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Allelopathic Effect of *Salvia plebia* R. Brown on Germination and Growth of *Zea mays* var. 30-25 Hybrid, *Triticum astivum* var. Pirsabak-04 and *Sorghum bicolor* L.

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ABSTRACT

Salvia plebeia R. Brown (family Lamiaceae) allelopathic effect was determined on *Zea mays* var. 30-25 Hybrid, *Triticum astivum* var. Pirsabak-04 and *Sorghum bicolor* L. Aqueous extracts from root, shoot, mulch and soil sample were used to run experiment both in laboratory and field conditions. The results were statistically analyzed by using SPSS 21 for Dunken Multiple test. The aqueous extract showed that it has strongly affected the germination, plumule growth, radical growth, chlorophyll content and fresh and dry weights. Phytotoxicity of extracts depended upon amount and soaking duration. Mulching experiments also proved that it have strong inhibitory effect on Wheat. The results showed that *S. plebeia* has strong inhibitory effect on wheat. From the results, it was concluded that pytotoxicity was depending on the concentration of the part used against germination.
KEY WORDS: *Salvia plebeia, Sorghum bicolor,* wheat, Maize, allelopathy, Phytotoxicity

INTRODUCTION

Salvia plebeia R. Brown (family Lamiaceae) is a cosmopolitan weed in Asia ranges from Afghanistan to China, Japan and southwards to east of India, west of New Guinea and Australia [1]. Weeds are defined as plants growing in places where they are not supposed to grow, particularly where man wants to have something else. Weeds strive with the crops for nutrients, water, space, carbon dioxide and hamper the healthy growth ultimately reducing the yield both qualitatively and quantitatively. Weeds growing in the field have unfavorable effects on the yield and growth of crops mainly due to their competition with the main crops. Weeds inhibit crop growth and development by releasing various allelochemicals [2,3,4, 5]. Apparently, pests are thought to be most ruinous hazard in crop production but research has shown that weeds pose a more serious problem worldwide than the other pests could bring. In Asia and other continents, around 33-53% crop produce is damaged if weeds are not controlled from the crop fields [6,7,8].

Generally plants have the same structural and morphological adaptations as they live in association, communities and various groups depending upon the ecological conditions. Plants compete with each other for various life requirements, whenever two or more plants occupy the same niche in nature. One of the better understood mechanisms in plant competition is the ability to exert biochemical inhibition. Such struggle for existence is termed as allelopathy. Plants propagate both inhibitory and stimulatory effect on each other by releasing chemical compound [9]. Allelopathy disturbs the quality, development and quantity of the product and plays a significant role in agroecosystems [10, 11].

Selection of allelopathic plants is a commonly used approach to identify the plants with biologically active natural products [12]. The readily visible effects of allelochemicals on the growth and development of plants include inhibited or retarded germination rate, darkened and swollen seeds, reduced root and shoot extension, swelling or necrosis of root tips, curling of the root axis, discoloration, lack of root hairs, increased number of seminal roots, reduced dry weight accumulation, and lowered reproductive capacity [13]. Allelopathy has a broad application prospects in increasing crop production, forest tending, plant protection, biological control, etc. *Salvia macrosiphon* Boiss. aqueous extract effect the seed germination (%) and growth of *Zea mays* L. [14]. According to Bajalan *et al.,* 2013 aqueous extract of aerial parts of *Salvia officinalis* L., has strong allelopathic effect on seeds germination of barley (*Hordeum vulgare*) and purslane (*Portulaca oleracea*) [15]. Oxygenated terpenoids from *S. elegans* and *S. Munzii* repressed germination and radical growth of *Raphanus sativus L.* and *Lepidium sativum* L. [16].

Salvia plebeia is one of the most important herb known for its medicinal potential. Natural herbs have provided the modern world with some of the very important lifesaving drugs used in the armamentarium of modern medicine.

This research was done in order to compare and analyze the allelopathic effect of the aqueous extracts of *Salvia plebeia* on test species. Till this date, modern science has not recorded any research study to evaluate the allelopathic potential of *S. plebeia*.

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MATERIALS AND METHODS

Collection & preparation of sample

Salvia plebeia R. Br. was collected at flowering stage near wheat fields of Badragga, Malakand Agency. The plant was identified according to the Flora of Pakistan [17,18,19,20]. Shoot and roots were dried at room temperature (25-30°C). The dried shoots and roots were mashed into fine powder (80 mashes) with the help of grinder and then stored in paper bags for extraction. After thorough washing with sterilized water glassware were sterilized at 121°C for 15 minutes. Seeds were surface sterilized in 5% MgCl for 5 minutes and then washed with distilled water.

1. Effect of aqueous extracts on seed germination

Extracts was prepared by soaking weighed amounts of air dried plant shoots and roots, 5, 10 and 15g per 100mL in distilled water at room temperature for 24, 48 and 72 hours. The extract was filtered through filter paper to get aqueous extracts. 1mL of the filtrate was added to every petri dish containing two folds of filter paper. Distilled water was used as control. Ten seeds of each *Triticum aestivum*, *Zea mays* and *Sorghum bicolor* were placed in petri dishes. Five replicates of each treatment was used and incubated at 30˚C for 72 hours. This temperature was chosen after preliminary seed germination experiments of the test species at different temperature and soaking period [21].

2. Soil Bioassays

(a)Soil bed bioassay: Rhizospheric soil of Salvia plebeia was collected and dried at room temperature. Soil without species was used as control. 1gm of Salvia and control soil was used as seedbed in petri dishes. Soil was topped with a single sheet filter paper. In each case, 5 replicates with 10 seeds were used. The petri plates were damped with 5 mL distilled water. After 3 days, seeds of test species was placed on filter papers and incubated for 72 hours at 30˚C for Sorghum and Zea mays, at 24˚C for wheat.

(b)Soil extract bioassay:5g of both test and control soil was dissolved in 100 mL distilled water for 24 hours and after filtration it was tested against the same test species as before. After 72 hours, plumule and radicle length were determined [22].

3.Effect of hot water extracts on seed germination

5g of dried plant parts were separately boiled in 100 ml of water for 5 minutes and filtered. The room cooled extracts were applied against the same test species as before**.**

4. Mulching Experiment

The dried Mulch of *S. plebeia* was spread over the soil and mixed in proper proportion. Five seeds of each *Triticum aestivum*, *Sorghum bicolor* and *Zea mays* was sown out of which 1 was control. It was kept at room temperature for one week. From the date of sowing till harvesting, the pots were watered of equal amount from time to time when needed. The plants of test species were pulled out along with root system after one week. It was washed with water to remove the soil particles and dried. Various parameters like fresh, dry biomass, length and number of root, shoot and leaves and chlorophyll content was recorded.

Statistical Analysis

All the statistical analyses were performed using SPSS 21.0.

RESULTS & DISCUSSION

1. Aqueous shoot extracts effect on seed germination

i. Plumule Length

Aqueous shoot extracts of *Salvia plebeia* at different concentration (5g, 10g and 15g) showed strong inhibitory effect on test species. The length of plumule was significantly decreased as compared to control (Fig.1, 2 and 3). Bars with different letters show significant difference (p=0.05) as determined by Duncan's Multiple Range Test. The extracts from the shoot adversely affect the growth of plumule of the test species in all combinations of concentrations and soaking periods. The inhibitory effect was dependent on concentration.

Studies demonstrated the presence of growth inhibitors produced by Salvia species were carried out on *S. leucophylla* and *S. apiana* [23]. Previous studies showed that crushed leaves cucumber seedlings inhibits growth of test species when placed near it. The inhibition was directly proportional to the amount of leaves [24]. *Salvia macrosiphon* aqueous extract effect the seed germination (%) and growth of *Zea mays* L. [14]. Aqueous extract of aerial parts of *Salvia officinalis* has strong allelopathic effect on seeds germination [15].

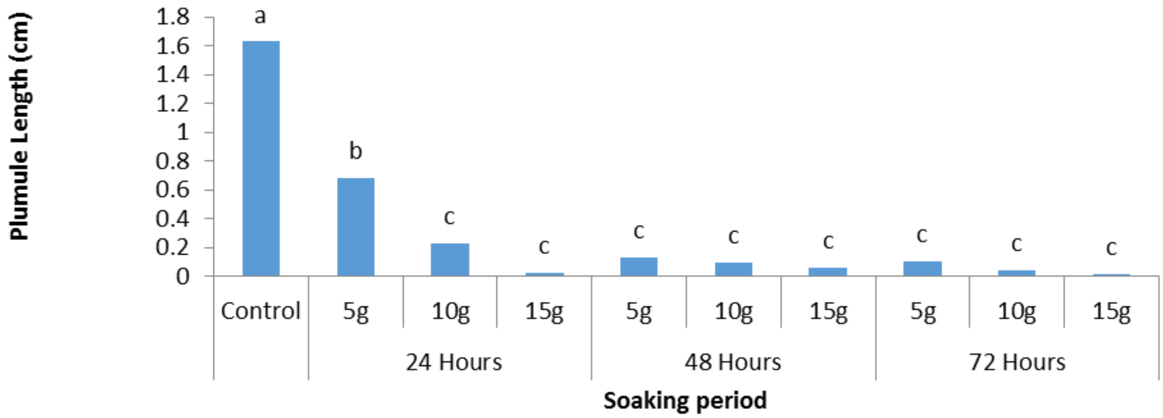


Fig. 1 *Salvia plebeia* aqueous shoot extracts different concentrations (5g, 10g, and 15g) effect on Plumule length of *Triticum aestivum* var. Pirsabak-04.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

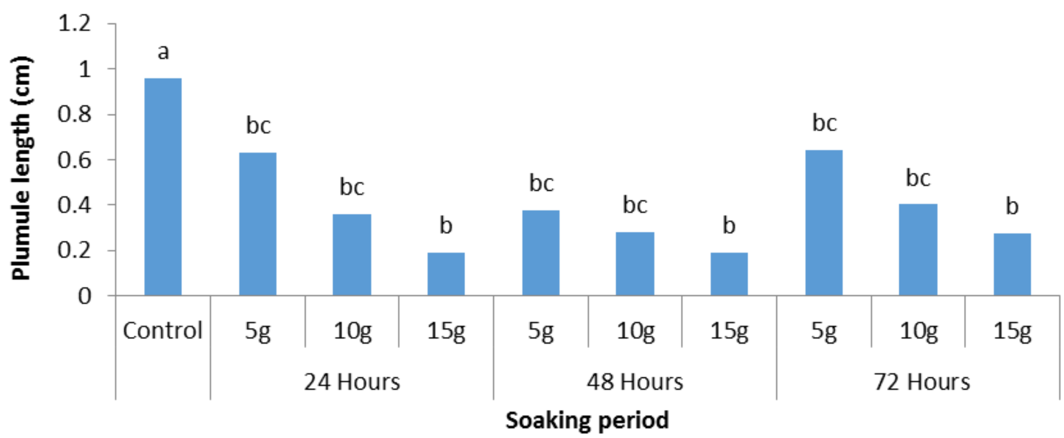


Fig. 2 *Salvia plebeia* aqueous shoot extract different concentrations (5g, 10g, and 15g) effect on Plumule length of *Zea mays* var. 30-25 Hybrid.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

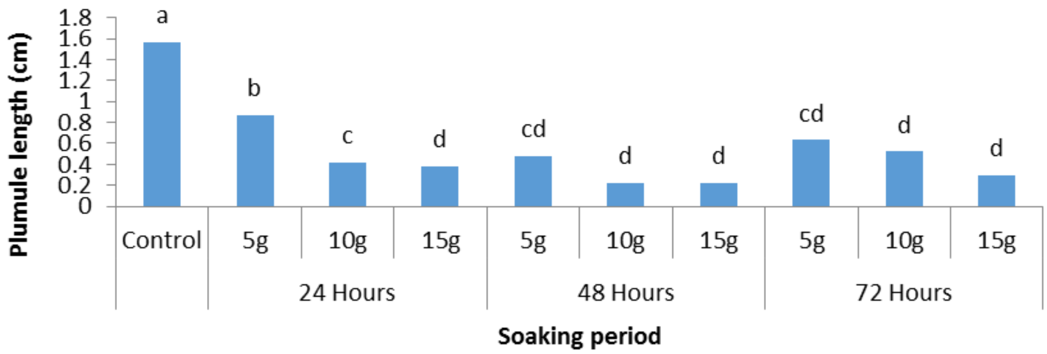


Fig. 3 *Salvia plebeia* aqueous shoot extract different concentrations (5g, 10g, and 15g) effect on Plumule length of *Sorghum bicolor*.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

ii. Radicle Length

The extracts from the shoot exhibited significant inhibitory effect on the radicle growth of the test species at all concentrations and in all soaking periods. Maximum inhibition was observed in 15gm concentration with 72 hours soaking period (Fig. 4, 5, 6). By increasing concentration, inhibition also increased [25,26]. The degree of inhibition increased with the extract concentration [27]. Chung and Miller [28] support these findings. *S. elegans* and *S. Munzii* oxygenated terpenoids inhibited germination and radical elongation [16]. According to Samreen et *al.* 2009 by enhancing soaking duration, phytotoxicity of aqueous extracts increase[29]. *Salvia hierosolymitana* and *Salvia multicaulis* essential oils have phytotoxic activity against seed germination and radical elongation of radish and garden cress [30].

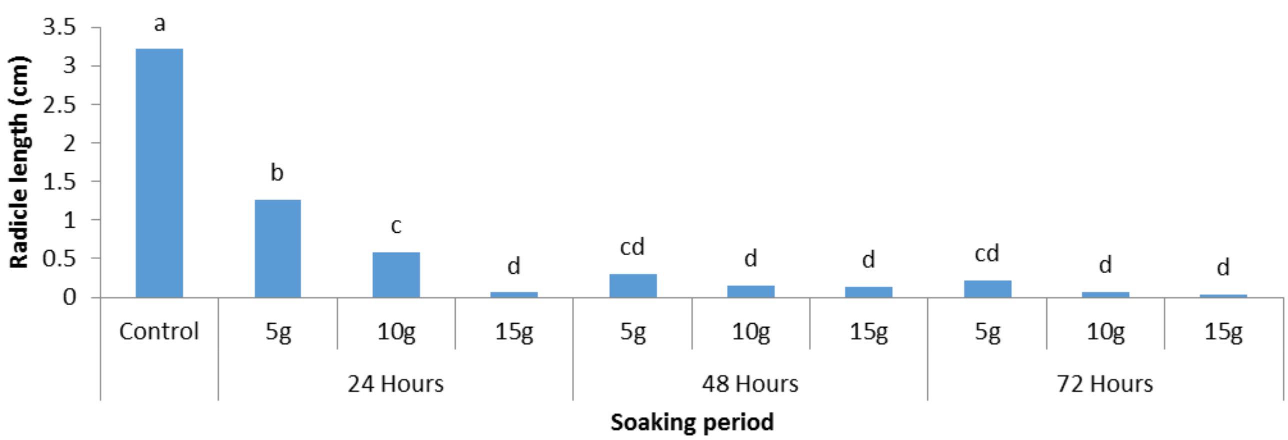


Fig. 4 *Salvia plebeia* aqueous shoot extract effect on Radicle length of *Triticum aestivum* var. Pirsabak-04.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

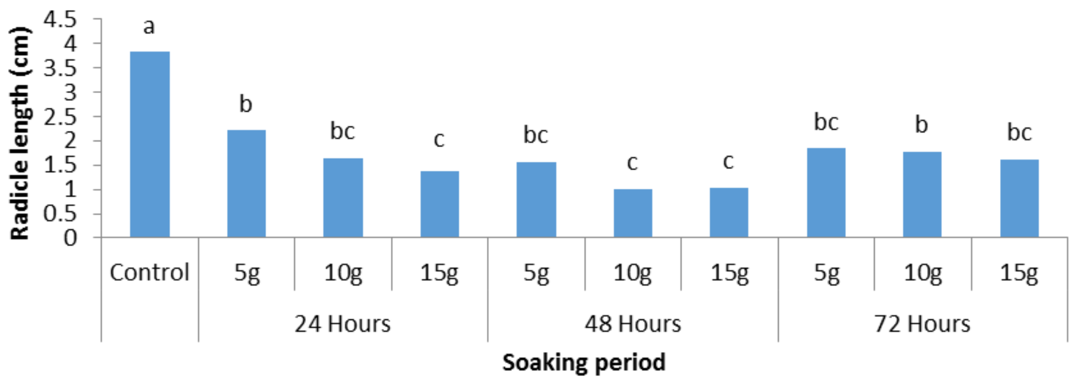


Fig. 5 *Salvia plebeia* aqueous shoot extract different concentrations (5g, 10g, and 15g) effect on radicle length of *Zea mays* var. 30-25 Hybrid.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

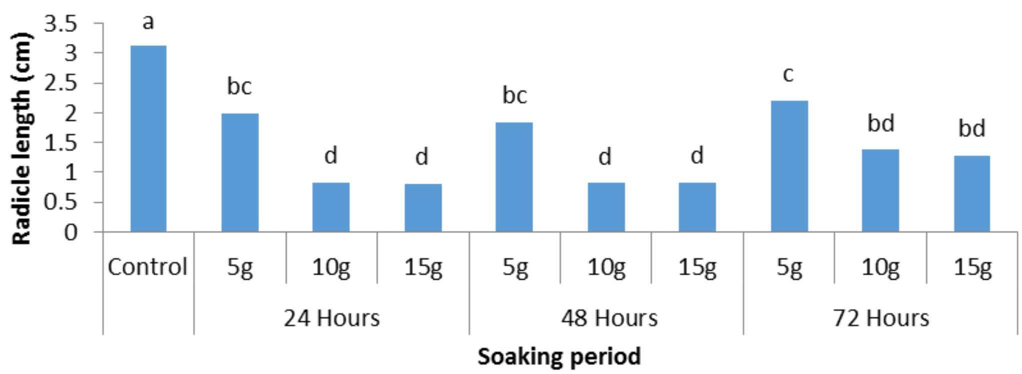


Fig. 6 *Salvia plebeia* aqueous shoot extracts different concentrations (5g, 10g, and 15g) effect on Radicle length of *Sorghum bicolor*.

Bars with different letters show significant difference (p=0.05) as determined by Duncan's Multiple Range Test. Bars with similar letters are not significantly different from each other.

Aqueous root extracts effect on seed germination

i. Plumule growth

Salvia root extracts significantly inhibit the growth of plumules in all the test species. By increasing concentration and soaking period inhibition also increased. Strong inhibitory effect was observed in *Triticum aestivum*, followed by *Zea mays* and *Sorghum bicolor*(Fig. 7, 8, 9).

Brassica oleracea, Daucus carota, Cucumis sativus, Cucurbita pepo, Allium cepa, Capsicum annum and *Lycopersicon esculentum* plumule and radicle and shoot dry weights were effected by root exudates of *Salvia syriaca* [31]. *Ageratum conyzoides* [32,33,34] *Cardaria draba* [35], *Brassica nigra* [36], *Raphanus raphanistrum* [37] and *Parthenium hysterophorus* [38,39], studied the allelopathic effect of aqueous extracts of the mentioned weeds.

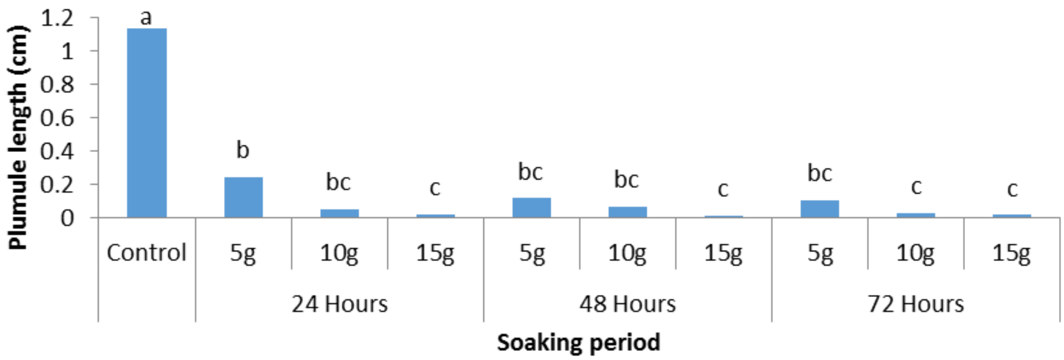
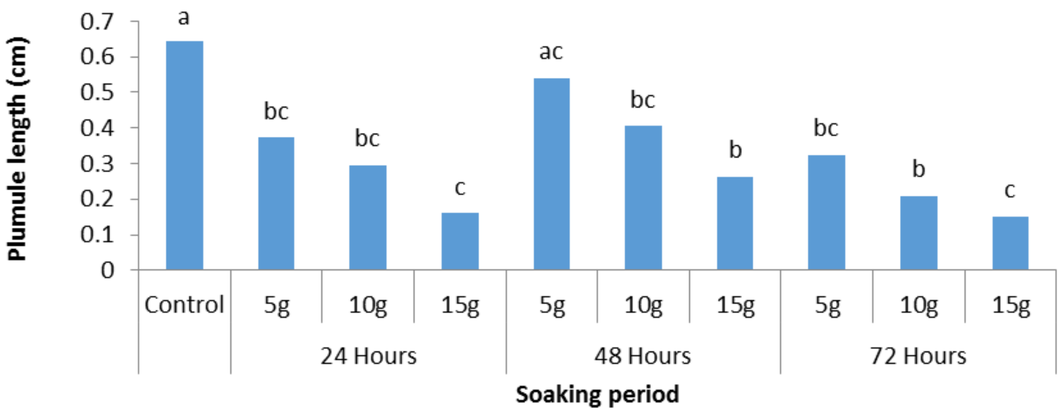


Fig. 7 *Salvia plebeia* aqueous root extract effect on Plumule length of *Triticum aestivum* var. Pirsabak-04.

Duncan's Multiple Range Testdetermined significant difference (p=0.05). Similar letters bars are significantly same.



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ig. 8 *Salvia plebeia* aqueous root extract different concentrations (5g, 10g, and 15g) effect on Plumule length of *Zea mays* var. 30-25 Hybrid.

Bars with different letters show significant difference (p=0.05) as determined by Duncan's Multiple Range Test. Bars with similar letters are not significantly different from each other.

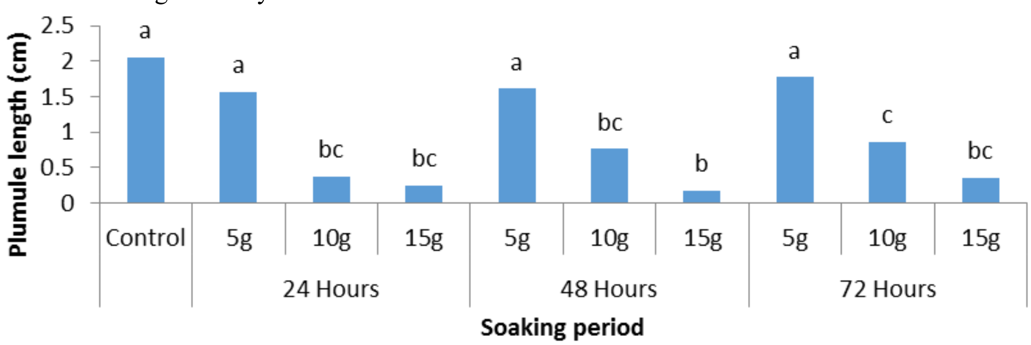

Fig. 9 *Salvia plebeia* aqueous root extracts different concentrations (5g, 10g, and 15g) effect on Plumule length of *Sorghum bicolor*.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

ii. Radicle growth

Root extracts of *S. plebeia* significantly decreased the radicle length of wheat. By increasing soaking period, inhibition also increased. The 5g aqueous extracts of *Salvia plebeia* obtained after 72 hour possess more inhibiting effect than 24 hour extracts (Fig. 10). Aqueous extracts with lowest concentration (5g) showed moderate inhibition in sorghum and maize (Fig. 11-12).

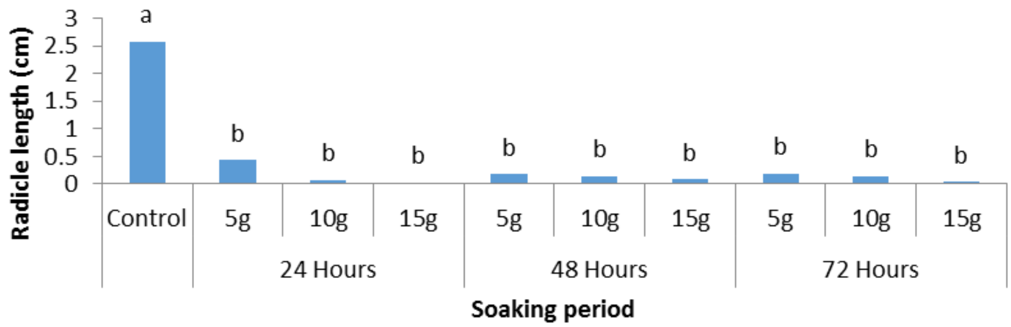

Fig. 10 *Salvia plebeia* aqueous root extract effect on Radicle length of *Triticum aestivum* var.Pirsabak-04.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

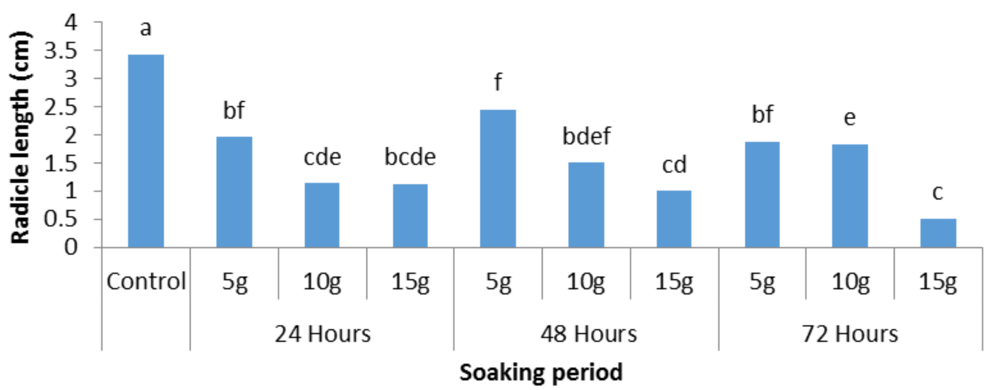

Fig. 11 *Salvia plebeia* aqueous root extract different concentrations (5g, 10g, and 15g) effect on radicle length of *Zea mays* var. 30-25 Hybrid.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

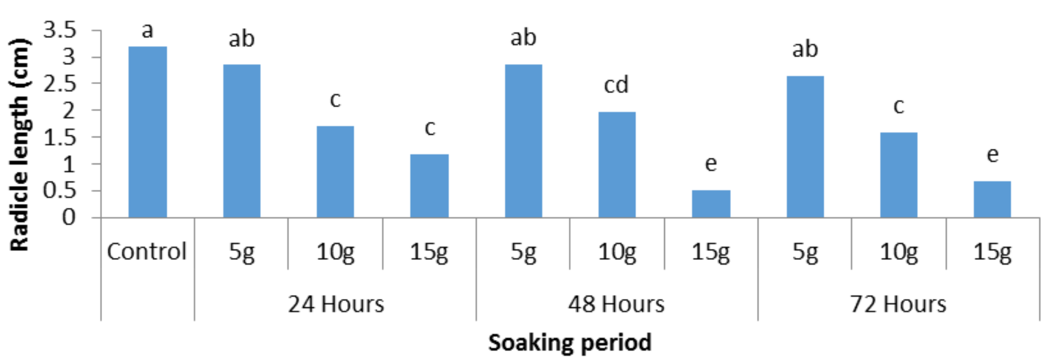

Fig. 12 *Salvia plebeia* aqueous root extracts different concentrations (5g, 10g, and 15g) effect on Radicle length of *Sorghum bicolor*.

Duncan's Multiple Range Test determined significant difference (p=0.05). Similar letters bars are significantly same.

2.Soil residual toxicity

While conducting soil bed bioassay, it was observed that it had no significant effect on radicle length. Similarly, soil extract showed no effect on radicle length of all the test species (Fig. 14). Soil bed has no effect on plumule length of *Zea mays*. Soil extract inhibited growth of plumule length of test species (Fig. 13). Soil collected from beneath *Acacia tortillus* and *Prosopis cineraria* had no significant inhibition [40].

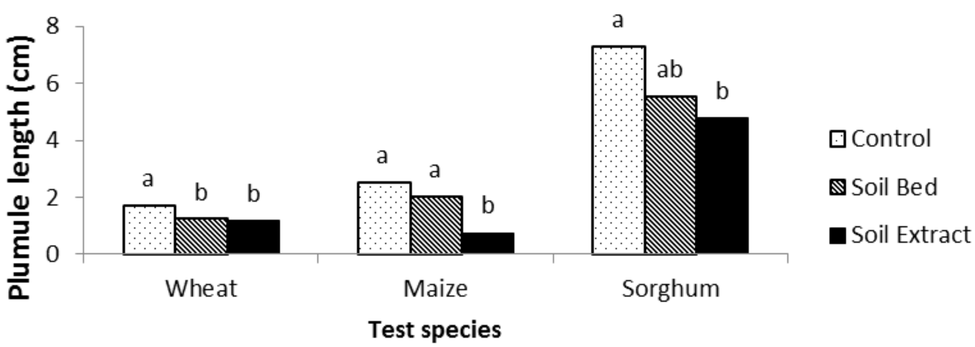

Fig. 13 Effect of Salvia-soil bed bioassay and Salvia-soil extract bioassay on plumule growth of *Sorghum bicolor, Zea mays* var. 30-25 Hybrid and *Triticum aestivum* var. Pirsabak-04.

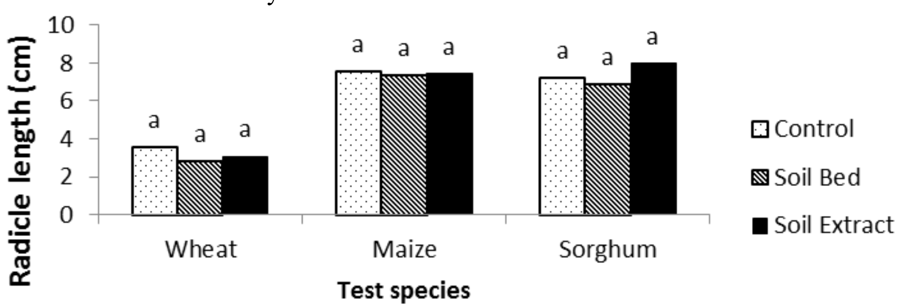

Fig. 14 Effect Salvia-soil bed bioassay and Salvia-soil extract bioassay on radicle growth of *Sorghum bicolor, Zea mays* var. 30-25 Hybrid and *Triticum aestivum* var. Pirsabak-0

3.Effect of Hot water extracts

The hot water extract reduced the germination in wheat and maize. Hot water root had no significant effect on Sorghum plumule (Fig.15). Radicle development of all the test species was declined by hot aqueous extract (Fig. 16). *Dodonaea viscosa* hot water extracts strongly inhibit the growth of *Pennisetum americanum*, *Setaria italica* and *Sorghum vulgare* [41]. Hot water significantly reduced the germination and are reported to show more inhibitory effect [42,43,44,45, 46].

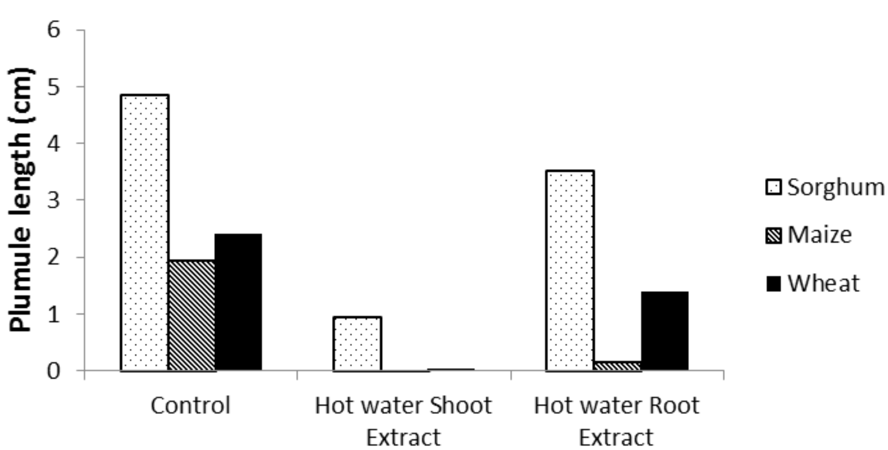

Fig.15 Effect of hot water shoots and root extracts on plumule growth of *Sorghum bicolor, Zea mays* var. 30-25 Hybrid and *Triticum aestivum* var. Pirsabak-04.

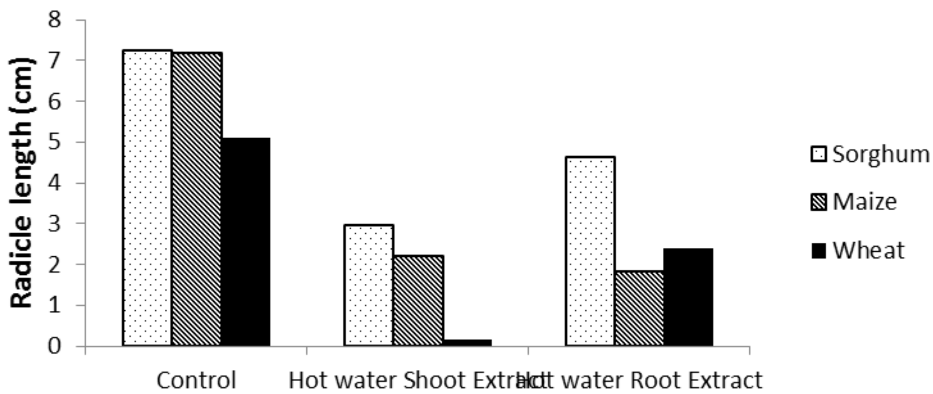

Fig. 16 Effect of hot water shoot and root extracts on radicle growth of *Sorghum bicolor, Zea mays* var. 30-25 Hybrid and *Triticum aestivum* var. Pirsabak-04.

4.Mulching effect on seed germination

The Mulch of *S. plebeia* showed strong inhibitory effect on test species. As compared to control the different parameters such as Plant height, root length, chlorophyll content, fresh and dry biomass. *Zea mays* plant height and chlorophyll content was reduced by *Salvia* mulch.

There was increase in dry biomass of maize as compared to control. However, mulch has no effect on germination percent of maize (Table 1). *Salvia plebeia* mulch showed strongest inhibitory effect on *Triticum aestivum*. Plant height (-78%), root length (-73%), germination percentage (-33.33%), chlorophyll content (-95%), fresh (-66.66%) and dry biomass (-39%) was significantly reduced in wheat (Table 2).

Table 1 Effect of Mulch of *Salvia plebeia* on plant height, root length (cm), percent germination, chlorophyll content, fresh and dry weight (g) on *Zea mays*. Mean of 5 replicates with 10 seeds is used below.

Treatment	Plant Height (cm)	Root Length (cm)	Germination %	Chlorophyll content	Fresh Biomass (g)	Dry Biomass (g)
Control						
Mean	27.4	23.6	100	26.36	1.783	0.156
SE	0.458	0.88	0	1.69	0.118	0.080
Treatment						
Mean	24.1	24.43	100	23.93	1.83	0.223
SE	1.69	1.31	0	1.46	0.097	0.016

PERCENT PROMOTION/REDUCTION

Treatment	Plant Height (cm)	Root Length (cm)	Germination %	Chlorophyll content	Fresh Biomass (g)	Dry Biomass (gm)
Control						
T1	-12.04	3.24	0	-9.23	2.62	42.5

Table 2 Effect of Mulch of *Salvia plebeia* on plant height, root length (cm), percent germination, chlorophyll content, fresh and dry weight (g) on *Triticum aestivum*. Mean of 5 replicates with 10 seeds is used below.

Treatment	Plant Height (cm)	Root Length (cm)	Germination %	Chlorophyll content	Fresh Biomass (g)	Dry Biomass (g)
Control						
Mean	14.33	12.26	100	33.66	0.28	0.027
SE	0.54	0.65	0	2.15	0.050	0.0063
Treatment						
Mean	3.083	3.22	66.66	1.59	0.093	0.016
SE	1.55	0.51	33.33	1.37	0.049	0.012

PERCENT PROMOTION/REDUCTION

Treatment	Plant Height (cm)	Root Length (cm)	Germination %	Chlorophyll content	Fresh Biomass (g)	Dry Biomass (g)
Control						
T1	-78.48	-73.71	-33.33	-95.27	-66.66	-39.024

Table 3 Effect of Mulch of *Salvia plebeia* on plant height, root length (cm), percent germination, chlorophyll content, fresh and dry weight (g) on *Sorghum bicolor*. Mean of 5 replicates with 10 seeds is used below.

Treatment	Plant Height (cm)	Root Length (cm)	Germination %	Chlorophyll content	Fresh Biomass (g)	Dry Biomass (g)
Control						
Mean	22.13	8.066	100	16.8	0.256	0.04
SE	1.33	1.38	0	1.81	0.057	0.0057
Treatment						
Mean	19.5	9.95	100	16.90	0.276	0.176
SE	0.714	0.99	0	2.24	0.038	0.111

PERCENT PROMOTION/REDUCTION

Treatment	Plant Height (cm)	Root Length (cm)	Germination %	Chlorophyll content	Fresh Biomass (g)	Dry Biomass (g)
Control						
T1	-11.89	23.45	0	0.65	7.79	34.6

Similarly reduction in plant height (-11.89%) of Sorghum was also observed. Root length, chlorophyll content, fresh and dry biomass was promoted (Table 3). Chlorophyll content of mustard seedlings showed decrease with increasing concentration of *Cassia tora* [47]. Chlorophyll content of soyabean leaves were significantly reduced by treatment with different allelopathic compounds [48]. *Gliricidia* pruning mulch caused leaf chlorosis in maize and cowpea but showed no effect on the yield [49]. *Adina cordifolia* and *Prunus cerasoides* mulch reduced the growth of *Glycine max* and *Hordeum vulgare* [50] the mulch of *S. plebeia* showed strong inhibitory effect on test species. As compared to control Plant height, root length, chlorophyll content, fresh and dry biomass was significantly decreased.

Salvia plebeia is an annual herb grows mostly near roadsides and wheat fields. Our results showed that aqueous extracts and mulch of *Salvia* had strong inhibitory effect on wheat, followed by Maize and sorghum. Due to excess of allelochemicals in it, *S. plebeia* should not be grown near the cultivating fields.

CONCLUSION

Salvia plebeia possess allelochemicals which effect the growth of test species. Aqueous extracts from different plant parts showed strong inhibitory effect on all test species. The growth of plumule and radicle of the test Wheat, Sorghum and Maize was strongly inhibited by almost all the extracts. The toxicity of the extracts varied from plant part to part and also with the concentration & soaking periods. The extracts with higher concentration and longer soaking periods were more inhibitory than those with low concentration and shorter soaking period. The extracts from roots proved to be more toxic to both radicle and plumule growth. Salvia litter significantly inhibits the germination, dry biomass and chlorophyll content of Triticum aestivum. Zea mays and Sorghum bicolor was also affected by the litter. The extracts from the shoot adversely affect the growth of plumule of the test species in all combinations of concentrations and soaking periods. The inhibitory effect was dependent on concentration. Results suggest that S. plebeia should not be grown near wheat fields and the litter must be removed before sowing the next crop.

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