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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 *Amaranthus viridis, Lantana camara* and *Achyranthes aspera* 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 effects on seed germination characteristics, seedling length and dry weight of Basmati IR-8 variety of rice 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, dry weight, leaf surface area and chlorophyll content of basmati IR-8 variety of rice plant. The leaf extract of *Amaranthus viridis* had the most inhibitory effect on test plant followed by *Lantana camara*. Interestingly, the effects of *Achyranthes aspera* varied 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

Weeds are the undesired plants that interfere with human activities and compete with main crops for nutrients, moisture, space, light thus reducing the growth and yield and are toxic to the germination and growth of the plants. The notion of weeds being undesired was born when agriculture started (Jabeen *et al.,* 2013). 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.

Rice is an annual grass cultivated in tropical and sub-tropical regions. Rice is the only cereal crop plant on which most of the allelopathic effect has been seen. Khanh *et al.* (2008) found that paddy soil infested with barnyard exerted a strong inhibitory effect on growth of rice. Biswas *et al.* (2010) observed that the weed debris at different concentrations of Parthenium reduced the seed emergence, plant height, leaf number; leaf area and seedling dry weight of rice. Seedling emergence, plant height, leaf number, leaf area, and dry weight were reduced by 25.40%, 20.98%, 20.02%, 33.85% and 22.78% respectively. Among all the parameters considered, leaf area was most affected than other parameters. The inhibitory effects on rice were positively related to the concentration of Parthenium weed debris in soil. The leaf litter and roots of weeds possessphytotoxic compounds that produce inhibitory effects on germination and growth of agricultural crops (Norhafizah *et al.,* 2012). Weeds possess some special features that have made weeds dominant throughout the world (Holm *et al*., 1977), threatening the native vegetation (Lee and Klasing 2004). Weeds forming only one percent of land plants, cause severe damage (Singh *et al*., 2006). 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 term Allelopathy is derived from two Greek words – "Allelon" which means "of each other" and "pathos" which means "to suffer". According to the International Allelopathy Society (1996), allelopathy is defined as "Any process involving secondary metabolites produced by plants, algae, bacteria, viruses and fungi that influences the growth and development of agriculture and biological systems (excluding mammals), including positive and negative effects" (Torres *et al*., 1996; Kruse *et al.,* 2000). Weeds are rich source of allelochemicals that modify the environment of surrounding plants (Nandan *et al.,* 1994). The allelochemicals are present in different concentrations in different parts of the plants *viz*., leaves, flowers, fruits, roots, stems, rhizomes, seeds and even pollens (Kruse *et al.,* 2000; Bertin *et al.,* 2003; Gatti *et al.,* 2004). These can also be found in the surrounding soil. There are enough indications that allelochemicals released from certain weeds into the soil reduce crop growth (Rice, 1979; Weston and Putnam 1985). Allelopathic efficacy of weeds on germination and seedling growth of crops vary from weed to weed (Hamayun *et al.,* 2005). The allelopathic effects of different parts of same weed also differ for their effects on germination and initial growth of plants (Veenapani, 2004; Aziz *et al.,* 2008). Allelopathic interactions between plants have been studied in both man-made and natural ecosystems. In agricultural systems allelopathy can be a part of interference between crops and between crops and weeds that affect the economic outcome of plant production. Both crop and weed species with allelopathic activity are known (Inderjit and Dakshini 1998; Inderjit and Foy 1999).

MATERIALS AND METHODS

To achieve 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 (*Amaranthus viridis* L., *Achyranthes aspera* L. and *Lantana camara* 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, 2022. Each experiment was laid out in a completely randomized block experimental design (RBD) with three replications. The study was focussed on seed germination characteristics, seedling growth, leaf surface area and chlorophyll content of rice.

For in vitro experiments, 100 g of weed leaves 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% concentration 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 ration 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

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:

$$\text{Inhibitory percentage} = 100 - \left(\frac{\text{Final germination\% in treatment}}{\text{Final germination\% in control}} \times 100\right)$$

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:

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

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

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

RESULTS AND DISCUSSION

From the experimental procedure it is evident that the weeds (*Amaranthus viridis, Lantana camara and Achyranthes aspera*) affected the various physiological parameters of Basmati IR-8, albeit to various degrees. It was found that *A. viridis* had the most inhibitory effects on various physiological parameters of Basmati in general, followed by *L. camara.* Interestingly, the effect of *A. aspera* was different in petriplates and test pots.

Amaranthus viridis. *A. viridis* species are among the worst weeds in the world competing with cereals and vegetables and produce great yield losses (Holm *et al.,* 1977; Kolar *et al*., 1980; Conn *et al*., 1987; Qasem, 1992a, b). Many workers have reported phytotoxicity of A. viridis spp. residues on several crop species including sorghum (*Sorghum bicolor*), cabbage (*Brassica oleracea*), carrot (*Daucus carota*) and onion (*Allium cepa*) (Menges, 1988).

In the lab study, the germination percentage of rice was reduced to 63.3% in case of *A. viridis*. Parameters such as GVI and SVI were also found to be highly influenced by the presence of the weed. The root and shoot length, leaf surface area and dried biomass of rice seedlings were also negatively influenced by the allelopathic effects of *A. viridis.* In fact, in hot water leachate treatments of *A. viridis* growth of test seedlings was strongly inhibited. Rahimzadeh *et al.* (2012) observed reduced germination percentage and seedling growth of linseed under the influence of goose foot (*C. album*), red-root amaranth (*A. retroflexus*) and field bindweed (*Convolvulus arvensis*). Mlakar *et al.* (2012) found that there was reduction in germination percentage and root length of garden cress in petriplates when treated with *A. viridis* leaves.

Chlorophyll a, chlorophyll b and total chlorophyll content in the rice leaves were highly inhibited by *A. viridis*. Interestingly, lower dose of the weed was found to be promotive while higher dose was found to be highly inhibitive. Research indicates that leaves and debris leaves, roots, pollen and flowers, stem of *A. viridis* have detrimental effects on germination and growth of different crop species (Tabrizi and Yarnia 2011). *A. retroflexus* reduces seed germination and initial growth of wheat cultivars (Shahrokhi *et al*., 2012). Sultana *et al.* (2012) reported that *A. viridis* dried and fresh plant extracts inhibited germination rate and root/shoot growth of seedlings of canola and rye grass in petriplates and the phytotoxicity or allelopathy of *A. viridis* extracts was linearly correlated with its concentrations. The workers further concluded that these phytotoxic chemicals or allelochemicals could be used as a potential natural herbicide resource but they must first be identified and their mode of action studies.

Lantana camara. *L. camara* 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 *A. viridis*. 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.

Rice seedlings showed reduction in growth (root and shoot length, biomass) on treatment with *L. camara*. Higher conc. of the weed was more effective in inhibiting growth. The leaf surface area of the test seedlings was also influenced by the presence of the weed. Higher dose was inhibitory while lower dose was actually promotive. The opposite was true for *L. camara* in green house as higher dose promoted shoot length and leaf surface area. The different parts of L. camara contain allelochemicals mainly aromatic alkaloids and phenolic compounds (Ambika *et al*., 2003) which can interfere with seed germination and early growth of many plant species (Sharma *et al*., 2005; Ahmed *et al.,* 2007). *L. camara* can also interfere growth of nearby plants by outcompeting for soil nutrients (Dobhal *et al*., 2010) and altering microenvironment (e.g. Light, temperature) by forming dense thickets (Sharma and Raghubanshi 2007). The weed is recognized as among the worst invasive alien species in the world (Baars and Neser 1999; Sharma *et al*., 2005; Zalucki *et al.,* 2007).

L. camara inhibited chlorophyll a, Chlorophyll b and total chlorophyll content in rice leaves. The effect was however of lower degree as compared to *A. viridis*. It was found that lower concentration of *L. camara* was found to be promotive while higher conc. was found to be inhibitive.

Hossain and Alam (2010) reported that *L. camara* leaf extract produced strong inhibitory effects in petriplates on both selected agricultural crops - *Oryza sativa, Triticum aestivum, Vigna sinensis, Cucurbita pepo, Abelmoschus esculentus, A. tricolor* and forest crops - *Acacia auriculiformis,* and *Albizia procera.* Adverse effects on germination, root and shoot elongation of *Trianthema portulacastrum* in petriplates have also been documented (Jawahar *et al.,* 2010). The study showed that *L. camara* aqueous extract contain inhibitors probably allelochemicals for germination and growth of *T. portulacastrum*. They suggested that further field investigations are needed with graded levels of *L. camara* on weed management in food crops, either in the form of compost or as green leaf manure (Jawahar *et al.,* 2010). The aqueous leaf extract of *L. camara* leaves had inhibitory effects on shoot and root elongation, leaf surface area and fresh and dry weight of *P. hysterophorus* (Mishra, 2014).

Achyranthes aspera. In the case of *A. aspera* 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 these extracts was different under laboratory and greenhouse conditions. In petriplates, *A. aspera* was found to inhibit germination percentage, GVI, root length, dried biomass but not SVI and shoot length. However, in the greenhouse study, it was found to be least effective of all weeds under investigation as it produced zero effect on all these parameters. The leaf surface area and chlorophyll (a, b, total) were least inhibited by *A. aspera*. Lower dose of *A. aspera* leaf residues in these three parameters were promotive while higher conc. were inhibitive.

While investigating the allelopathic influence of *L. camara* and *A. aspera* on the germination and growth behavior of *Oryza sativa* in laboratory conditions, Sharma and Satsangi (2012) demonstrated that different concentrations (25%, 50%, 75% and 100%) of leaf extracts of *A. aspera* and *L. camara* caused significant inhibitory effect on germination, root and shoot elongation of paddy seeds. The germination percentage decreased with increase in extract concentrations. It was concluded that *A. aspera* showed more inhibitory effect in comparison to *L. camara*. However, in the present findings, *L. camara* was categorized above *A. aspera* based on the overall findings.

Tadele (2014) reported that *L. camara* leaf extracts produce stimulatory effects on early growth of maize and finger millet and inhibitory effects on teff growth under laboratory conditions. Growing teff however, may not be promising in areas where *L. camara* invasion occurs due to allelopathic interference though the allelochemicals causing reduction in growth of teff may not cause the same effect in the field since the concentration of these substances is probably greater in aqueous extracts than under natural conditions in the field (Rice, 1984) or they may be bound and made unavailable by soil particles (Dalton *et al.*, 1983) or decay may reduce the allelopathic effects of leaf litter (May and Ash 1990). Hussain *et al.* (2011) also showed strong allelopathic effects of aqueous extracts of all parts of *L. camara* on the test species while the soil collected underneath *L. camara* thickets had no allelopathic effects. The result coincides with findings of Sahid and Sugau (1993); Achhireddy *et al.* (1985) who reported that *L. camara* affected soil did not influence the crop.

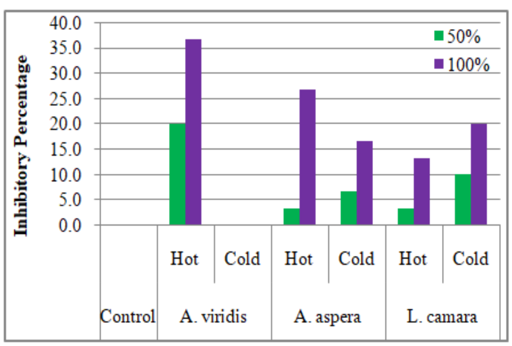

Fig. 1. Effect of aqueous extracts of weeds on inhibitory percentage of Basmati rice variety IR-8 (petriplates).

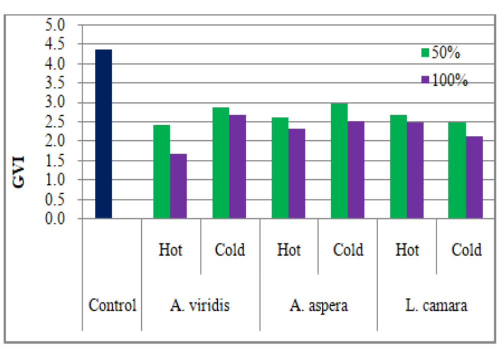

Fig. 2. Effect of aqueous extracts of weeds on Germination Velocity Index of Basmati rice variety IR-8 (petriplates).

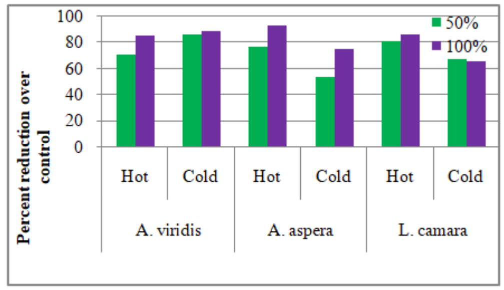

Fig. 3. Percentage reduction in root length of Basmati rice variety IR-8 due to aqueous extracts of weeds (petriplates).

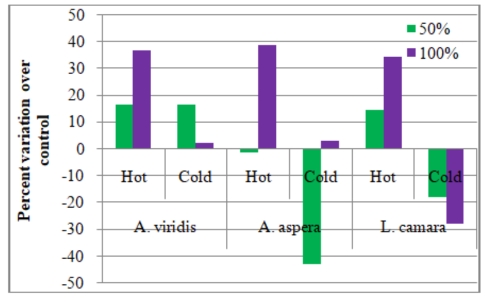

Fig. 4. Percentage variation in shoot length of Basmati rice variety IR-8 due to aqueous extracts of weeds (petriplates).

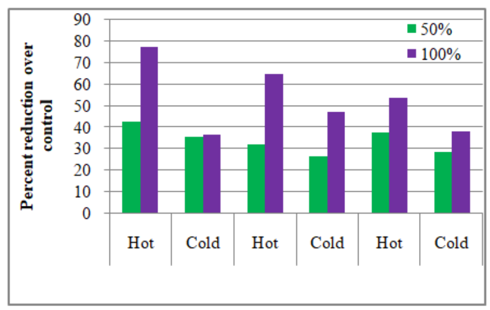

Fig. 5. Percentage reduction in biomass of Basmati rice variety IR-8 due to aqueous extracts of weeds (petriplates).

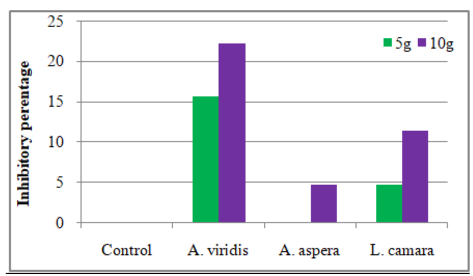

Fig. 6. Effect of dried leaf residues of weeds on Inhibitory percentage of Basmati rice variety IR-8 (pots).

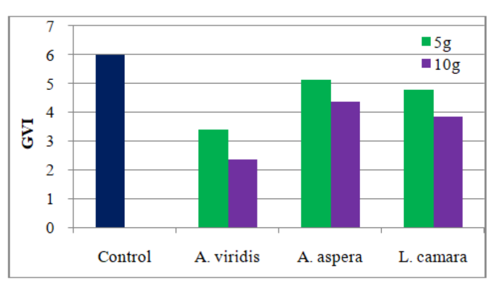

Fig. 7. Effect of dried leaf residues of weeds on Germination Velocity Index of Basmati rice variety IR-8. (pots).

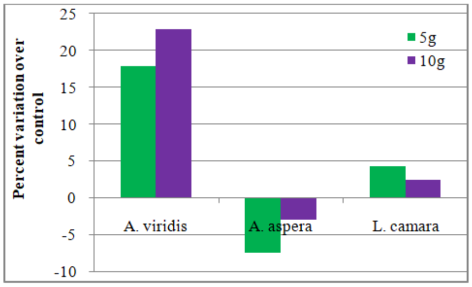

Fig. 8. Percentage reduction in Leaf Surface Area of Basmati rice variety IR-8 due to dried leaf residues of weeds (pots).

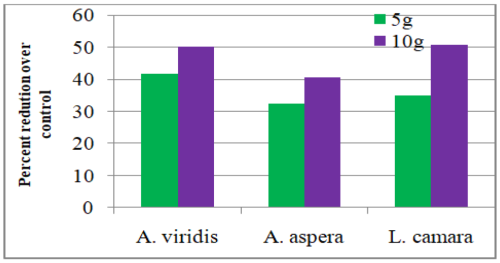

Fig. 9. Percentage reduction in root length of Basmati rice variety IR-8 due to dried leaf residues of weeds (pots).

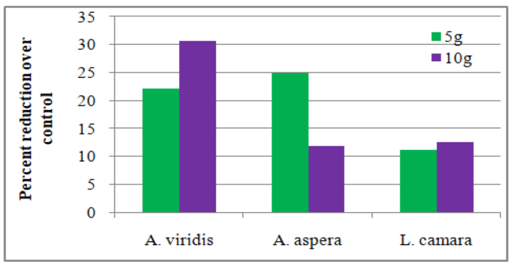

Fig. 10. Percentage reduction in shoot length of Basmati rice variety IR-8 due to dried leaf residues of weeds (pots).

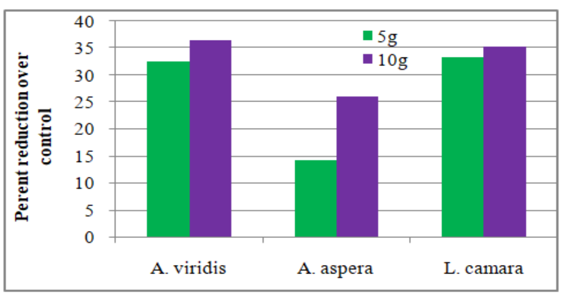

Fig. 11. Percentage reduction in biomass of Basmati rice variety IR-8 due to dried leaf residues of weeds (pots).

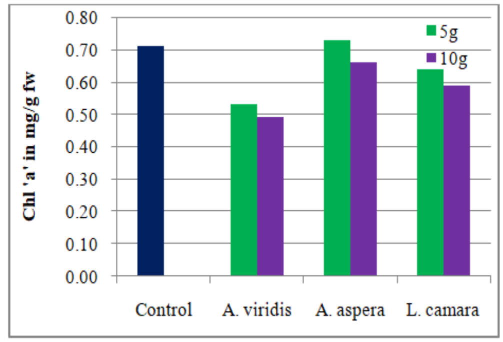

Fig. 12. Effect of dried leaf residues of weeds on chl 'a' Content of Basmati rice variety IR-8 (pots).

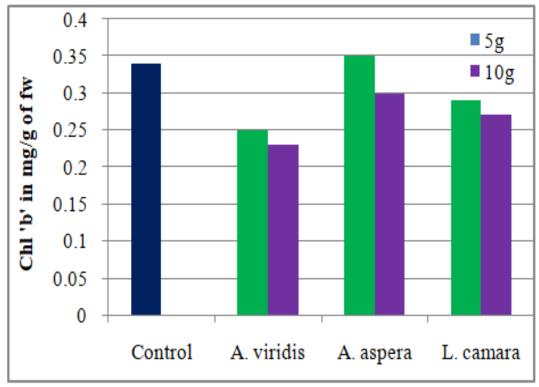

Fig. 13. Effect of dried leaf residues of weeds on chl 'b' content of Basmati rice variety IR-8 (pots).

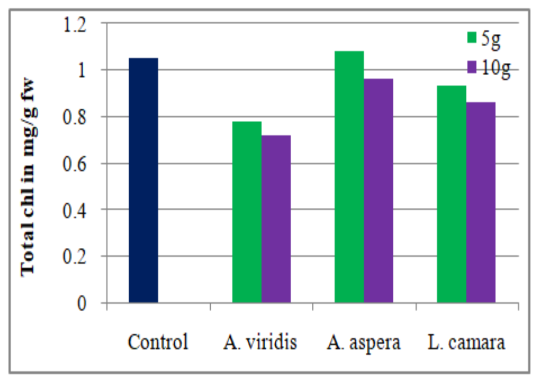

Fig. 14. Effect of dried leaf residues of weeds ontotal Chlorophyll content of Basmati rice varietyIR-8 (pots).

Table 1: Effect of aqueous extracts of weeds on Germination, Seedling Vigour Index and growth 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.	*A. viridis*	Hot	**50%**	80±17.3	5.45±1.56	3.70±0.72	6.83±1.76	4.72±0.71
			100%	63.3±15.3	3.42±0.85	1.93±1.36	5.20±0.95	1.83±0.60
		Cold	**50%**	100±0	8.00±0.41	1.83±0.29	6.83±0.29	5.27±0.40
			100%	100±0	6.83±0.24	1.50±0.50	8.00±0.50	5.18±0.68
3.	*A. aspera*	Hot	**50%**	93.3±11.5	8.53±1.05	3.90±0.62	9.17±1.05	5.50±0.52
			100%	83.3±5.8	5.01±0.23	1.80±0.53	6.00±1.22	5.43±1.72
		Cold	**50%**	93.3±5.8	8.81±1.79	7.83±2.08	9.37±1.72	8.13±0.90
			100%	90±17.3	5.39±0.86	5.07±2.14	6.07±0.50	5.10±0.14
4.	*L. camara*	Hot	**50%**	96.7±5.8	6.72±0.69	2.50±0.50	7.00±1.32	5.10±1.17
			100%	86.7±11.5	4.67±0.68	1.83±0.29	5.37±0.32	3.80±0.85
		Cold	**50%**	90±10	8.70±0.92	4.17±0.76	9.67±0.58	5.83±1.03
			100%	80±10	8.46±1.70	4.43±2.24	10.5±1.38	5.07±0.90

Table 2: Effect of leaf residues of weeds on Germination, Seedling Vigour Index and growth 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.	*A. viridis*	5g	84.4± 1.4	19.0± .86	4.73±0.50	23.7±1.58	16.8±3.11
		10g	77.8 ± 3.9	18.4± .85	4.07±0.15	23±0.58	15.8±3.15
3.	*A. aspera*	5g	100 ± 0	22.7± .37	5.50±1.32	23.7±5.52	21.3±2.14
		10g	95.4 ± 8.0	21.3±3.21	4.83±0.31	21.7±2.55	18.4±2.76
4.	*L. camara*	5g	95.4 ± 8.0	21.5± .29	5.30±0.26	19.3±0.19	16.6±0.40
		10g	88.7 ± 2.2	17.0± .32	4.00±0.10	22.7±0.51	16.1±1.05

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

Sr. No.	Weed	Conc.	LSA (cm2)
1.	**Control**	–	3.40 ± 0.26
2.	*A. viridis*	5g	2.79 ± 0.29
		10g	2.62 ± 0.32
3.	*A. aspera*	5g	3.65 ± 0.35
		10g	3.50 ± 0.50
4.	*L. camara*	5g	3.25 ± 0.25
		10g	3.32 ± 0.20

CONCLUSIONS

Amaranthus viridis inhibited the germination and growth of rice variety IR-8 the most followed by *Lantana camara.* 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 *Achyranthes aspera*.

Thus, it is of vital importance to corroborate any in vitro findings with the 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 further 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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