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Allelopathic Effect of Leaf Extracts of Some Common Weeds on Seed Germination Characteristics and Growth of *Oryza sativa* L.

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ABSTRACT: The present study was conducted to investigate the allelopathic effects of *Chenopodium album, Cannabis sativa* and *Solanum nigrum* on seed germination and seedling growth of basmati rice (*Oryza sativa L.*). Aqueous leaf extracts of weeds at 50% conc. and 100% conc. were applied to determine their effect on seed germination characteristics, seedling length and seedling dry weight of test plant under in vitro conditions. In field experiment, 5g/kg and 10g/kg of the weeds' leaf powder was used to study the above mentioned parameters along with the leaf surface area and chlorophyll content. The aqueous extracts of the weeds under study caused inhibitory effects on seed germination characteristics, seedling length and seedling dry weight, leaf surface area and chlorophyll content of basmati IR-8 variety of rice plant. The leaf extract of *Chenopodium album* had the most inhibitory effect on test plant while that of *solanum nigrum* produced least effects. Interestingly, the effects of *Cannabis sativa* were the most varied and found to be changed with the growth matrix. Hot water extracts of the weeds were found to be more effective than cold water extracts and inhibitory effects was linearly proportional to the conc. of the weeds as it increased with the increase in the conc. of the weeds.

Keywords: Allelopathy, weeds, seed germination characteristics and growth.

INTRODUCTION

All weed species found in natural environment have become an obstacle for the crops (Baker 1965, 1991; Aldrich 1984). Weeds that grow among crop plants, adversely affect the yield and quality of the harvest and increase production costs, resulting in high economic losses. Soils of weed infected areas have less organic matter. In spite of modern weed management technology, weeds continue to cause annual losses of about 10% in agricultural production of the world (Jabeen *et al.,* 2013). The inhibitory effects of weeds on germination and growth of agricultural crops is due to the presence of phytotoxic compounds that are released from leaf litter and roots (Norhafizah *et al.,* 2012). These may be released from leaves, stems, rhizomes, roots, flowers, fruits and seeds. These chemicals are known as allelochemicals and this phenomenon is called allelopathy.

Allelopathy is a biological process characteristic of plants and microbes in which an organism produces one or more biochemicals that influence the growth, survival and reproduction of other organisms. These biochemicals can have beneficial (positive allelopathy) or detrimental (negative allelopathy) effects on the target organisms (Stamp, 2003; Willis, 2007). Weeds are the rich source of allelochemicals modifying the environment on surrounding plants (Nandan *et al.,* 1994).

Paddy (*Oryza sativa* L.) ranks second of the most growing crop in the world and India is the second largest producer of rice in the world following China. The average yield of paddy in India is less due to several factors; the major factor in the loss of production is weeds. According to Mani *et al.* (1968) weeds cause 9-51 % reduction in paddy yield.

Allelopathic potential of weeds on germination and seedling growth of crops vary among weeds (Hamayun *et al.,* 2005). Weeds exhibit allelopathy by releasing water-soluble allelochemicals from various parts such as leaves, stems, roots, rhizomes, flowers, fruits and seeds (Kruse *et al.,* 2000; Bertin *et al.,* 2003; Gatti *et al.,* 2004) that affect various biochemical and physiological mechanisms such as inhibition of respiration, cell division, pollen germination, nutrient uptake, photosynthesis and specific enzyme function, sporophytic growth and reproduction (Ferguson *et al.,* 2013). Weeds have made themselves dominant throughout the world because of some peculiar characteristics that are threatening the native vegetation (Holm *et al.,* 1977; Dukes and Mooney 1999; Tilman 2000; McNeely 2001; Heutte and Bella 2003; Lee and Klasing 2004; Jeschke and Strayer 2005). There is a variation among weeds in their competitive potential in reducing the yield of crops (Weaver and Ivany 1998).

MATERIALS AND METHODS

To fulfill the objectives of the present research, two types of experiments, in vitro and greenhouse experiments, were set up to assess the allelopathic effects of three common weeds (*Chenopodium album* L., *Solanum nigrum* L. and *Cannabis sativa* L.) on Basmati rice (*Oryza sativa* L.), variety IR-8, at the Department of Agriculture and Life Sciences, Desh Bhagat University, Punjab during the months of April – August, 2023. Each experiment was laid out in a completely Randomized Block Design (RBD) with three replications. The study was focused on seed germination, seedling growth and physiology and chlorophyll contents of rice.

For in vitro experiments, 100 g of weeds leaf leachates were rinsed thrice with distilled water, air dried to remove water and placed in an air tight conical flask (1L) containing 500 ml distilled water and kept in a refrigerator. After 5 days, the leachates were filtered thrice using muslon cloth and Whatman No. 1 filter paper. This was used as 100 % concentration extract for the experiments. The solution was diluted appropriately to obtain 50% conc. by mixing equal amount of distilled water.

In the experiment, seeds of Basmati IR-8 variety were first sterilized with bavistin (2.5 g/100 ml distilled water) and 0.1 % mercuric chloride (0.10 g/80 ml distilled water), washed with distilled water and then soaked between two filter papers and then placed on Whatman No. 1 filter paper in petriplates sterilized with 90% ethanol and designated by different solutions. Ten seeds of the test plant were placed in each petridish. The seeds were treated with both concentrations (50% and 100%) of cold and hot water extracts of all six weeds as and when required. The seeds were allowed to germinate for ten days at a temperature of 23±2ºC maintained in the B.O.D. Three replicates of each treatment were maintained. Distilled water was used as control. The number of germinating seeds was recorded daily during the period of ten days. After ten days of germination, samples were taken from each treatment and their root and shoot lengths and biomass were measured.

For greenhouse experiment, leaves of the weeds were air dried in shade for about 15-20 days and then powdered. Pots were prepared with soil (soil sand ratio 3:1) previously sterilized in an autoclave at 121ºC and 12-14 psi for about 25 min. Leaf powder of each weed was applied separately to pots in two concentrations – 5 g/kg and 10 g/kg soil in triplicate. All pots were maintained in random block design and watered with tap water. Seeds of the target crop were sown and observed for three weeks. No leaf powder was added to control pots. During this period, the numbers of seeds germinated was recorded every day. After three weeks, samples were taken from each treatment and their root and shoot lengths and biomass were measured.

PARAMETERS STUDIED

The parameters assessed in the two studies were— Germination Percentage, Germination Velocity Index [GVI], Inhibitory percentage, Seedling Vigour Index [SVI], Leaf Area, Root/shoot length and dry biomass and Chlorophyll content.

Germination percentage. Seed germination was recorded daily for ten days after the start of experiment. Seeds were considered germinated when radicle growth was 2 mm in length. The germination percentage was calculated using the formula given in the hand book of Association of Official Seed Analysis (AOSA, 1990).

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Germination Velocity Index. The GVI was calculated by using the formula described by the Association of Official Seed Analysis (AOSA, 1983).

$$\text{GVI} = \sum \frac{\text{Number of germinated seeds}}{\text{Number of days}}$$

Inhibitory percentage

Percentage inhibition of germination of test seeds in response to the weed treatments was calculated by the following formula:

Inhibitory percentage = 100 – (Final germination % in treatment)/(Final germination% in control) × 100

Seedling Vigour Index (SVI). SVI was calculated according to Abdul-baki and Anderson (1973).

$$\text{SVI} = \frac{\text{Seedling shoot length} \times \text{Germination \%}}{100}$$

Seedling shoot length was taken in cm after ten days of germination.

Leaf Area. Leaf area was determined by using standard graph paper method. Leaves were outlined on graph paper and the area of squares covered was measured (Taghipour and Saheli 2008). A mean of three leaves was taken as the reading.

Root and shoot length. Length of root and shoot were measured with the help of a scale. Readings were taken from both treated and control seedlings.

Root and shoot dry biomass. To obtain root and shoot dry biomass the samples were first washed thoroughly with water and then dried on blotting paper. These were then placed in oven for 48 hours at 60ºC or till their weight became constant.

Chlorophyll estimation. Chlorophyll content of each plant was estimated by Arnon (1949). 40 mg fresh leaves were soaked in 10 ml of 80% acetone and kept in a bottle covered with black carbon paper to prevent the entry of light and kept in a refrigerator for 4-5 days.

The bottle was sealed to prevent evaporation of acetone. Care was taken to ensure that leaves of same age were taken from each treated plant. After 5 days, optical density was calculated by spectrophotometer at 663 and 645 nm. The amount of chl 'a', chl 'b' and total chlorophyll (mg g^{-1} fw) in leaf samples was calculated using the following formulae:

$$\text{Chl 'a'} = \frac{(9.78 \times OD\ 663) - (0.99 \times OD\ 645) * V}{1000 * \text{Fresh Weight}}$$

$$\text{Chl 'b'} = \frac{(21.4 \times OD\ 645) - (4.65 \times OD\ 663) * V}{1000 * \text{Fresh Weight}}$$

Total Chl = Chl 'a' + Chl 'b'.

RESULTS

From the experimental procedure it is evident that the weeds affected the various physiological parameters of Basmati IR-8, albeit to various degrees. It was found that *C. album* had the most inhibitory effects on the physiological parameters of Basmati in general, followed by *S. nigrum*. Interestingly, the effect of *C. sativa* was different in petriplates and test pots. In the petriplate experiments, hot water extracts of the weeds were more effective compared to cold water extracts except *C. sativa* where cold water extracts were better. In the greenhouse study, higher dosage of weed residues was more effective in most cases.

In the lab study, the germination percentage of rice was reduced to 50% at 100% hot extract concentration in case of C. album. Parameters such as GVI and SVI were also found to be highly inhibited by the weed. The weed showed the least SVI of 0.87 among the three weeds. The root, shoot length, leaf surface area and dried biomass of rice seedlings were also negatively influenced by the allelopathic effects of C. album. There was no root emergence at all at hot 100% extract conc. of the weed, showing 100% reduction when compared to that of control. The shoot length of the test plant was highly reduced as least shoot length among all the weeds was observed at hot 100% extract conc. (1.80 cm) of the weed, showing a reduction of 78% compared to control. The dried biomass of the test plant also showed the same trend and at the same conc. of the weed the least biomass (0.12 mg) was observed, showing a reduction of 98.6%. In pots, the weed being the most effective showed 29.1% reduction in case of leaf area and 53.1% reduction in case of biomass over control. The chlorophyll a, chlorophyll b and total chlorophyll content in the rice leaves were also highly inhibited by C. album. Interestingly, lower doses of the weed were found to be promotive in both petriplates and pots while higher doses were found to be highly inhibitive.

S. nigrum was also found to have marked inhibitory effects on Basmati IR-8; however the effect was found to be of lower degree as compared to *C. album.* Lower concentrations of the weed promoted the germination, GVI and SVI of basmati in the laboratory as well as in green house. Higher conc. however, showed inhibitory effects. The weed reduced the germination of basmati to 73.3% at hot 100% extract conc. GVI and SVI at hot 100% extract conc. were the least with values (1.81) and (3.63) respectively. Rice seedlings showed reduction in growth (root and shoot length, biomass) on treatment with *S. nigrum*. Higher conc. of the weed was more effective in inhibiting growth. The shoot of the test plant showed an increase in length under the weed at lower conc. in petriplates with 12.2% (cold 50% conc. extract) and 14.6% (hot 50% conc. extract) increase respectively. The weed produced lesser effect on biomass with cold 50% conc. extract showing the least effect of 0.80%. The leaf surface area of the test seedlings was also influenced by the presence of *S. nigrum*. Lower dose of the weed enhanced the leaf surface area (3.42 cm^2). Higher dose was inhibitory while lower dose was actually promotive in both petriplates and pots. *S. nigrum* also inhibited chlorophyll a, Chlorophyll b and total chlorophyll content in rice leaves. The effect was however of lower degree as compared to *C. album* and *C. sativa.* It was found that higher dose of *S. nigrum* was inhibitory while lower dose was actually promotive.

In the case of *C. sativa* leaf leachates, the results were found to depend not only on the concentrations of the extracts but also on the growth matrix. The response of rice seeds/seedlings to the weed extracts was different under laboratory and greenhouse conditions. It showed almost no effect on germination as 100% germination was observed in petriplates treated with *C. sativa* while in pots, 5.56% inhibition was seen. GVI and SVI were least effected in petriplates. In the present study, the results of *C. sativa* extracts were the most varied. It was the second most effective weed after *C. album* against shoot length of rice seedlings in both petriplates and pots but it inhibited GVI, leaf surface area and chlorophyll content (a, b, total) in pots only. On the other hand, it showed the most inhibitory effects on root length in pots while it was not effective in petriplates. It was the second most effective weed after *C. album* in inhibiting the SVI and biomass in pots while it showed least inhibitory effects on dried biomass in petriplates. In most cases, lower doses of the weed were promotive in both petriplates and pots except for shoot length, dried biomass and leaf surface area in pots where higher doses were found to be promotive.

DISCUSSION

***Chenopodium album,* the most effective weed.** *C. album* species are among the worst weeds in the world that compete with cereals and vegetables and produce great yield losses (Holm *et al.,* 1977). A reduction in germination percentage, seedling vigour index, root length, shoot length and dried biomass of rice seedlings when compared to control is due to various allelochemicals present in *C. album,* Experiments have proven that it has alkaloids, aldehyde, flavonoids, apokartonoyd, gezylozyd and saponins (Rezaei *et al.,* 2008). Results were supported by the findings with the same weed on germination and growth of wheat and jute (Roy *et al.,* 2006) and safflower (Rezaie and Yarnia, 2009), Sorghum cultivars (Bagheri *et al.,* 2013; Bagheri *et al.,* 2014) and *Brassica juncea* (Dabgar and Patel 2014). Mallik *et al.* (1994) reported inhibited germination and growth of radish and wheat under aqueous air-dried extract of *C. album*.

Reduction in root, shoot length and dried biomass of rice seedlings is also supported by the findings that soil infested with *C. album* residual material reduced the growth of wheat (Bhatia *et al.,* 1984), lucerne (Muminovic, 1990), lettuce (Souto *et al.,* 1990). Anaya *et al.* (1987) reported that *C. album* has inhibitory effect on radicle growth of corn and beans.

***Solanum nigrum*, the intermediate effective weed.** *Solanum nigrum* is a common and troublesome annual weed in many parts of the world. It is the most widespread species of the family Solanaceae. Reduction shoot length is supported by the findings of (Shen *et al.,* 2005) who observed that *S. nigrum* produced strong allelopathic effects on wheat, cucumber and radish seedlings and reduced their shoot length. The inhibitory effects on yield parameters are directly proportional to increase in the concentration used and might be due to the presence of some alkaloids. According to Girija and Gowri (2008), the leaf extracts of *S. nigrum* contains alkaloids that may suppress the germination, radicle length and total protein content of *Pisum sativum* and *E. caracona*. Ismaiel and Salama (2021) found that there was direct negative relationship between germination test and plant parts used, including their concentrations. The reduction in germination, growth and yield of beans increased with higher concentrations of roots and leaf aqueous extracts. The strongest allelopathic effect was caused by the root extracts of the highest concentrations.

***Cannabis sativa*, the variable effective weed.** *C. sativa* is an effective weed that suppresses crop plants known for centuries (Willis, 2007) but the nature of its dominance and influence is not well documented. The significant inhibitory effect on germination percentage, seedling vigour index, root/ shoot length and dried biomass of basmati may be due to some water soluble compounds present in *C. sativa* and is supported by the findings of Tanveer *et al.* (2010) who observed the same effect of *Euphorbia helioscopia* on wheat, chickpea and lentil.

The efficacy of toxins in soil depends upon a number of factors including texture, accumulation capability and microbial activity. The toxins must accumulate to a physiological active level for exhibition of allelopathy. Verma and Rao (2006) reported that natural conditions are more complicated than laboratory bioassays.

Therefore, field experiments are necessary before any final conclusions are made on allelopathic effect of these weed species.

Inhibition in Chl a, Chl b and total Chl content of the test plant may be due to the allelochemical stress as has been reported previously (Ervin *et al.,* 2000; Moradshahi *et al.,* 2003; Singh *et al.,* 2009). Yang *et al.* (2002) stated that decrease in chlorophyll content of rice is due to the phenolic acids. Siddiqui (2007) reported that reduced chlorophyll content of *Vigna mungo* could be due to the allelochemicals present in black pepper leachate which possibly target enzymes responsible for the conversion of porphyrin precursors.

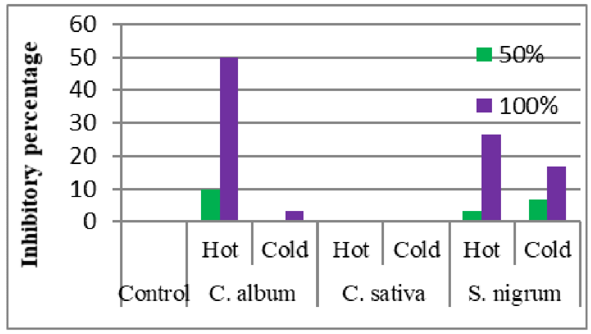

Fig. 1. Effect of aqueous extracts of weeds on inhibitory percentage of Basmati rice variety IR-8 (petriplates).

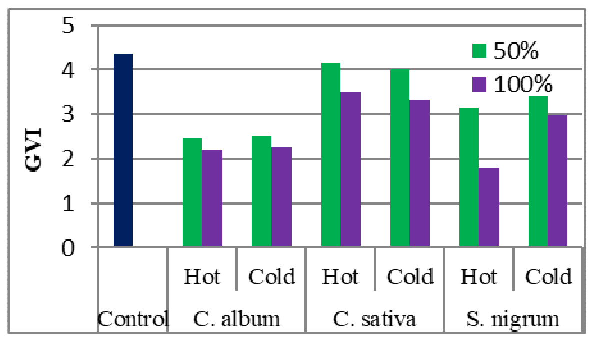

Fig. 2. Effect of aqueous extracts of weeds on Germination Velocity Index of Basmati rice variety IR-8 (petriplates).

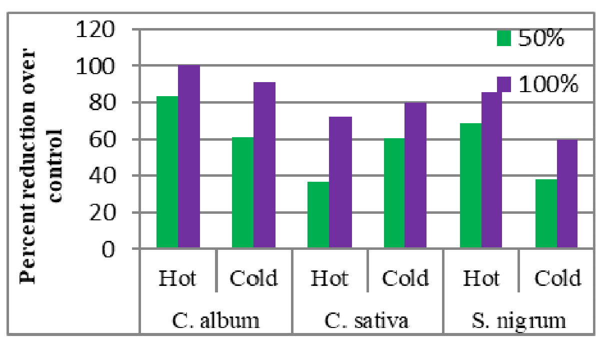

Fig. 3. Percentage reduction in root length of Basmati rice variety IR-8 due to aqueous extracts of weeds. (petriplates).

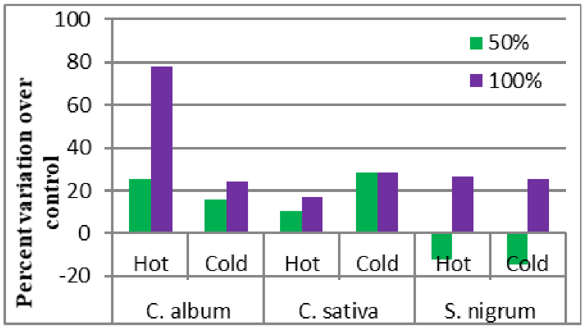

Fig. 4. Percentage variation in shoot length of Basmati rice variety IR-8 due to aqueous extracts of weeds (petriplates).

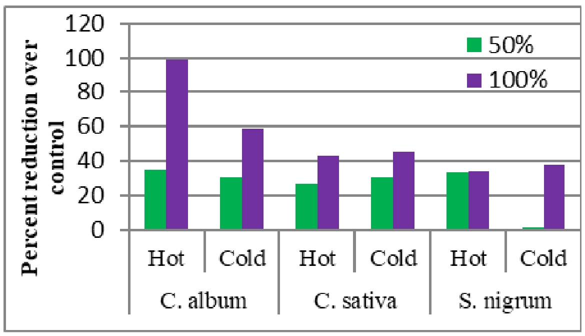

Fig. 5. Percentage reduction in biomass of Basmati rice variety IR-8 due to aqueous extracts of weeds (petriplates).

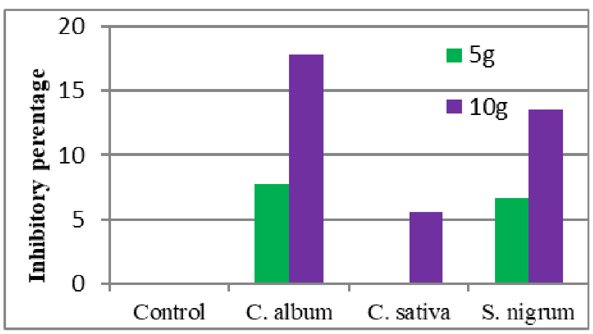

Fig. 6. Effect of dried leaf residues of weeds on Inhibitory percentage of Basmati rice variety IR-8 (pots).

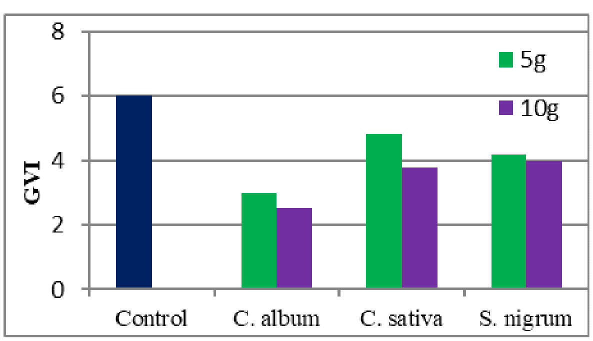

Fig. 7. Effect of dried leaf residues of weeds on Germination Velocity Index of Basmati rice variety IR-8 (pots).

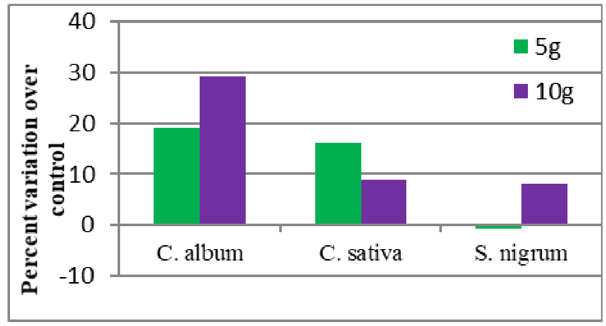

Fig. 8. Percentage reduction in Leaf Surface Area of Basmati rice variety IR-8 due to dried leaf residues of weeds (pots).

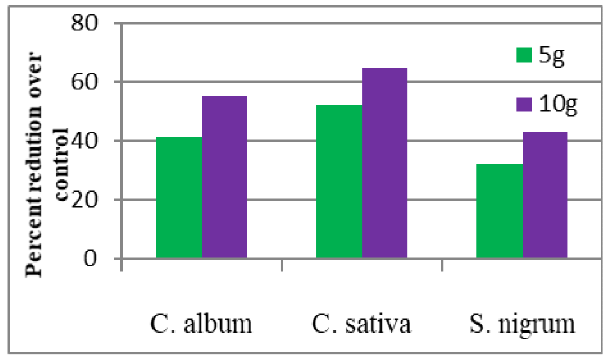

Fig. 9. Percentage reduction in root length of Basmati rice variety IR-8 due to dried leaf residues of weeds (pots).

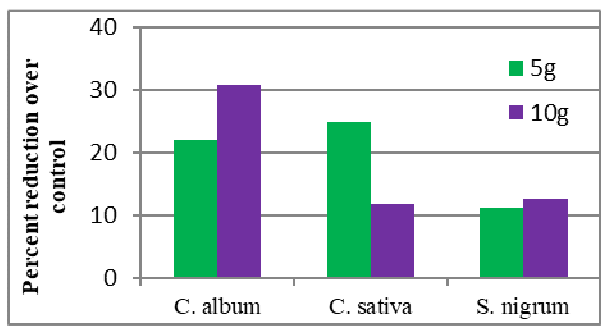

Fig. 10. Percentage reduction in shoot length of Basmati rice variety IR-8 due to dried leaf residues of weeds (pots).

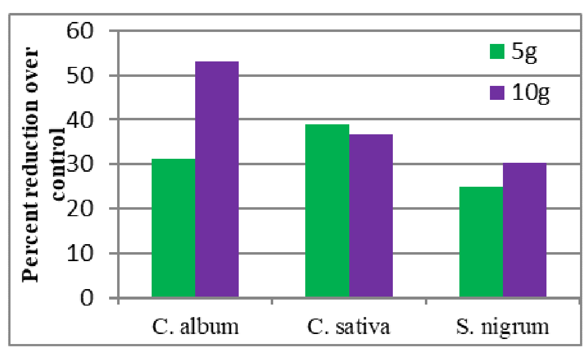

Fig. 11. Percentage reduction in biomass of Basmati rice variety IR-8 due to dried leaf residues of weeds (pots).

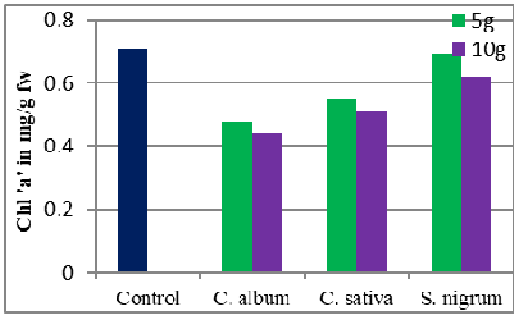

Fig. 12. Effect of dried leaf residues of weeds on chl 'a' Content of Basmati rice variety IR-8 (pots).

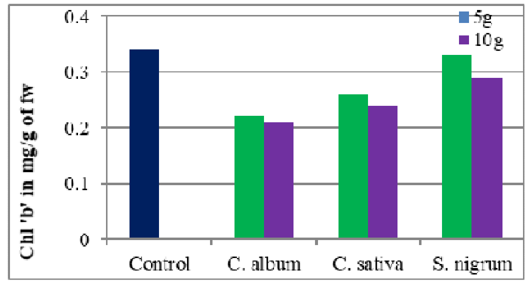

Fig. 13. Effect of dried leaf residues of weeds on chl 'b' content of Basmati rice variety IR-8 (pots).

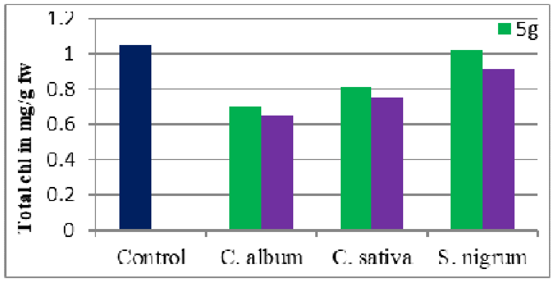

Fig. 14. Effect of dried leaf residues of weeds on total chlorophyll content of Basmati rice variety IR-8 (pots).

Table 1: Effect of leaf residues of weeds on Germination percentage, Seedling Vigour Index, root length, shoot length and dried biomass of Basmati Rice variety IR-8 (Petriplates).

Sr. No.	Weed	Extract	Conc.	G. %	SVI	R.L (cm)	S.L (cm)	D.B (mg)
1.	**Control**	—	—	100±0	8.17±0.74	12.63±2.97	8.17±0.90	8.20±1.48
2.	***C. album***	**Hot**	**50%**	90±10	5.50±0.89	2.07±0.59	6.10±0.87	5.32±0.56
			100%	50±17.3	0.87±0.13	0	1.80±0.26	0.12±0.03
		Cold	**50%**	100±0	6.87±0.49	4.90±2.08	6.87±0.60	5.73±0.59
			100%	96.7±5.8	5.91±0.76	1.13±0.06	6.17±1.36	3.42±0.35
3.	***S. nigrum***	**Hot**	**50%**	96.7±5.8	7.97±0.86	3.00±1.50	8.30±1.57	5.58±0.71
			100%	73.3±20.8	3.63±0.82	1.00±0.20	5.03±0.83	2.87±.064
		Cold	**50%**	93.3±5.8	10.85±1.00	5.87±0.23	11.7±1.95	6.00±1.28
			100%	83.3±20.8	6.31±0.67	3.17±2.04	7.93±2.30	4.33±0.55
4.	***C. sativa***	**Hot**	**50%**	100±0	7.40±0.62	8.00±1.68	7.33±0.75	6.00±0.72
			100%	100±0	6.80±0.71	3.47±0.96	6.80±0.87	4.67±1.02
		Cold	**50%**	100±0	5.87±0.47	4.97±1.55	5.87±0.58	5.73±1.05
			100%	100±0	5.83±0.34	2.57±1.40	5.83±0.42	4.47±0.98

Table 2: Effect of leaf residues of weeds on Germination percentage, Seedling Vigour Index, root length, shoot length and dried biomass of Basmati Rice variety IR-8 (Pots).

Sr. No.	Weed	Conc.	G. %	SVI	R.L (cm)	S.L (cm)	D.B (mg)
1.	**Control**	—	100 ± 0	24.4 ± 1.44	8.13±1.03	24.4±1.06	24.9±2.81
2.	***C. album***	**5g**	92.2 ± 6.94	17.3 ± 1.22	4.77±0.46	19.0±1.00	17.1±2.62
		10g	82.2 ± 10.2	16.5 ± 3.14	3.63±0.49	16.9±1.46	11.7±1.40
3.	***S. nigrum***	**5g**	93.3 ± 11.5	19.9 ± 1.88	5.53±0.51	21.7±1.50	18.7±1.61
		10g	86.5 ± 6.67	19.0 ± 2.65	4.63±1.43	21.3±2.04	17.3±1.18
4.	***C. sativa***	**5g**	100 ± 0	19.1 ± 1.63	3.90±0.26	18.3±2.39	15.2±3.83
		10g	94.4 ± 5.09	17.9 ± 1.11	2.87±0.95	21.5±1.61	15.7±0.64

Table 3: Effect of leaf residues of weeds on Leaf Surface Area of Basmati Rice variety IR-8 (Pots).

Sr. No.	Weed	Conc.	L.S.A (cm^2)
1.	**Control**	—	3.40±0.26
2.	***C. album***	**5g**	2.75±0.25
		10g	2.41±0.19
3.	***S. nigrum***	**5g**	3.42±0.28
		10g	3.12±0.12
4.	***C. sativa***	**5g**	2.85±0.26
		10g	3.10±0.20

CONCLUSIONS

To conclude, *Chenopodium album* inhibited the germination and growth of rice variety IR-8 the most followed by *Solanum nigrum.* These findings are significant as inhibition of germination and growth translates into decreased productivity. Hence, such weed-crop interactions need to be taken into consideration also. The results of petridish and pot experiments varied for *Cannabis sativa*. Thus, it is of vital importance to contradict any in vitro findings with subsequent greenhouse and field trials which take into account diverse natural conditions which plants face while growing without human interference. Furthermore, there is need to work on the allelopathic stress of such species under varied climatic and soil conditions and also to quantify the allelopathic compounds.

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