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Biochemical Actions of Two Medicinal Plants (Spinach and Pomegranate) On Germination and Growth of Some Plant Species

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Abstract

This study was conducted to examine allelopathic actions of Spinach (*Spinacia oleracea*) and Pomegranate (*Punica granatum*) aqueous leaf extracts on germination of seeds and some growth parameters of wheat (*Triticum aestivum*) and rapeseed (*Brassica napus*). The experiment was performed in sterilized petri dishes for one week at 22 °C. The experiment was arranged for completely randomized design. The concentrations were chosen for this study as (0%, 3%, 6% and 9%). The results indicated that the higher concentrations (6% and 9%) of both extracts of Spinach (*Spinacia oleracea*) and Pomegranate (*Punica granatum*) caused significant reduction in seed germination whereas the lowest concentration caused the least negative impact on seed germination. Other growth parameters such as shoot and root length and shoot and root oven dry weight were significantly inhibited by the application of both extracts at concentrations (6% and 9%) in wheat *T. aestivum* and rapeseed *B. napus*. However, spinach leaf extracts at concentration (3%) caused more significant reduction of germination and seedling growth than pomegranate extracts. dicot *B. napus* turned out to be more effected than monocot *T. aestivum.* The findings of this study suggest that Spinach (*Spinacia oleracea*) and Pomegranate (*Punica granatum*) aqueous shoot extracts can be recommended to utilize as bio-herbicide to supress seed germination and growth of weeds.

1. Introduction and Literature Review

The definition of allelopathy is any direct or indirect suffer or advantageous effect of a plant or a the effect of a microorganism on other plants by releasing allelochemicals to the environment [1-3]. The phenomenon of plants which have influence on other nearby plants through delivering chemicals was originally addressed by Theophrastus in 370 BC [4]. Molisch [5] was the first who mentioned the term Allelopathy. According to the previous scientific research, there are significant numbers of secondary metabolite of plants that are considered to have allelopathic influences. For instance, some plant natural products, for example, phenolics and alkaloid compounds play crucial role in natural activities of plants for instance, seed germination development and plumule growth [2, 6-8].

Plants, which are able to have phytochemical activities, must have ability to produce bio-compounds called allelochemicals, that must be delivered into the neighbouring plants which must be reachable for the process of transportation in order to be taken by another plant [3]. Allelopathic compounds are delivered into environment through various of mechanisms as

they involve root exudation process, leaching, plant leaf volatile as well as decomposition of plants [3, 9].

Based on some recent scientific investigations, utilizing chemical herbicides causes many risks to environment which negatively affect human health and reduces quality of water, as well as it has the negative influence on soil microorganisms [10]. In addition, there are about 470 genotype weeds which application of synthetic herbicides to them are no longer effective. (Heap, 2017). Therefore , to escape from these expecting issues, plants which have capability of producing bio-active compounds might be useful to utilize them as bioherbicides for terminating unwanted plants, to such an extent that allelopathy may be considered to be a possible implement to reduce weed existence and increase crop production [11-14].

Shehata [15] examined the allelopathic activities of *P. oleracea* L. seed extracts on germination and growth of *Cichorium endivia* L., *Lactua sativa* L., *Echinochloa crus-galli* L., and *Brassica tournefortii.* The results illustrated that the extracts caused a significant reduction in germination and seedling growth with the increase of concentration. Another study by Rashidi, Reza Yousefi [16] indicated that seeds of *P. oleracea* L. has inhibitory allelopathic reduction in growth parameters of some crop species such as common bean onion, sugar beet, broad bean, and pea.

The germination tests of extracts of the fruit of pomegranate (*Punica granatum*), noni (*Morinda citrifolia*) and leaves of eucalyptus (Eucalyptus spp.), with commercial seeds of lettuce (*Lactuca sativa*), tomato (*Solanum lycopersicum*) and pepper (*Capsicum baccatum*) showed to have allelopathic inhibitory effects on germination and delayed its development [27]

Materials and Methods

2.1 Sample Collection

Spinach and Pomegranate plants were collected in Akre in Spring- 2020. The plants were separated and cut into 5 cm pieces. The chopped plants then left air-dried for 14 days. The air-dried samples were delivered to Agriculture Lab at Salahaddin University-Erbil, where they were ground into fine particles and were getting ready for the test.

2.2 Aqueous extract preparation

Leaf water extracts of dried plants were prepared via mixing 10 g of shoot with 100 ml deionised water separately and then put into a shaker then left overnight. The shoot and root

extracts were centrifuged (1000 rpm) for 10 min after filtration using filter paper. The supernatant was filtered through a micropore filter (0.45 μm). The resultant extracts were stored at 4°C until required for the germination tests.

2.3 Experiment of seed germination

For this experiment, seeds of wheat (*Triticum aestivum*) and rapeseed (*Brassica napus*) were utilized. The seeds were obtained from College of Agricultural and Engineering Sciences, Salahaddin University-Erbil. To avoid contamination, sodium hypochlorite (10%) was used for seed sterilization and washed for 15 min and then rinsed 3 times in deionized water. This process was performed to prevent contamination with pathogens during their germination.

2.4 Bioassay

Ten seeds of wheat and rapeseed were separately placed in 9 cm diameter petri dishes lined with filter paper. Five ml of three different Spinach and Pomegranate shoot extracts were applied to the petri dishes of both seed samples as these were the treatment petri dishes. The non- treatment petri dishes (control) received 5 ml distilled water only. There were 3 replications of each seed species (wheat and rapeseed) including test and control treatments. Petri dishes were incubated in a growth chamber at 22°C. After one week, seed germination percentage, shoot length, root length, shoot dry weight and root dry weight of germinated seedlings were recorded.

2.5 Statistical Analysis

Results of experiments were analyzed using ANOVA general linear model (Minitab software, version 17) for a completely randomized design (CRD) with three replications. Tukey's test ($P \leq 0.05$) was used to calculate significant differences between means.

2. Results

3.1 Effect of Spinach and Pomegranate on seed germination

Figure 1 shows the effects of various concentrations of Spinach and Pomegranate aqueous extracts on germination of wheat (*Triticum aestivum*) and rapeseed (*Brassica napus*). Results indicate that shoot aqueous extracts of both plants significantly inhibited seed germination of

both *T. aestivum* and *B. napus* (Figure 6 and 7). The highest reduction turned out to be at concentrations (6% and 9%).

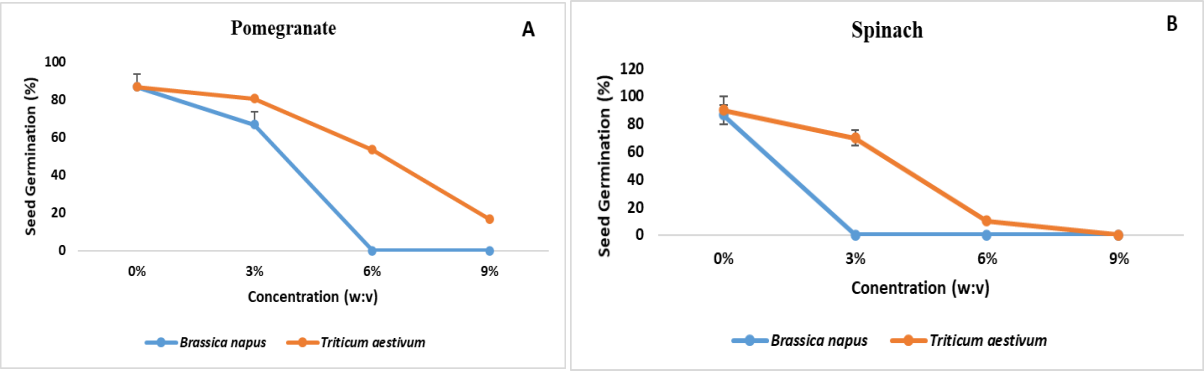

Figure 1 Effect of Spinach and Pomegranate aqueous extract on seed germination of *Brassica napus* and *Triticum aestivum*. The results are means of three replications. Error bars stand for standard errors. Tukey's test ($P \leq 0.05$) was used to calculate significant differences between means.

3.2 Effect of Spinach and Pomegranate on shoot length

The results from Figure 2 shows that Spinach and Pomegranate ground leaf aqueous extract at all concentrations (3%, 6% and 9%) significantly reduced shoot length of dicot *B. napus* and monocot *T. aestivum* except Pomegranate extracts did not significantly reduce shoot length of wheat.

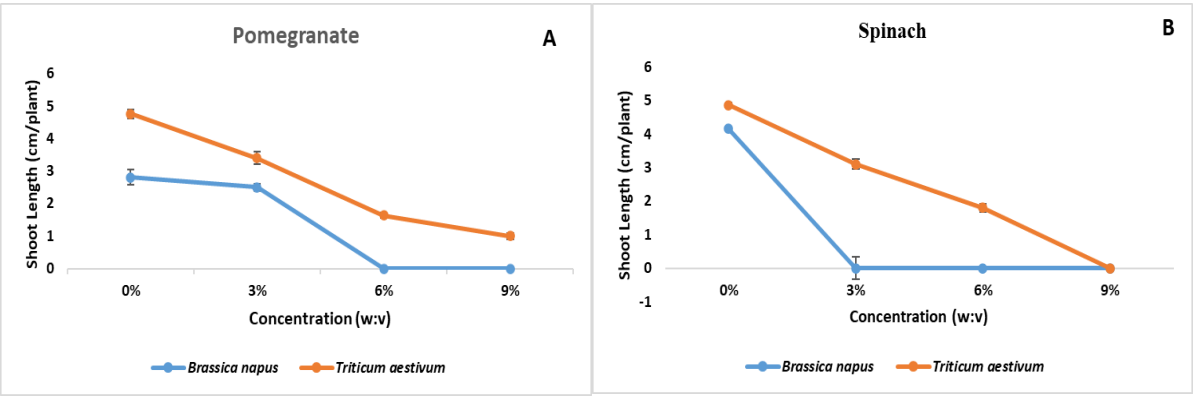

Figure 2 Effect of Spinach and Pomegranate aqueous extract on shoot lenght of *Brassica napus* and *Triticum aestivum*. The results are means of three replications. Error bars stand for standard errors. Tukey's test ($P \leq 0.05$) was used to calculate significant differences between means.

3.3 Effect of Spinach and Pomegranate on root length

Root length of *B. napus* and *T. aestivum* were significantly reduced by the application of Spinach and Pomegranate shoot aqueous extracts (Figure 3) at all concentrations (3%, 6%, 9%). In addition, shoot length of *B. napus* was more significantly reduced compared with *T. aestivum*.

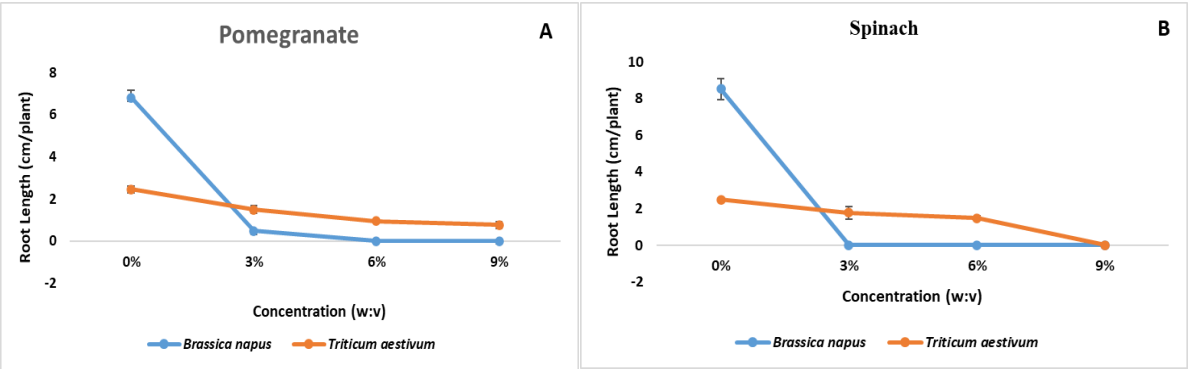

Figure 3 Effect of Spinach and Pomegranate aqueous extract on root length of *Brassica napus* and *Triticum aestivum*. The results are means of three replications. Error bars stand for standard errors. Tukey's test ($P \leq 0.05$) was used to calculate significant differences between means.

3.4 Effect of Spinach and Pomegranate on shoot dry weight

Figure 4 shows the effects of different concentrations of both Spinach and Pomegranate aqueous extracts on shoot dry weight of *B. napus* and *T. aestivum*. *B. napus* at concentrations (3%, 6% and 9%) completely affected by the application of aqueous shoot extracts ($P < 0.001$) in comparison with *T. aestivum* which had less significant reduction.

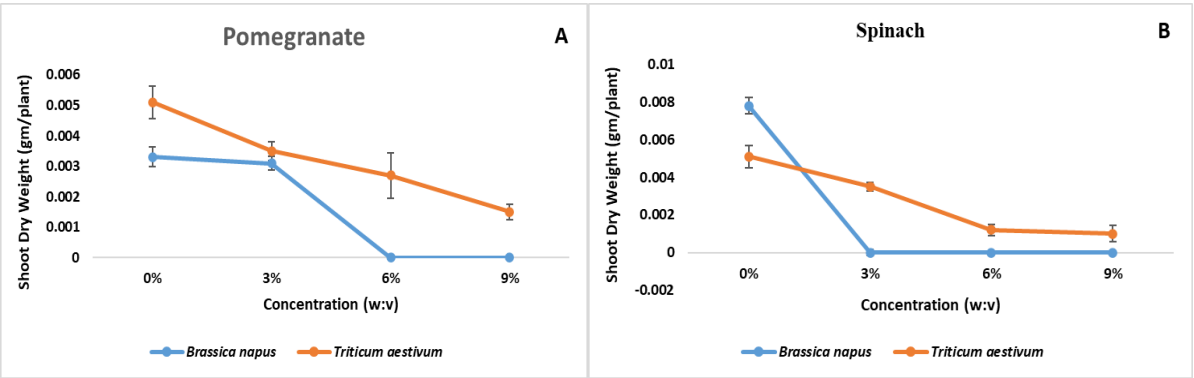

Figure 4 Effect of Spinach and Pomegranate aqueous extract on shoot dry weight of *Brassica napus* and *Triticum aestivum*. The results are means of three replications. Error bars stand for standard errors. Tukey's test ($P \leq 0.05$) was used to calculate significant differences between means.

3.5 Effect of Spinach and Pomegranate on root dry weight

Root dry weights of *B. napus* and *T. aestivum* were significantly ($P < 0.001$) inhibited by 6% and 9% concentrations of aqueous leaf extracts of Spinach and Pomegranate. Also, the results indicate that root growth of *B. napus* dicot turned out to have more growth reduction than *T. aestivum* monocot plant (Figure 5).

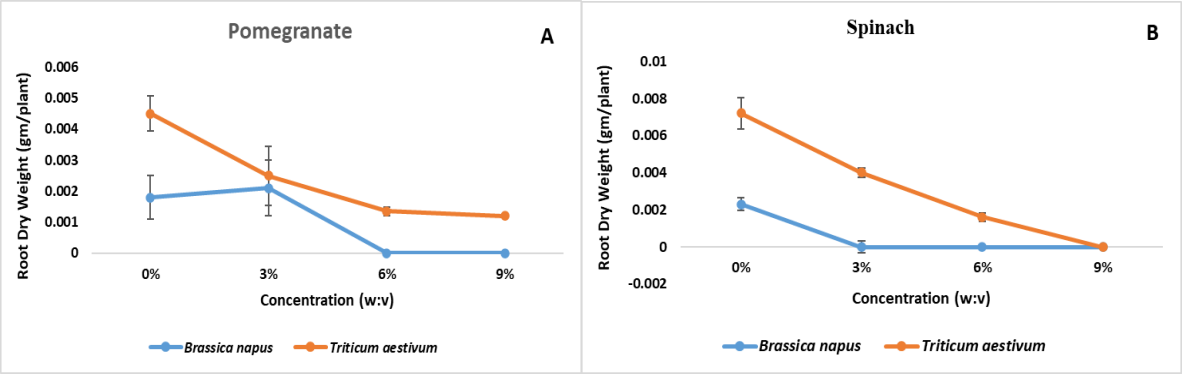

Figure 5 Effect of Spinach and Pomegranate aqueous extract on root dry weight of *Brassica napus* and *Triticum aestivum*. The results are means of three replications. Error bars stand for standard errors. Tukey's test ($P \leq 0.05$) was used to calculate significant differences between means.

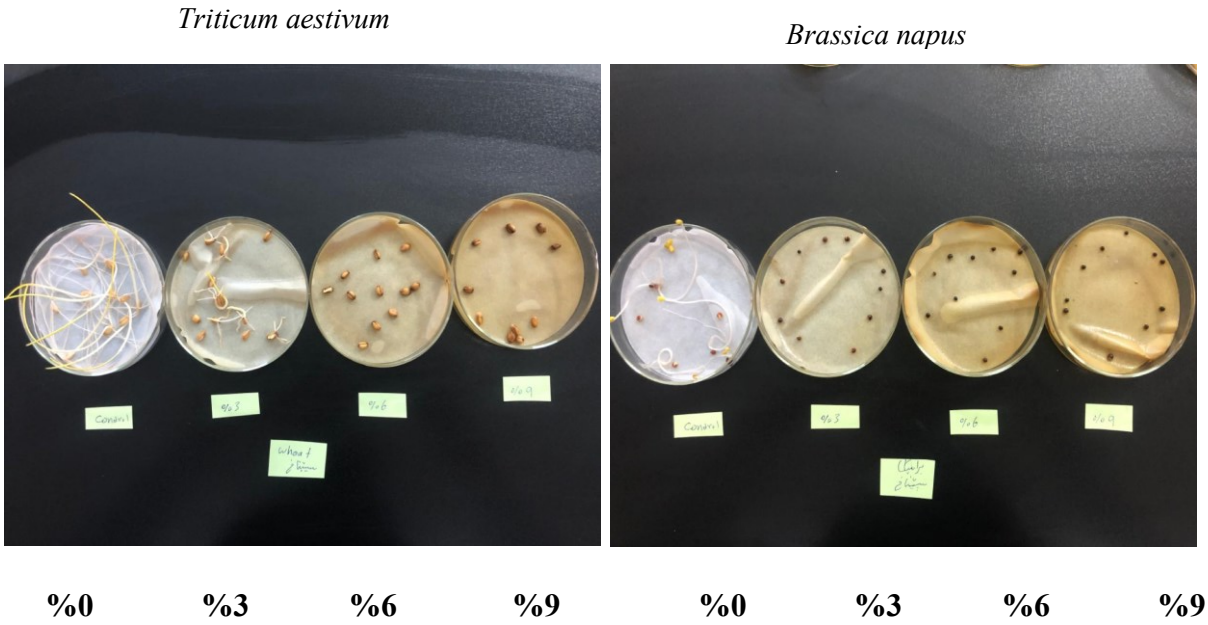

Figure 6 Effect of Spinach aqueous extract on seed germination and growth of *Brassica napus* and *Triticum aestivum*.

Triticum aestivum *Brassica napus*

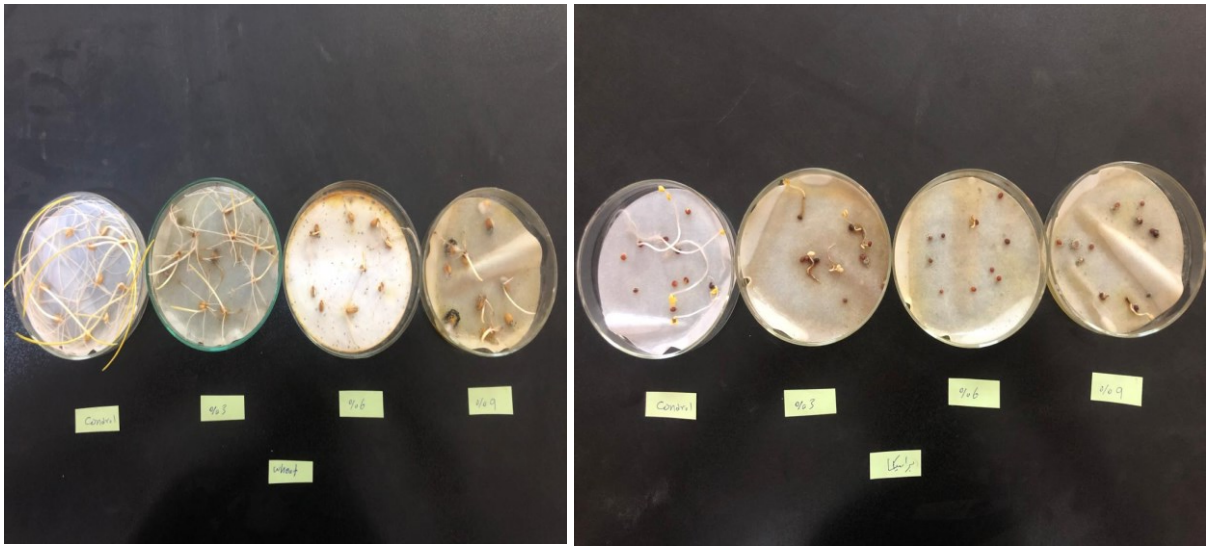

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Figure 7 Effect of Pomegranate aqueous extract (3%, 6%, 9%) on seed germination and early growth of *B. napus* and *T. aestivum*.

3. Discussion

The allelopathic actions of Spinach and Pomegranate leaf extracts at different concentrations (0%, 3%, 6% and 9%) on germination and early growth of *B. napus* and *T. aestivum* were examined. The reduction of seed germination and growth, shoot and root length, and shoot and root oven dry weight of the examined plant species may be due to the effect of biochemicals present in Spinach and Pomegranate extracts [18]. The response of germinating seeds and early growth to phenolic compounds have been reported in previous studies which illustrates that phenolic compounds contribute with the plant growth system and could affect seedling growth of plants through affecting plant hormones[19]. The results are in agreement with Çam et al [18] who isolated some phenolic compounds from pomegranate extracts which they play a crucial role in allelopathy [20, 21].

The findings about the allelopathic activities of different concentrations (3%, 6% and 9%) of Spinach and Pomegranate extracts on seed germination and early growth of *B. napus* and *T. aestivum* showed that the negative impact increased with increasing concentration of the extract. 6% and 9% aqueous shoot and root extracts recorded the most allelopathic influence on seed germination and early growth of the plants. These results are similar to the

findings obtained by Sharma and Satsangi [22], who indicated that higher concentrations (50-100%) of sunflower aqueous shoot extracts had more negative impacts than extracts with low concentrations on *Amaranthus viridis* and *Parthenium hysterophorus*.

The reduction of seed germination and growth parameters of *B. napus* and *T. aestivum* may be because of allelochemicals present in Spinach and Pomegranate extracts which could have negative impacts on cell division and physiological activities. Additionally, during the seed germination process, biochemicals may be the reason for changing in cell membrane permeability of the examined plant and weed species [23]. They may change respiration and reduce the concentration of RNA and ATP or disturb the secondary messengers functions that are required for germination of seeds and growth development [24].

The findings of this study has indicated that Spinach and Pomegranate extracts have more allelopathic influences on germination, shoot and root length and shoot and root oven dry weight than aqueous root extract [25, 26]. Moreover, our findings are similar with Turk and Tawaha [27], who showed that for black mustard the greater allelopathic effects can be released through leaf extracts. This might be because water soluble allelochemicals have greater inhibitory effect from shoot extracts than root extracts. It is concluded that both Spinach and Pomegranate leaf aqueous extracts have inhibitory allelopathic effects on seed germination and growth of both monocot and dicot studied species which could lead to be alternative to using of herbicides and it can be a production of natural herbicides in the future.

4. Acknowledgment

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