A comparative study of the allelopathic effects of *Euphorbia helioscopia* on growth and germination of *Brassica campestris* and *Triticum aestivum*

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Abstract

The allelopathic potential of *Euphorbia helioscopia* L. was investigated against *Brassica campestris* L. and *Triticum aestivum* L. in the laboratory. Fresh and dry leaves of *Euphorbia helioscopia* of 5g, 10g and 15g weight were soaked in 100ml of distilled water. These materials were filtered after 24 and 48 hours. The extract of fresh and dry leaves of *Euphorbia helioscopia* was applied in 5g, 10g and 15g concentration on *Brassica campestris* and *Triticum aestivum* in order to investigate the allelopathic impact on radicle and plumule length and germination percentage after 72hrs of incubation period at 26°C. The results showed that the dry and fresh leaves extract of *Euphorbia helioscopia* significantly reduced plumule and radicle length of both test species while stimulated the germination percentage in *Brassica campestris* and *Triticum aestivum* as compared to the control. The inhibitory effect of dried leaves on the test plant was more conspicuous than the fresh leaves. The plumule length was inhibited more in *Brassica campestris* as compared to *Triticum aestivum* by dry leaves and was recorded 95.49% and 61.57% in 24 hours soaking extract at 10g concentration and 94.83% and 54.43% in 48 hours soaking extract at 15g concentration. The radicle length *B. campestris* as compared to *Triticum aestivum* was effected more by 24 hours soaking extract and was recorded 93.57% in 10g fresh leaves and 15g dry leaves extract. The germination percentage of *Triticum aestivum* was stimulated by 24- and 48-hours soaking extract at all concentrations of fresh and dry leaves extract. As compared to *Triticum aestivum*, *Brassica campestris* was more effected by all treatments. From the result, it was concluded that the dry and fresh extract of *Euphorbia helioscopia* negatively affected the growth of *Brassica campestris* and *Triticum aestivum* and stimulated the germination of *Triticum aestivum* which suggests that some allelochemicals might be present in *Euphorbia helioscopia*.

Keywords: Allelopathy; *Brassica campestris*; *Euphorbia helioscopia*; Germination percentage; Inhibitory effect; Plumule length; Radicle length; *Triticum aestivum*
Introduction

*Euphorbia helioscopia* also known as Lun spurge belongs to family Euphorbiaceae which consist of 300 genera and 5000 species. It is cosmopolitan having strong representation in humid tropics and subtropics of both hemispheres. It is smooth annual weed mainly grow with wheat and mustard. It is small plant having erect stem ranging from 8-12 inches in height. The branches as well as main stem end in a special type of inflorescence known as cyathium. The leaves are oblong shape scattered all over the stem. Its latex possesses inflammatory, antiarthritic, antiamoebic, hepatoprotective, antitumor, vermifugal and febrifugal activities. It is also used for the treatment of bronchitis, rheumatism, cancer, tumor and warts [1]. *Brassica campestris* commonly known as mustard belongs to family Brassicaceae is cultivated in winter and harvested in March in Pakistan. It is cosmopolitan and commonly found in Central Asia, Europe and Mediterranean region. It is annual herb having 18-48 inches height. *B. campestris* consist of tap root and stem is herbaceous, erect, branched and consist of nodes and internodes. Leaves are palmate compound and flowers are small, yellow, pedicellate, ebracteate, actinomorphic, hypogynous, cruciform and tetramerous. The fruit of *B. campestris* is glabrous known as siliquas and seeds are small, dark brown and smooth. It is common crop of Pakistan and cultivated for production of oil producing seeds [2]. *Triticum aestivum* is a cereal belongs to family poaceae grown all over the world. It is an annual grass, is small, erect, hollow, glabrous, up to 1.2 cm tall; leaves are flat and narrow, about 1.3 cm broad; spikes are long, slender, dorsally compressed. Wheat is the mostly produced crop around the world after maize and rice. It is the most important crop cultivated for it grains which are the source of bread, alcoholic beverages, beer etc. [3]. Allelopathy is derived from the Greek word derived from two words allel on ‘to suffer’ and pathos ‘of each other’ coined by Hans Molisch in 1937 for the first time in the book ‘Der Einfluss einer Pflanze auf die André’ – Allelopathy (The Effect of Plants on Each Other). “Any direct or indirect, harmful or beneficial effect of one plant as a donor plant on another as a recipient plant through the production of chemical compounds that escape into the environment is known as allelopathy”. Putnam described allelopathy as a Positive plant response mediated through chemicals produced by another plant”, instead of only negative or inhibitory effect, he also considered that release of chemicals not only from living, but even from dead plant parts can affect nearby surroundings. So, allelopathy is both beneficial and detrimental effects of chemicals derived from living or dead plant parts over another in present surrounding [4]. Secondary metabolites and non-nutritional primary metabolites which are released from plants as a byproduct during different physiological process is known as allelochemicals. Allelochemicals can stimulate or inhibit plant germination and growth [5]. Allelochemicals are classified into 14 chemicals groups on the basis of chemical similarity including terpenes, coumarins, flavonoids, terpenoids, steroids, phenolic acids, tannins, lignin, fatty acids, strigolactones, benzoquinones, benzoic acid, triketones, purines, and non-protein amino acids [6]. Tanveer [7] studied the allelopathic effect of *Euphorbia helioscopia* water extract of root, stem, leaves and soil infested with *Euphorbia helioscopia* on germination and seedling growth of chickpea, lentil and wheat. From the results it was concluded that seedling emergence, seedling vigor index and total dry weight of these plants were significantly decreased. He also oobserved that in shoot length of lentil and root length of lentil and wheat are significantly decreased by *Euphorbia helioscopia* infested
soil. Anwer [8] studied allopathic effect of *Euphorbia helioscopia* on *Triticum aestivum*, *Rumex dentatus*, *Helianthus annuus*, *Zea mays* and *Avena fatua*. The concluded that *Euphorbia helioscopia* has the potential to be used as a source of weed management. Tongma [9] studied the allelopathic effect of *T. diversifolia* and from the results it was concluded that *T. diversifolia* decrease shoot and root growth of test plant species. Walia [10] studied the allelopathic effect of neem leaf leachate and extract in different concentrations on germination and early growth of Rabi crops namely Wheat, Barley and reported that germination and early growth of seedling of both the test crops were reduced significantly. It was also found that the inhibitory effect of leachate and extract were more on Barley in comparison to Wheat.

**Materials and methods**

**Collection, Drying, Crushing and Storage of plant material**

Fully grown mature leaves of *Euphorbia helioscopia* were collected. From the collected leaves half were dried in shade while half were directly crushed and soaked in distil water for extract.

**Relative toxicity of plant part**

Aqueous extracts were prepared by soaking 5g, 10g and 15g of fresh and dry leaves separately in 100ml of distilled water for 24 and 48 hours at room temperature. The extract was filtered and were stored in a refrigerator for future use. Distilled water was used as a control treatment. The apparatus used in the research was Beaker, Filter paper, Petri dishes, Titration flasks, Incubator, Refrigerator, Digital balance, Funnels, stirrer, Iron stand and Mortar and pestle. Petri dishes were lined with doubly folded Whatman No.1 filter paper. The viability of mustard and wheat seeds were checked. Each Petri dish was provided with 5 healthy seeds of wheat and mustard separately and were placed at equal distance on the filter paper. The seed bed was moistened with 4-5 drops of plant extract and in case of control plant, was moistened with distilled water. These petri dishes were placed in incubator at 26°C for 72 hours. Each treatment and control had 3 replicates. After 72 hours of incubation plumule length, radicle length and germination percentage were recorded.

**Statistical analysis**

The experiment was performed using three replicates per treatment. The data was statistically analyzed by using one-way ANOVA followed by LSD test at P=0.05.

**Results**

**Effect of 24 hours soaking duration extract**

In (Fig. 1) showed effect of 24 hours soaking duration extract of *Euphorbia helioscopia* on plumule length of *Brassica campestris* and *Triticum aestivum*. It was observed from the figure that plumule length was inhibited in all concentrations of fresh and dry leaves extract as compared to control. As compared to control maximum inhibition in plumule length of *Brassica campestris* was observed at 10g dry leaves extract which showed 95.49% inhibition whereas minimum inhibition 61.03% was observed in fresh leaves extract. In *Triticum aestivum* maximum inhibition in plumule length 61.57% was observed in 10g dry leaves extract while minimum inhibition was observed 14.41% fresh leaves extract as compared to control.

**Effect of 48 hours soaking duration extract**

The seeds of *Brassica campestris* and *Triticum aestivum* were applied with *Euphorbia helioscopia* fresh and dry leaves 48 hours soaking extract and its effect was studied on plumule length. From the results it was observed that the plumule length was reduced in all concentrations of fresh and dry leaves extract. As compared to control maximum reduction in plumule length of *Brassica campestris* and *Triticum aestivum* was observed 94.83% and 54.43% in 15g dry
leaves extract and minimum reduction in plumule length of *Brassica campestris* and *Triticum aestivum* was observed 61.24% and 8.01% in 5g fresh leaves extract (Fig. 2).

**Effect of 24 hours soaking duration extract**

Fig. 3 showed that *Euphorbia helioscopia* fresh and dry leaves extract decrease the radicle length of *Brassica campestris* and *Triticum aestivum* in all concentrations after 24 hours soaking duration as compared to control. Maximum decrease in *Brassica campestris* radicle length was observed 93.57% in 10g fresh leaves and 15g dry leaves extract while maximum decrease in radicle length of *Triticum aestivum* was observed 96.38% in 15g dry leaves extract. As compared to control minimum decrease in radicle length of *Euphorbia helioscopia* and *Triticum aestivum* was observed in 15g fresh leaves extract 81.78% and 15.88%.

**Effect of 48 hours soaking duration extract**

*Euphorbia helioscopia* fresh and dry leaves extract decrease the radicle length of *Brassica campestris* and *Triticum aestivum* in all concentrations after 48 hours soaking duration as compared to control. As compared to control maximum decrease in radicle length of *Brassica campestris* and *Triticum aestivum* was observed 91.87% and 96.44% at 15g dry leaves extract whereas minimum decrease was observed 42.75% in *Brassica campestris* at 15g fresh leaves extract and 17.43% *Triticum aestivum* at 10g fresh leaves extract (Fig. 4).

![Graph showing effect of 24 hours soaking duration extract](image)

**Figure 1.** Effect of 24 hours soaking duration extract of *Euphorbia helioscopia* fresh and dry leaves at 5g, 10g and 15g fresh on Plumule length of *Brassica campestris* and *Triticum aestivum*. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars with different letters represents significance difference at P=0.05
Figure 2. Effect of 48 hours soaking duration extract of *Euphorbia helioscopia* fresh and dry leaves at 5g, 10g and 15g fresh on Plumule length of *Brassica campestris* and *Triticum aestivum*. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars with different letters represents significance difference at P=0.05.

Figure 3. Effect of 24 hours soaking duration extract of *Euphorbia helioscopia* fresh and dry leaves at 5g, 10g and 15g fresh on Radicle length of *Brassica campestris* and *Triticum aestivum*. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars with different letters represents significance difference at P=0.05.
Figure 4. Effect of 48 hours soaking duration extract of *Euphorbia helioscopia* fresh and dry leaves at 5g, 10g and 15g fresh on Radicle length of *Brassica campestris* and *Triticum aestivum*. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars with different letters represents significance difference at P=0.05

Effect of 24 hours soaking duration extract
Fresh and dry leaves extract of *Euphorbia helioscopia* stimulated the germination in all concentrations except 15g fresh leaves extract in *Brassica campestris* in which the germination percentage was decreased 11.16% as compared to control after 24 hours of soaking. Maximum increase in germination percentage of *Brassica campestris* was observed 22.16% in 10g dry leaves extract whereas maximum increase in germination percentage of *Triticum aestivum* was observed 15.47% in 5g fresh leaves extract as compared to control (Fig. 5).

Effect of 48 hours soaking duration extract
Germination percentage was stimulated by *Euphorbia helioscopia* extract in all concentrations except in *Brassica campestris* at 5g fresh leaves extract and 10g dry leaves extract in which the germination percentage was reduced to 11.16% as compared to control. Maximum increased in germination percentage of *Brassica campestris* was recorded 33.33% in 15g fresh leaves extract and maximum increase in germination percentage of *Triticum aestivum* was recorded 99.65% as compared to control (Fig. 6).
**Figure 5.** Effect of 24 hours soaking duration extract of *Euphorbia helioscopia* fresh and dry leaves at 5g, 10g and 15g fresh on Germination percentage of *Brassica campestris* and *Triticum aestivum*. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars with different letters represents significance difference at P=0.05

**Figure 6.** Effect of 48 hours soaking duration extract of *Euphorbia helioscopia* fresh and dry leaves at 5g, 10g and 15g fresh on Germination percentage of *Brassica campestris* and *Triticum aestivum*. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars with different letters represents significance difference at P=0.05

**Discussion**

*Euphorbia helioscopia* L. significantly reduced plumule length, radicle length and germination percentage in *Brassica campestris* L. and *Triticum aestivum* L. the results indicate that *Euphorbia helioscopia*
The aqueous extract contain leached phytotoxins which effects the growth of test species as compared to control [11]. The current work was carried out to look for any potential allelopathic impact of this plant on *Brassica campestris* and *Triticum aestivum* germination and growth. The overall results are surprising, as almost the treatments, that were aqueous extracts, were having a potential to reduce the germination percentage and growth of the *Brassica campestris* and *Triticum aestivum*. These parameters were inhibited by extracts of almost all soaking durations, which clearly indicated that the chemicals are quickly released into the environment, irrespective of the duration of soaking [12]. The radicle length was inhibited with increasing concentration and soaking time. The dry leaves extract showed more inhibition in radicle length as compared to fresh leaves extract. The increased inhibition in radicle length was recorded in 10g and 15g dry leaves extract. It was also reported that fresh leaves were having less potential to reduce germination percentage, as compared to dry leaves, that may be due to the possible maturity of allelochemicals that are processed once the leaves are separated from the parent plant [13]. Another interesting phenomena was reported that out of 3 different concentration of extracts (5g, 10g and 15g), 10g extract was most inhibitory for germination percentage and growth of the plants, that was contrary to the general idea that with increase in concentration, negative impacts increase; rather the highest concentration extract i.e. 15g was stimulatory in its nature, as reported by some earlier workers. The effect of inhibition was enhanced with increasing plant material. The results showed similarity with results of various researches [14-17]. It’s a general trend that allelochemicals may reverse its impact as per the concentration, as reported many times that increasing concentration some time cause positive effects, then negative [18, 19].

**Conclusion**

From the result, it was concluded that leaves extract of *Euphorbia helioscopia* contain some kind of allelochemicals that caused reduction in growth parameter while stimulated germination percentage in both test plant i.e. mustard and wheat. The allelopathic effect was dependent on the concentration as well as on soaking duration and was maximum in case of 10g followed by 15 g and 5g. The results also revealed that dry leaves extract showed maximum inhibitory effect than fresh leaves. In both test plants the most affected part was plumule followed by radicle. From the observations and the results, it is evident that *Euphorbia helioscopia* as significant allelopathic potential inhibiting the plumule and radicle growth of *Brassica campestris* and *Triticum aestivum* so *E. helioscopia* should be removed near their field. As *Euphorbia helioscopia* is a medicinal plant and economically very important, therefore further studies should be done in order to find out in which environment it can grow best and in which habitat it well not affect other plants and vice versa. Furthermore, studies should be done in order to identify and evaluate the specific allelochemicals present in *Euphorbia helioscopia* which stimulated the germination percentage.

**Authors’ contributions**

Conceived and designed the experiments: S Khalid, Performed the experiments: S Zaman, F Khisro & H Shumail, Analyzed the data: SIU Haq & M Asghar, Contributed materials/analysis/ tools: S Khalid, S Zaman & F Khisro, Wrote the paper: S Khalid & SIU Haq.

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