

Research Article

The allelopathic effects of *Rumex dentatus* and *Dalbergia sissoo* on growth and germination of *Brassica campestris* L.

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Citation

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Abstract

The present research work was conducted to acquire the allelopathic effect of *Rumex dentatus* and *Dalbergia sissoo* in relevance to germination and growth of *Brassica Campestris*. Fresh and dry leaves of *R. dentatus* and *D. sissoo* (5g, 10g and 15g) were soaked for 24, 48 and 72 hours in 100ml of water and was filtered by using Whatman filter paper no. 1. The prepared extract was used against the test plant by observing its impact on the germination percentage, radicle and plumule length after 72 hours of incubation period at 26°C. The results showed that the extract of *D. sissoo* effected the radicle length more as compared to plumule length while *R. dentatus* significantly effected the radicle length, plumule length and germination percentage. Moreover, the results also illustrated that for *D. sissoo* the inhibitory effects of fresh leaves on the test plant were more pronounced than the dry leaves extract whereas the inhibitory effects of dried leaves extract of *R. dentatus* on the test plant was more evident than the fresh leaves. The application of *R. dentatus* dry leaves extract completely suppressed the germination percentage, whereas in the case of fresh leaves minimum germination percentage was recorded 20% in 5g (24hrs soaking duration) as compared to control (95%). In the case of *D. sissoo*, the fresh and dry leaves extract showed a lesser effect on germination percentage while the application of fresh leaves extract was more inhibitory and showed more reduction in radicle and plumule length. The maximum reduction in the radicle length was recorded (0.12cm) and plumule length (0.05cm) as compared to control 3.98cm and 1.6cm in 24hrs soaking duration. From the results, it was concluded that fresh and dry leaves extract of *D. sissoo* and *R. dentatus* significantly reduces germination and growth of *B. campestris* in comparison to control. The reported inhibitory effects assumed that *D. sissoo* and *R. dentatus* had allelopathic potential which inhibited the growth and development of *B. campestris*.

Keywords: Allelopathy; Extract; *Brassica campestris*; *Dalbergia sissoo*; *Rumex dentatus*; Germination percentage; Inhibitory effect; Plumule length; Radicle length

Introduction

The term allelopathy was first coined by physiologist Hans Molish (1937) in his book 'Der Einfluss einer Pflanze auf die Andre (The Effect of Plants on Each

Other)'. It is a Greek word consisting of two words allelon meaning "mutual" and pathos means "suffering or harmful effects on each other [1, 2]. Allelopathy is a biological phenomenon according to which

one organism produces one or more biochemicals that affects the rate of germination, growth, reproduction and survival of other organisms negatively or positively [3]. Ironically the term Allelochemicals was first used by two scientists Whittaker and Feeny in 1971. According to them “allelochemicals are biochemicals that are of major significance in adaption of species and organization of communities”. They have both beneficial (positive allelopathy) or detrimental (Negative allelopathy) effects on the target organisms and community and are responsible for causing allelopathy [3, 4]. They are produced by releasing secondary metabolites and are not required for direct metabolism of allelopathic organisms [5, 6]. On the basis of chemical similarities, allelochemicals are distributed from simple hydrocarbons to complex pyrolic compounds. The Allelochemicals are concentration dependent. If present in low concentrations, they may stimulate rather than inhibit the growth. Chemicals with allelopathic potential are present in all plants and in many tissues, such as leaves, flowers, fruits, buds, stems and root [7]. Allelochemicals with negative allelopathy effects are important part of plant defense against herbivory [8]. Pakistan is a country with its economy greatly dependent on forestry and agriculture. *Dalbergia sissoo*, commonly known as North India Rosewood, is an evergreen rosewood tree extensively found in Punjab Province. It is also known as weed of bush lands at road side [9]. It is found in tropical to subtropical climates in forests mainly near streams, rivers [10, 11]. In Pakistan, *Dalbergia* is renowned for its ornamental purposes and quantity of timber [12]. It has a strong root system which helps it to control erosion and stabilize the disturbed area [11]. It grows naturally under subtropical climatic condition [13]. *Rumex*

dentatus Linn. is a common weed of cultivated fields is also found extensively in Pakistan. It is ubiquitous in Sindh, Balochistan, Punjab, Rawalpindi and KP region. It usually grows throughout the year but sometimes it is biannual. The seeds are grown by the mid of November and it reaches its maturity by the end of December. The well-developed root system of *Rumex dentatus* provides an edge over other plants. The leaves are small in the seedling stage which become extremely well in later stage [14]. Brassica oil seeds crops are cultivated on 23 million hectares annually which give over 36 million tones production to the world [15]. It is best known for its important agricultural and horticultural crops and includes a number of weeds. Mustard is the second most important source of oil in Pakistan that contribute to 17% of the total domestic production of edible oil. [16]. In Pakistan it is cultivated in winter and harvested in March. Further it is cultivated in the alkaline soil and raised in full sun light. It tolerates a PH from 4.8 to 8.3. *Brassica campestris* is characterized by high amount of Sulphur and nitrogen compound. *Brassica campestris* is influential modulator of immune system with potent antiviral, antibacterial, anticancer and antiandrogen [17].

Materials and Methods

Collection and mechanical processing of plant material

Fully grown mature leaves of *Rumex dentatus* and *Dalbergia sissoo* were collected from areas around the Botany Department of Islamia College Peshawar, Pakistan. From each, half of the leaves were washed thoroughly with distilled water and were placed in a room to dry them with temperature ranging from 14°C to 20°C. The other half of them were crushed using mortar and pestle. The aqueous extracts are made by soaking them in distilled water.

The extracts were stored at 5-10°C when not in use. However, the extracts were used within 24, 48 and 72 hours of its extraction.

Apparatus used

The apparatus used in the research were: Filter paper, Petri dishes, Incubator, Refrigerator, Beaker, Digital balance, Titration flasks, Funnels, Iron stand, stirrer and Mortar and pestle. All above apparatus were thoroughly washed with tap water, rinsed with distilled water and then finally sterilized in the autoclave machine at 160°C for at least 4 hours.

Procurement of seeds of test species

Brassica campestris variety BGE-5 was used as test specie. The seeds of the test plant were obtained from the institute of Biotechnology and Genetic Engineering, the University of Agriculture, Peshawar.

Standard filter paper bioassay

After sterilization of Petri dishes, Whatman no.1 filter paper and cotton were used as the seed-beds in these experiments. Firstly, cotton was spread and placed in the Petri dishes and then Whatman no.1 filter paper was folded twice and placed in the Petri dishes. These filter papers seed-beds were moistened with distilled water for control and with the respective plant extracts (i.e. leaves) for making tests. Excess of extract or distilled water was strictly avoided to reduce the effect of waterlogging on germination and growth of the seedling. Each Petri dish was provided with 5 healthy seeds that were placed at equal distance on the filter paper. The Petri dishes were always sealed with large size Petri dishes or polythene sheets to retain moisture and humidity. Loaded Petri dishes were incubated at 26°C.

Preparation of aqueous extract

Five, 10g and 15g fresh and dry leaves of *R. dentatus* and *Dalbergia sissoo* each were separately soaked in 100ml distilled water for 24, 48 and 72 hours at 26°C and then filtered to get aqueous extract. These

extracts were used against *B. campestris* as test species by using the “standard filter paper bioassay” [18, 19]. The filter papers were moistened with the aqueous extracts, while distilled water was used as a control. Each treatment had 3 replicates, each with five seeds. Petri dishes were incubated at 26°C. Germination, radicle and plumule growth were recorded after 24, 48 and 72 hours.

Standard germination

The percent germination of the test species was recorded according to Scott [20].

(Germination Percent = no of germinated seeds / total no of seeds x 100)

Statistical analysis

Each experiment was performed in a completely random design block and results obtained were the average of three replicates. The results were statistically analyzed through significantly different from control at alpha = 0.050 according to one way ANOVA followed by the student's t-test.

Results and Discussion

Germination percentage (%)

Reduction was observed in case of germination percentage that was duration and concentration dependent. The percent germination of *Brassica campestris* was inhibited by the aqueous dry and fresh leaves extract of *Rumex dentatus* and *Dalbergia sissoo*. The study indicated that the aqueous dry leaves extract of *Rumex dentatus* in all treatments showed inhibitory effects on percent germination as compared to fresh leaves extract and fresh and dry leaves extract of *D. sissoo*. According to the mean value of germination of *B. campestris* the highest percent (95.3%) was noted in 72hrs followed by 48hrs (94%) and 24hrs (87%) of soaking duration in case of *D. sissoo* respectively. However, the lowest percent germination of *B. campestris* was found to be (46.7%) in 24hrs followed by 48hrs (74.3%) and 72hrs (86%) as

compared to control (100%) inhibited by *R. dentatus*. Moreover, hours x concentration mean of the germination of *B. campestris* indicated that under control condition lowest inhibition (20% and 60%) at 5g was recorded in soaking duration of 24hrs in case of *R. dentatus* while, the highest inhibition (100%) at 5g and 10g was noted extraction duration (48hrs and 72hrs) of *D. sissoo*. Even then the result revealed that all treatments (i.e. 5g, 10g and 15g) of dry leaves extract of *R. dentatus* in all duration was negatively affected by the germination percentage to (0%) as compared to control (100%). Finally the result apprises us that extraction of *R. dentatus* showed complete inhibition whereas *D. sissoo* showed least inhibition in the growth and development of germination percentage (Table 1; Fig. 1). Similarly, the aqueous dry and fresh shoot and root extracts of *Rumex dentatus* spp. *Klotzschianus* significantly inhibited the seedling growth and germination percentage of test species as compared to control [21]. The current work was also carried out to look the allelopathic effect of *Clerodendrus infortunatum* L. leaf extract on seed germination and seedling growth of some agricultural crops such as *Triticum aestivum*

(wheat), *Brassica campestris* (mustard), *Vigna radiata* (mung bean) etc. It was found that aqueous leaf extract of *C. infortunatum* L. completely inhibited the germination percentage of tested crops [22]. The effect of aqueous extract from *Calotropis procera* on the growth of *Brassica* specie. The effect of extract was studied against germination percentage, seedling growth and dry biomass. Result concluded that higher concentration extracts significantly reduced germination percentage, radicle length and plumule length of *Brassica* seedling as compared to control [23]. Likewise, another work also evaluated that aqueous extract of walnut leaf significantly inhibited seed germination and seedling growth of *B. campestris*. Hence seed germination and seedling of mustard were affected negatively by walnut leaf extract [24]. Parallely, some other experiment also evaluated the allelopathic potential of leaf aqueous extract obtained from *Cynara candunculus* L. plant species on germination of *B. campestris* used as a test specie on average the aqueous leaf extracts significantly reduced the final percentage of seed germination as compared to control [25].

Table 1. Effect of 24, 48 and 72 hours soaking duration extract of *Rumex dentatus* and *Dalbergia sissoo* at 5g, 10g and 15g fresh and dry leaves on Germination percentage of *Brassica campestris* L. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars represents significance difference at P=0.05

Incubation period	<i>Rumex dentatus</i>						<i>Dalbergia sissoo</i>					
	24hr		48hr		72hr		24hr		48hr		72hr	
Treatments	FL E	DL E	FL E	DL E	FL E	DL E	FL E	DL E	FL E	DL E	FL E	DL E
Control	95	0	100	0	100	0	98	98	100	100	100	100
5g	20	0	60	0	86	0	98	98	100	100	100	100
10g	60	0	83	0	86	0	98	98	100	100	100	100
15g	60	0	80	0	86	0	65	65	82	82	86	86
Mean	46. 7	0.0	74. 3	0	86	0	87	87	94	94	95. 3	95. 3

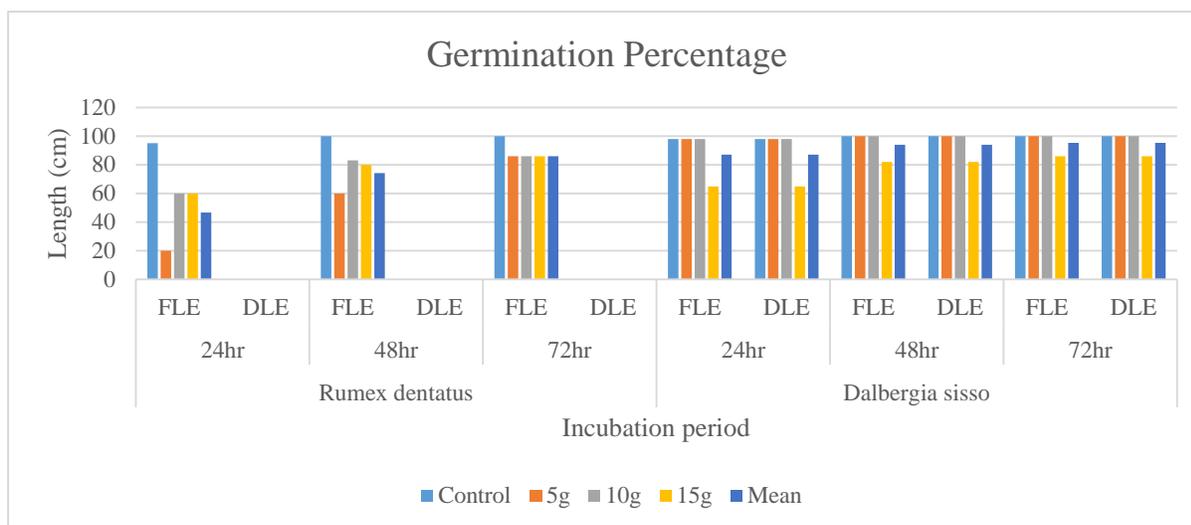


Figure 1. Effect of 24, 48 and 72 hours soaking duration extract of *Rumex dentatus* and *Dalbergia sissoo* at 5g, 10g and 15g fresh and dry leaves on Germination percentage of *Brassica campestris* L. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars represents significance difference at P=0.05

Radicle length (cm)

The statistical analysis of radicle length illustrated the significant deviation in duration and concentration mean. The results clearly showed that all the treatments of fresh and dry leaves extract of *R. dentatus* and *D. sissoo* considerably affected the radicle length of *B. campestris*. Further it was observed that the dry leaves extract of *R. dentatus* stands out to be more inhibitory in all treatments. It is so because it completely suppresses the radicle length of *B. campestris*, comparative to fresh and dry leaves extract of *D. sissoo* (Table 2; Fig. 2). Moreover the results gave the same maximum radicle length value of (0.81) cm inhibition in 72hrs of soaking duration as well as in 48hrs of soaking duration respectively but for 24hrs of soaking time the radicle length was recorded as (0.50) cm inhibition in *B. campestris* for dry of *D. sissoo*. The minimum inhibition was observed in case of *R. dentatus* which reduced the radicle length to (0.51) cm at 24hrs duration followed by (0.54) cm in

48hrs and (0.80) cm in 72hrs respectively. The extracts obtained from 15g fresh leaves of *D. sissoo* was more significant for radicle length of *B. campestris* which reduced the radicle length to (0.12) cm after 24hrs of incubation followed by 48hrs (0.23) cm except for 72hrs duration in which the *R. dentatus* showed more inhibition i.e. (0.73) cm length. The results showed that 5g dry leaves of *D. sissoo* was least inhibitory as it contracted the radicle length by (1.19) cm in 72hrs followed by (1.5) cm in 48hrs and (0.98) cm in 24hrs of time subsequently. The overall result proved that *R. dentatus* is comparatively more inhibitory in comparison to *D. sissoo*. In the same fashion the Hulless barley (*Hordeum vulgare*) and its 66 varieties were tested. Its two varieties (Qing 0039 and Qing 0415) significantly reduced the root length in *B. campestris*. Results revealed that inhibition was drastic (100% inhibition) in radicle length [26]. Similarly, the allelopathic influence of *Terminalia belerica* Roxh on the *Brassica campestris* seed. The fresh and dry aqueous

extract of leaves and fruits from *T. belerica* were tested on *B. campestris* that affects the radicle length of the seed. Percent germination was also inhibited to aqueous extract of the leaves but the radicle length was more significantly reduced as compared to control [27]. The aqueous leaf extract of *A. conyzodes* L. completely inhibited the germination and radicle extension of *Brassica campestris* [28]. Similarly the root and shoot growth of two winter crops (*Triticum aestivum* and *Brassica campestris*) and some associated weeds had been significantly reduced by the extract obtained from *Artemisia dubia* [29]. Similarly the allelopathic effect of *Parthenium hysterophorus* leaves extract against some cultivated and wild herbaceous spp. They reported that the *P. hysterophorus* leaves extract completely reduced the germination and root elongation of seedling in cereal and shoot elongation in crucifers and wild members Asteraceae [30].

Plumule length (cm)

After statistical analysis the plumule length portrays significant differences between duration and concentration mean. The results showed that all the treatments of fresh and dry leaves extract of *R. dentatus* and *D. sissoo* significantly effected the plumule length of *B. campestris*. Moreover, it was also found that all treatments of dry leaves extract of *R. dentatus* in all duration completely suppressed the plumule length of

B. campestris in comparison to control, fresh and dry leaves extract of *D. sissoo* (Table 3; Fig. 3). According to mean value, the plumule length of *B. campestris* was observed for dry leaves extracts of *D. sissoo* as relative maxima which gives an inhibition of (1.36) cm in 72hrs of soaking duration followed by (1.25) cm inhibition in 48hrs and (1.03) cm inhibition in 24hrs of soaking time respectively. Contrarily to it minimum inhibition was noticed in case of *R. dentatus* which reduced the plumule length to (0.29) cm at 24hrs followed by (0.48) cm in 48hrs and (0.90) cm in 72hrs of soaking time.

Furthermore the extract obtained from 15g of fresh leaves of *D. sissoo* was found out to be more significant for plumule length of *B. campestris* which also reduced the plumule length to (0.05) cm after 24hrs of incubation followed by (0.18) cm in 48hrs and (0.23) cm in 72hrs of soaking duration respectively. Finally, the results demonstrate that 5g and 10g dry leaves extract of *D. sissoo* was least inhibitory in the afore mentioned parameters which also gives a reduced plumule length of (1.61) cm in 5g and (1.92) cm in 10g as compared to control. However, the entire results justify that the dry extracts of *R. dentatus* showed more inhibitory effects on plumule length of *B. campestris* as compared to *D. sissoo* which also seems to more radicle and operative in plants.

Table 2. Effect of 24, 48 and 72 hours soaking duration extract of *Rumex dentatus* and *Dalbergia sissoo* at 5g, 10g and 15g fresh and dry leaves on Radicle length of *Brassica campestris* L. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars represents significance difference at P=0.05

Incubation period	<i>Rumex dentatus</i>						<i>Dalbergia sissoo</i>					
	24hr		48hr		72hr		24hr		48hr		72hr	
Treatments	FLE	DLE	FLE	DLE	FLE	DLE	FLE	DLE	FLE	DLE	FLE	DLE
Control	3.98	3.98	4.15	4.15	4.38	4.38	3.98	3.98	4.15	4.15	4.38	4.38
5g	0.93	0	1.08	0	1.17	0	0.17	0.98	0.33	1.5	0.45	1.19
10g	0.25	0	0.22	0	0.51	0	0.43	0.36	0.67	0.58	0.82	0.81
15g	0.36	0	0.33	0	0.73	0	0.12	0.16	0.23	0.34	0.31	0.44
Mean	0.51	0.00	0.54	0.00	0.80	0.00	0.24	0.50	0.41	0.81	0.53	0.81

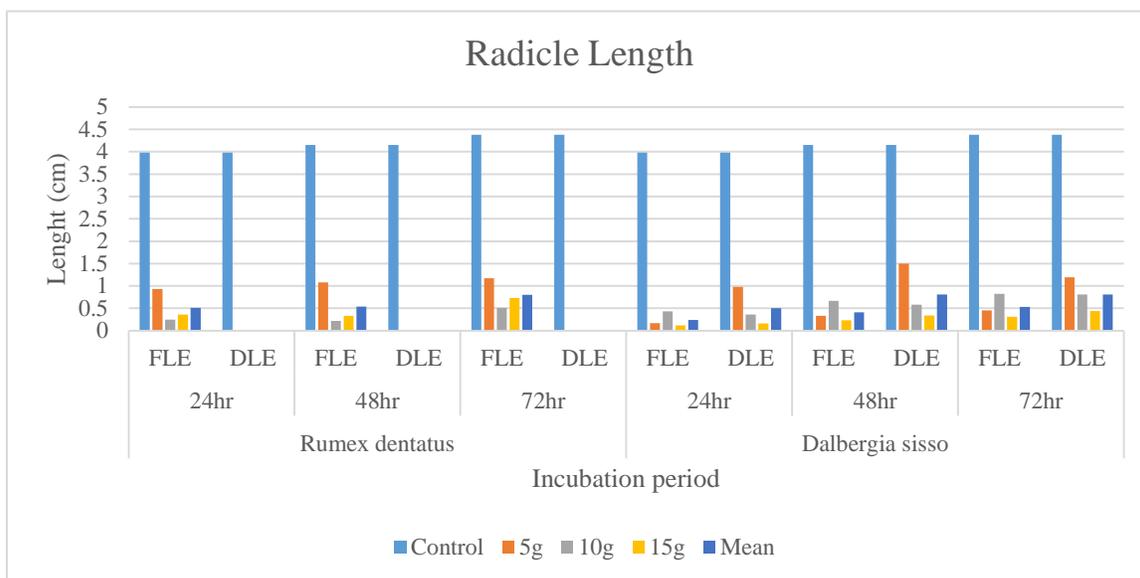


Figure 2. Effect of 24, 48 and 72 hours soaking duration extract of *Rumex dentatus* and *Dalbergia sissoo* at 5g, 10g and 15g fresh and dry leaves on Radicle length of *Brassica campestris* L. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars represents significance difference at P=0.05

In the same manner laboratory work were performed to investigate the allelopathic potential of *Forskeolea tenacissemata*. He used *B. campestris* as test specie. The aqueous fresh and dry leaves and stem extract from *F. tenacissemata* was used against *B. campestris*. Aqueous extract was used to notice the effect of plumule length and seedling of the seed. Result indicated that aqueous extract invariably reduced plumule length of test specie. Leaves extract prove more toxic than stem, as it significantly inhibits plumule length [31]. It was also observed that when aqueous dried and fresh leaves extract of *Quercus glauca* Thunb and *Leuconrichophora* A. Camus were applied on three crops such as, *Triticum aestivum*, *Brassica campestris* and *Len culinaris*, as a result they suppressed the germination, plumule and radicle length of all test crops [32]. Likewise, an experiment performed to evaluate the

allelopathic potential of fresh and dry extract from leaves and inflorescence of *Parthenium hysterophorus* on *B. campestris*. A marked reduction in plumule length indicated toxicity of extract in the leaves. Germination percentage was also inhibited but as compared the plumule length was more significantly reduced as compared to control [33]. Same result was also found that the aqueous leaf and root extract of *Cassia tora* extremely suppressed the seed germination and growth of *B. campestris*. There are some earlier workers which reported that, The effect of inhibition was enhanced with increasing plant material. The results showed similarity with results of various researches [34]. It's a general conception that allelochemicals may reverse its effect as per the concentration, as reported many times that enhanced concentration some time cause positive effects, then negative.

Table 3. Effect of 24, 48 and 72 hours soaking duration extract of *Rumex dentatus* and *Dalbergia sissoo* at 5g, 10g and 15g fresh and dry leaves on Plumule length of *Brassica campestris* L. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars represents significance difference at P=0.05

Incubation period	<i>Rumex dentatus</i>						<i>Dalbergia sissoo</i>					
	24hr		48hr		72hr		24hr		48hr		72hr	
Treatments	FLE	DLE	FLE	DLE	FLE	DLE	FLE	DLE	FLE	DLE	FLE	DLE
Control	1.6	1.6	2.33	2.33	2.91	2.91	1.6	1.6	2.33	2.33	2.91	2.91
5g	0.23	0	0.59	0	0.77	0	0.56	1.23	0.89	1.45	0.97	1.61
10g	0.41	0	0.42	0	1.12	0	1.05	1.48	1.14	1.83	1.26	1.92
15g	0.23	0	0.26	0	0.52	0	0.05	0.39	0.18	0.46	0.23	0.54
Mean	0.29	0.00	0.42	0.00	0.90	0.00	0.55	1.03	0.74	1.25	0.82	1.36

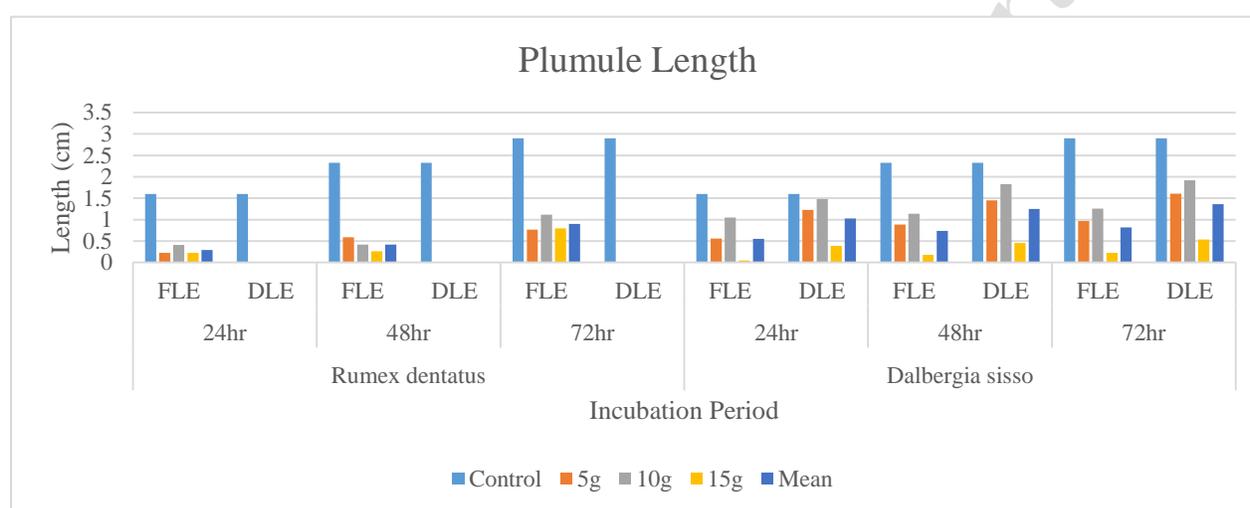


Figure 3. Effect of 24, 48 and 72 hours soaking duration extract of *Rumex dentatus* and *Dalbergia sissoo* at 5g, 10g and 15g fresh and dry leaves on Plumule length of *Brassica campestris* L. FLE= Fresh leaves extract, DLE= Dry leaves extract. Bars represents significance difference at P=0.05

Conclusion and Recommendations

The results concluded that both fresh and dry leaves extract of *Rumex dentatus* and *Dalbergia sissoo* contain allelochemicals, which significantly reduces the germination percentage, radicle and plumule length of *B. campestris*. It is observed that the dry leaves extract of *R. dentatus* is more inhibitory than the fresh leaves, while fresh leaves extract of *D. sissoo* was found to be more toxic than dry leaves extract. The toxicity of both plants (*D. sissoo* and *R. dentatus*) is dependent upon the soaking duration as well as concentration. The

results evaluate that 15g extract is found to be more strenuous followed by 10g and 5g substantially. This investigation indicated that the most affected part of *B. campestris* is plumule length is found followed by radicle length. From the experiment and the result, it is far observed that a more inhibitory effect was shown by *R. dentatus* and the least inhibition in the case of *D. sissoo*. The aqueous leaf extract of *R. dentatus* has more allelochemicals than *D. sissoo* and could be evaluated as an allelopathic species. From the overall result, it is concluded that *R. dentatus* and *D.*

sissoo leaves have potential allelochemicals that negatively affects germination percentage and growth parameter of the *B. campestris*. According to the results, it is recommended that *Dalbergia sissoo* and *Rumex dentatus* have strong allelopathic impacts on *Brassica campestris*. Due to this reason both plants should not be cultivated near the *B. campestris* field and further studies should be done to investigate and identify the specific allelochemicals.

Authors' contributions

Conceived and designed the experiments: S Khalid, M Naseem & M Sajjad, Performed the experiments: S Khalid, U Ibrahim & H Shumail, Analyzed the data: S Khalid & SIU Haq, Contributed materials/ analysis/ tools: M Naseem, M Sajjad, U Ibrahim & H Shumail, Wrote the paper: S Khalid & SIU Haq.

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