties or substances that interfere with hormones that can stimulate or affect the allocation of biomass in the plants (Andresen and Cedergreen, 2010). Thus for optimum germination and seedling growth, 10µg/ml of *Sansevieria* leaves saponins was ideal for treating a seed before germination.

In conclusions, the use of saponins extracted from *Sansevieria* leaves at low concentrations (ppm) is very safe and has no toxic effect on the germination and the growth rate. These active compounds possess clear activity similar to the effect of the plant growth regulator gibberellin on increasing seeds germination and promoting growth of seedling, could consider as a natural growth regulator and could be further exploited in agricultural applications in the future.

Table 1: Phytotoxic effect of saponins extract on seed germination (%) and root length (cm) after 3 days of culture.

Concentration (µg/ml)	Seed germination %	Root length (cm)
0	83.3	4.6 ab
10	100	10 a
100	80	5.67 a
1000	86.6	5.1 b
LSD ($p \leq 0.05$)		0.671

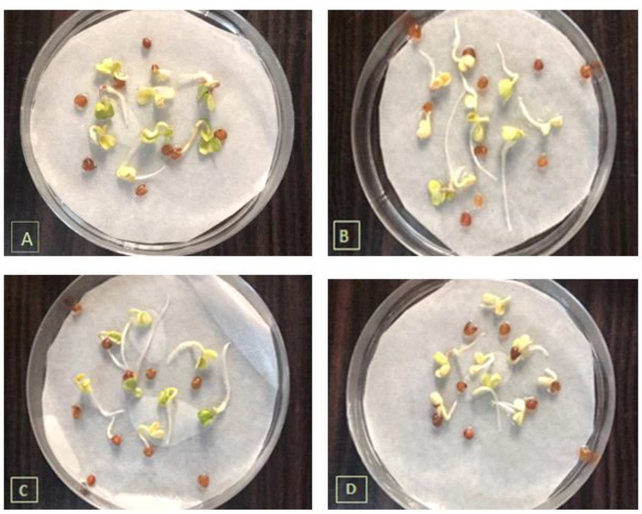

Fig. 2: Phytotoxic effect of saponins extracted from *Sansevieria* leaves on radish seed germination and root length at different concentration after 3 days: A control; B (10µg/ml); C (100 µg/ml); D (1000 µg/ml).

Table 2: Effect of saponins extracted from *Sansevieria hyacinthoides* leaves on percent germination of anise seeds, shoot and root length after 4 and 7 days.

Concentration (µg/ml)	Seed germination (%)		Root length (cm)		Shoot length (cm)
	4 days	7 days	4 days	7 days	7 days
0	16.6	60	0.400 d	0.482 d	0.428
1.25	23.3	56.6	0.617 cd	0.633 c	0.582
2.5	26.6	60	0.700 bc	0.783 c	0.647
5	29.3	60	0.900 b	1.00 b	1.108
10	33.3	100	1.300 a	2.3 a	2.190
LSD ($p \leq 0.05$)			0.274	0.302	

Fig. 3: Comparative germination images of anise seeds treated with different concentrations of saponins extract after 7 days of culture: (A) control; (B) 1.25µg/ml; (C) 2.5µg/ml; (D) 5µg/ml; (E) 10µg/ml.

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