"Allelopathic effects of *Argemone mexicana* L. on physiology and growth of wheat"

Research Project submitted to

Department of Botany, Vivekanand College, Kolhapur (Autonomous)

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Submitted by

Miss. Shravani P. Aswale and Mr. Swapnil A. Khamkar

Under the guidance of

Miss. Pradnya B. Shelar
M.Sc., MH-SET

Assistant Professor

Department of Botany, Vivekanand College, Kolhapur(Autonomous)

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DEPARTMENT OF BOTANY

VIVEKANAND COLLEGE, KOLHAPUR (AUTONOMOUS)

CERTIFICATE

This is to certify that the dissertation entitled "**Allelopathic effects of *Argemone mexicana* L. on physiology and growth of wheat"**submitted by **Miss Asawale Ashwini Popatrao and Khamkar Swapnil Anil** B.Sc. Botany in partial fulfillment for the award ofdegree Bachelor of Science in Botany is a bonafide record of the research work carried out by the under the supervision and guidance of **Miss. Pradnya B. Shelar,** Department of Botany, Vivekanand College, Kolhapur(Autonomous).

Head
Department of Botany

Head
Department of Botany
Vivekanand College
Kolhapur

KOLHAPUR

DECLARATION

I hereby declare that the project work entitled, **"Allelopathic effects of *Argemone mexicana* L. on physiology and growth of wheat''** submitted by **Miss Ashwini P. Aswale and Mr. Swapnil A. Khamkar** of B.Sc. Botany in partial fulfillment for the award of degree Bachelor of Science in Botany, Vivekanand College, Kolhapur (Autonomous). This work represents bonafide record of the research work carried out by students under the supervision and guidance of **Miss. Pradnya B. Shelar**, Assistant Professor, Department of Botany, Vivekanand College, Kolhapur (Autonomous).

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CONTENTS

INTRODUCTION

Allelopathy is a biological phenomenon in which plant synthesizes one or more allelochemicals that influence the germination, growth, metabolism, development, survival and reproduction of other organisms (Hossain *et al.,* 2012). Allelochemicals have beneficial or harmful effects on the target organisms and community (Mali and Kanade, 2014). Weeds can also affect a crop's growth by releasing allelochemicals into the growing environment (Edrisi and Farahbakhsh, 2011).Many workers reported beneficial as well as harmful allelopathic effects of weeds and plants on seed germination (Khan *et al*., 2008 and Ghodake *et al*., 2012), root-shoot growth (Dessalegne *et al*., 2013; Mali and Kanade, 2014), fresh and dry weight of seedlings (Hossain *et al*., 2012 and Mujawar *et al*., 2016), photosynthetic pigments (Sarkar *et al*., 2012 and Salgude *et al*., 2015), total proteins (Verma and Rao, 2006), enzyme activities (Muhammad *et al*., 2013), carbohydrates (Gulzar and Siddiqui, 2014), phenolic compounds (Iannucci *et al*., 2012), mineral contents, phytohormones, anthocyanin and amino acids (Balah and Latif, 2013), yield (Hossain *et al*., 2012), herbicidal potential (Marzieh *et al*., 2013), antibacterial and antioxidant properties (Milena *et al*., 2012), seed-borne mycoflora (Shafique *et al*., 2007), soil microbial populations (Souto *et al*., 2001) and soil pH (Wang *et al*., 2009).

Wheat is important winter crop in Baramati area of Pune district of Maharashtra and *Argemone mexicana* L. is found as a common weed in wheat fields. The objectives of this study is to determine the allelopathic effect of root, stem and leaves leachates of *A. mexicana* on seed germination, root-shoot length, biomass, chlorophyll and protein content in wheat (*Triticum aestivum*L.) seedlings.

CHAPTER - I

Review of Literature

1. Systematic position of Wheat:

Kingdom: Plantae

Division: Angiosperms

Class: Monocotyledonae

Order: Poales

Family: Poaceae

Genus: *Triticum*

Species: *aestivum* L.

Wheat (*Triticum aestivum* L.) is the first most important and strategic cereal crop for the majority of world population. It is the most importantstaple food about two million people. It is important source of carbohydrates. It is the leading source of vegetal protein in human food having a protein content of about 13%, which is relatively high compared to other major cereals. Wheat is a source of multiple nutrients and dietary fiber.

2. Systematic position of *Argemone mexicana* L.:

Kingdom: Plantae

Division: Angiosperms

Class : Dicotyledonae

Order : Papaverales

Family: Papaveraceae

Genus: *Argemone*

Species: *mexicana* L.

***Argemone mexicana* L.**

Argemone mexicana L. is a principle weed of cereals *A. mexicana* has an inhibitory effect on germination and seedling growth of vegetables and weed residues may affect. Grazing animals generally avoid this weed but can be poisoned if it is consumed in hay or chaff. *A. mexicana* has had a major impact on human health in the Indian subcontinent. Edible vegetable oil either accidently contaminated with *A. mexicana* or intentionally adultered by unscrupulous traders has resulted in economic dropsy.

2. Origin of the research problem:

Many crop and weed species have been observed to have allelopathic properties. Over 240 weed species have been reported to be allelopathic to other nearby plants of the same species (autotoxicity) or other crop and weed species. The use of Allelopathy to favor the crop over weeds has been investigated in three aspects

:i) as an allelopathic winter cover crop that suppresses weeds prior to the cropping season, ii) as a living mulch during the cropping season to reduce weed interference and iii) as an isolated compound from an allelopathic plant, applied as an herbicide (Colquhoun,2006)

3. Interdisciplinary relevance of the topic:

Allelopathy can affect many aspects of plant ecology, including occurrence, growth, plant succession, the structure of plant communities, dominance, diversity and plant productivity. Initially many of the forestry species evaluated had negative allelopathic effects on food and fodder crops, but in the 1980s research was beganto identify species that had beneficial, neutral or selective effects on companion crop plants.

Commonly cited effects of allelopathy include reduce seed germination and seedling growth. Like synthetic herbicides, there is no common mode of action or physiological target site for all allelochemicals. However, known sites of action for some allelochemicals include cell division, pollen germination, nutrient uptake, photosynthesis and specific enzyme function (Ferguson*et al.*, 2013).

4. International Status of the topic:

Lot of study carried out on allelopathy on cereal plants emphasizes useful as well as harmful effects. Stimulation in radical growth of kidney bean by treatment of 1 ppm concentration of mimosine was reported by Towata and Hongo (1987). Favouring results in seed germination of *Solanum* and *Capsicum* by allelopathic effect of leachates of *Parthenium* was reported by Dhawan*et al.*(1998).

Allelopathic effects of aqueous extracts of *Amaranthus hybridus, Parthenium hysterophorous, Datura stramonium* and *Argemone mexicana* leaf, stem and root on germination and growth of wheat studied by Gella *et al.*(2013) and observed that, all weed extracts markedly reduce the germination. In addition, leaves extract of *A. hybridus*, *P. hysterophorus*, weed species gave the highest negative effect on radical and plumule elongation of the seedlings. They advised to farmers that, farmers shall be give species attention in avoiding or minimizing those weed species from farm to contain their adverse effects on the crop.

Study of allelopathic effects of sorghum water extract on seed germination and seedling growth of *Trianthema portulacastrum* (one of the serious weeds of cotton and maize crop in Pakistan) carried out by Randhawa*et al*.,(2002). They concluded that, sorghum water extract at higher concentration (100%) reduced the germination of *T. portulacastrum* by 15 to 20% Root and shoot length of *T. portulacastrum* was also significantly suppressed at higher concentrations (i.e.75 and 100%) of sorghum water extract. While lower concentration (25%) of the extract promoted shoot length.

5. National Status of the topic:

The effect of aqueous extract of *Portulaca oleracea* L. on *sorghum vulgare*Pers. Was studied by Dhole *et al.* (2011) and found stimulatory effect towards seed germination, root-shoot length and seedling growth. Mubeen*et al*. (2012) reported stimulatory effect on seed germination and seedling growth of rice by the allelochemicals of jowar and sunflower. Dhawan *et al*.(1998) reported the effect of leachates of *Parthenium* on stimulation of seed germination of *Solanum* and *Capsicum.* Oudhia and Tripathi (1999) studied the effect of aqueous extracts of root, stem and leaf tissues of *Lantana camera on* rice seeds and reported greatest seed germination.

On the contrary, much work emphasizes on negative effect of allelopathic chemicals on seed germination and seedling growth. Nasira Jabeen and Moinuddin Ahmed (2009) observed the effect of *Asphodelus tenuifolius* and *Fumaria Indica* on maize seeds and reported inhibition of germination.100% concentration of root extract of *Euphorbia thiamifolia* inhibited seed germination by 31% in *Vigna uriculata* and 18% in *Vigna radiate* noticed by Dabgar and Kumbhar (2010).

6. Significance of the study in the context of current status:

The basic approach used in allelopathic research for agricultural crops has been to screen both crop plants and natural vegetation for their capacity to suppress weeds. To demonstrate allelopathy, plant origin, production of identification of allelochemicals must be established as well as persistence in the environment over time in concentrations sufficient to affect plant species. In the laboratory, plant extracts and leachates are commonly screened for their effects on seed germination, with further isolation and identification of allelochemicals from greenhouse tests and field soil confirming laboratory results. Interaction among allelopathic plants, host crops and other non-target organisms must be considered. Furthermore, allelochemistry may provide basic structures or templates for developing new synthetic herbicides.

CHAPTER - II

Material and Methods

1. Procurement of wheat seeds:

The wheat seeds are brought from the Mahalakshmi Agro Seeds, Baramati.

2. Procurement of *Argemone mexicana* L. plant material:

Fresh and healthy *A. mexicana* L. plant material was collected from wheat fields of Tandulwadi area of Baramati.

3. Preparation of root, stem and leaves leachates of *A. mexicana* and wheat seed treatment:

A. mexicana plant parts were separated into leaves, stems and roots. Leachates were prepared by soaking 10gm fresh plant material pieces into 100ml distilled water for 24 hrs. Then it was filtered through Whatman No.1 filter paper. These solutions were treated as stock solutions and 5, 10, 15,20% concentrations of all stock solutions were prepared for treatments. 15 wheat seeds were kept in sterile petriplates over filter paper at room temperature and treated with 5, 10, 15,20% concentrations of all leachates separately and control were made by using distilled water. The treatment solutions were supplied to the seedlings as and when required. The seed germination was observed after 24 hours of seed germination, root-shoot length was measured upto 96 hours, chlorophyll contents were measured from 144 hours old seedlings by the method of Arnon (1949) and protein was estimated from 192 hours old seedlings (Lowry *et al*., 1951).

4. Estimation of Chlorophyll (Arnon, 1949):

Procedure:

1 gm of wheat seedlings (Leaves along with green shoot) were homogenized in pre-chilled mortar with the 10-20ml chilled 80% acetone. While crushing added a pinch of magnesium carbonate to neutralize liberated acids. After this, extract was filtered through Buchner funnel using Whatman No. 1 filter paper. Residue washed 2-3 times with 5-10 ml 80% acetone and collected the filtrate in 100ml volumetric flask. Total volume of extract was made 100ml using 80% acetone and stored in conical

flask well protected from light (wrapped with black carbon paper). Absorbance of solution was read at 645 and 663 nm wavelengths.

Calculation :

Calculated the amount of chlorophyll as mg chlorophyll per g fresh tissue using the following equations :

mg chlorophyll a/g fresh tissue = $12.7(A_{663}) - 2.69\,(A_{645}) \times \dfrac{V}{1000 \times W}$

mg chlorophyll b/g fresh tissue = $22.9(A_{645}) - 4.68\,(A_{663}) \times \dfrac{V}{1000 \times W}$

mg total chlorophyll /g fresh tissue = $20.2(A_{645}) + 8.02\,(A_{663}) \times \dfrac{V}{1000 \times W}$

Where,

A = absorbance at specific wavelengths.

V = Final volume of chlorophyll extract in 80% acetone.

W = Fresh weight of tissue extracted.

5. Protein Estimation (Lowry *et al.*, 1951):

Preparation of reagents :

1) **Reagent A :** 2% Na_2CO_3 in 0.1 N NaOH (Dissolve 400 mg of NaOH in 90 ml distilled water. Make final volume 100 ml and to this add 2 gm Sodium carbonate).
2) **Reagent B :** 0.5% $CuSO_4$, $2H_2O$ in 1% sodium potassium tartarate (Dissolve 1 gm of sodium potassium tartarate and 50 mg of $CuSO_4$ in 100 ml distilled water).
3) **Reagent C :** Mixed 49 ml of reagent (A) and 1 ml of reagent (B) just before use.
4) **Reagent D :**Folin-ciocalteau reagent 1 part + distilled water 1 part.

5) **Standard protein solution :** Dissolved 10mg of bovin serum albumin in 1 ml of 0.1N NaOH and make final volume 10ml with distilled water. Thus in this stock solution, the standard protein is 1 mg/ml.

Procedure :

Preparation of plant extract :

0.5g of wheat seedlings (Leaves along with green shoot) were homogenized with mortar and pestle in 10ml Reagent - A. Collected the crude extract in centrifuge tube and centrifuged at 5000rpm for 10min. Collected the supernatant in test tube and store in ice bath. This was served as protein source from crude plant extract.

Assay :

0.2, 0.4, 0.6, 0.8 and 1ml standard protein solutions were taken in five test tubes and 0.5ml plant extract in separate test tube. To this added the volume of distilled water in such a way that final volume has become 1ml. Prepared 1 blank with 1 ml distilled water.To these test tubes added 5ml of fresh reagent C including blank. Mixed well and allowed to stand for 10min.Quickly added 1ml Folin-Ciocalteau reagent (Reagent - D). Shake well and allowed to stand for 10min. Absorbance for standard protein as well as plant extract was read at 660nm wavelength with using prepared blank.Plotted the graph of concentrations of standard proteins against absorbance at 660nm with the reading of plant extract using standard curve.

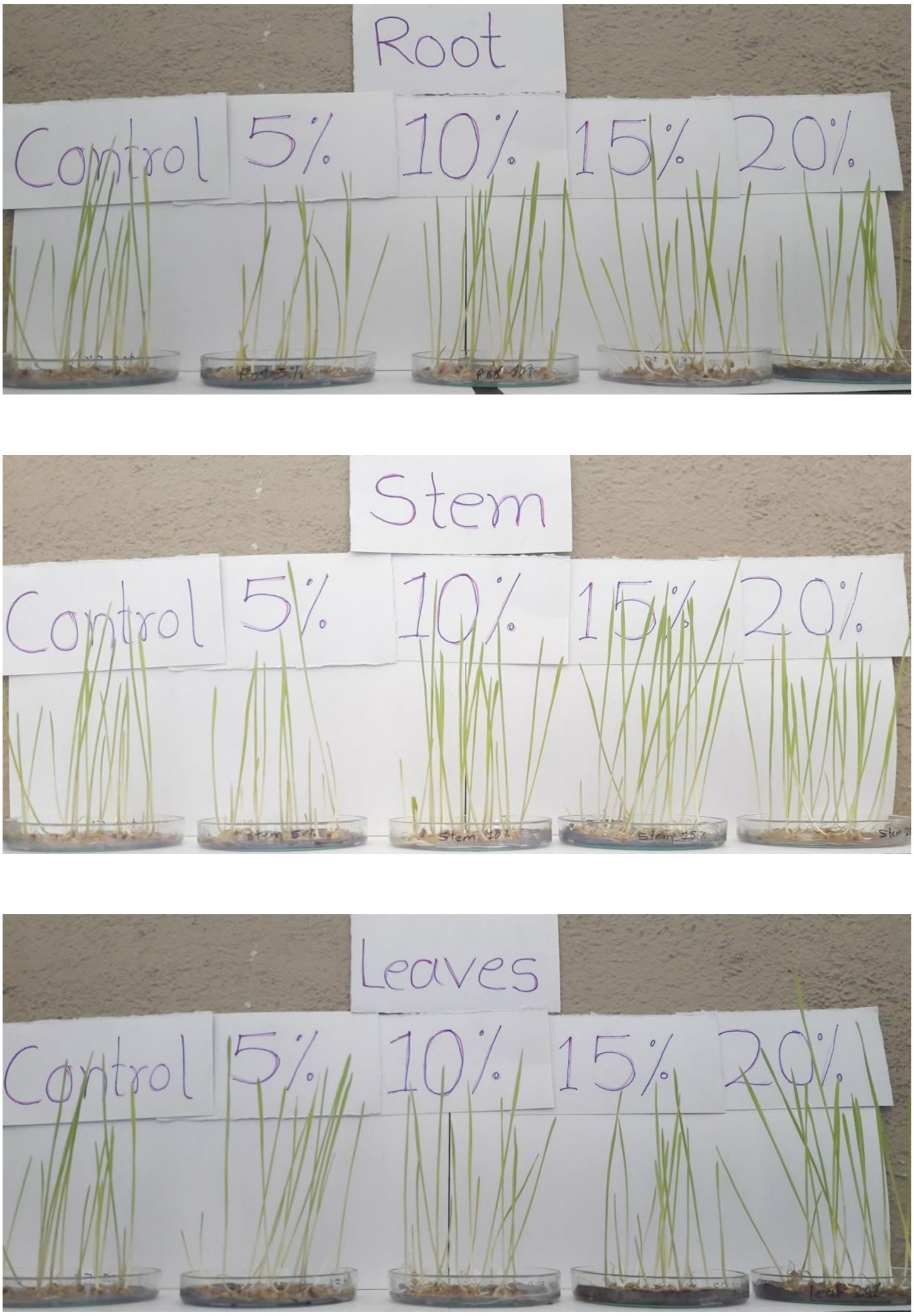


Plate -1: Root leachate, Stem leachate and Leaves leachate treatments.

CHAPTER - III

Results and Discussion

Effect of *A. mexicana* root, stem and leaves leachates showed favourable response to seed germination of wheat. It is interesting to note that lower concentration (5%) of all treatments noticed 100% seed germination. Along with seed germination, the root and shoot length also increased favourably. Maximum root length (5.6cm) observed in 10% stem leachates next to 15% root leachates (4.9cm) and 20% leaves leachates (4.6cm). The shoot length was highest in 15% root and 5% leaves leachates (6.2cm) then 5% stem leachates i.e. 5.3cm. General observation found that highest concentration (20%) of root, stem and leaves leachates recorded decreased trend of shoot length. The fresh biomass was reduced at higher concentrations (20%) of stem and leaves leachate treatments on the other hand 20% root and 5% of leaves leachates appreciably stimulated the biomass of seedlings compared to control. Stem leachates could not respond for weight gaining of seedlings (Table-1).

The photosynthetic pigments chl.a, chl.b and total chlorophylls were studied after 144 hours old seedlings. Usually photosynthetic pigments were stimulated in lower concentration of treatments i.e. 5 and 10% of all leachates. On the contrary they were inhibited at higher concentrations (15 and 20%) of root and stem leachates. It is interesting to note that higher concentration of leaves leachates promisingly enhance the chlorophyll contents (Table-2). Allelopathic impact of all leachate concentrations noticeably raised protein content of wheat seedlings compared to control. The maximum amount of protein was reported from 20% concentration of root leachates (Table-3).

Effect of aqueous extract and powder of lemon balm (*Melissa officinalis*) on the germination and seedlings growth of wheat, pea and safflower were investigated by Pour and Farahbakhsh (2012) and they reported inhibition in wheat and pea seed germination noticeably and found stimulatory effect on safflower germination. Mali and Kanade (2014) studied effect of *Alternanthera sessilis.* [L] R.Br. and *Cynadon dactylon*[L]. Pers. on seed germination of jowar and found stimulatory effect on seed germination as compared to control. According to Ghodake *et al*. (2012) aqueous extracts of *Euphorbia geniculata* Ort., *E. hirta* L. and *E. microphylla* Heyne exhibited inhibition in wheat seed germination in initial stage and later on it was stimulatory. Khan *et al*. (2008) noticed that aqueous extracts of *Eucalyptus camaldulensis* L. at a concentration of 10, 15 and 20% had inhibitory effect on wheat seed germination and effect was found significantly higher than control treatment. Effect of aqueous extracts of *Ammi majas*(Khella), *Guiera senegalensis* (Globefish) and *Salix* spp. (Safsaf) reduce total germination percentage of *Sorghum bicolor* L. and botanical extracts exhibited extra inhibitory effects on radicle emergence than on plumule growth were studied by Hassan *et al*., (2012). Seyed *et al*. (2011) investigated effect of *Artemisia annua* aqueous extracts on germination of *Plantago ovate* (Isabgol) and noticed a significant decreasing effect on germination percentage. *Parthenium hysterophorus* and *Amaranthus hybridus* leaf and stem extracts consistently causes reduction in seed germination percent of wheat and highest negative effect on radicle and plumule elongation of seedlings were reported by Dessalegne*et al*. (2013). Dhole *et al.* (2013) worked on effect of aqueous and ethanolic extract of some common 10 weeds *viz. Alternanthera sessilis* (L.) R.Br.ex Dc, *Amaranthus tricolor* L., *Cardiospermum helicacabum* L., *Corchorus olitorus* L., *Euphorbia hirta* L., *Phyllanthus amerus* Schumach. And Thann, *Portulaca oleraceae* L., *Vicoaindica* L. (Dc.) on seed germination and seedling growth of maize and noticed stimulatory effect on seed germination, seedling emergence and root and shoot length. Effect of *Moringa oleifera* on growth and productivity of mungbean investigated by Hossain *et al.* (2012) and reported that the different concentrations of root extracts have negative effect on the biomass performance.

Muhammad *et al*. (2013) investigated that, genetically modified maize aqueous extracts decreased chlorophyll-a, increased chlorophyll-b and significantly increase in protein and sugar content in wheat. Effect of powders of *Crocus sativus, Ricinus communis, Nicotiana tobaccum, Datura inoxia* and *Nerium* influenced on the dry weight, height, leaf area as well as chlorophyll and carotenoid contents of *Sorghum vulgare* (Marzieh *et al*., 2013). Sarkar *et al*. (2012) studied effect of *Cassia tora* aqueous extracts on the chlorophyll content of mustard and determined extracts causes reduction in the chlorophyll content. The effect of aqueous leaf leachates and organic fractions of *Eclipta alba* (L.) Hassk reduced chlorophyll and protein content of *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L. were studied by Gulzar and Siddiqui (2014). Verma and Rao (2006) investigated that effect of

Ageratum conzoides L., *Cynadon dactylon* (L.) Pers., *Parthenium hysterophorous* L., and *Solanum nigrum* L. aqueous extracts increased growth and protein content of *Glycine max* (L.) Merrill.

Table -1: Effect of *Argemone mexicana* L. root, stem and leaves leachates on seed germination, root-shoot length and biomass of fresh wheat seedlings

Time (after germination)	Part used	Treatments				
		Control	5%	10%	15%	20%
Germination percentage (%)						
24hrs	Root	99	100	100	100	100
	Stem	99	100	99	99	99
	Leaf	99	100	100	100	99
Root length (cm)						
72hrs	Root	3.64	3.9	4.0	4.0	4.3
	Stem	3.6	4.4	3.6	3.5	3.0
	Leaf	3.6	4.2	3.9	3.8	4.1
96hrs	Root	4.1	4.4	4.6	4.9	4.5
	Stem	4.0	4.5	5.6	5.5	4.6
	Leaf	4.0	4.4	4.4	4.5	4.6
Shoot length (cm)						
72hrs	Root	3.82	4.0	4.5	4.8	4.4
	Stem	4.2	3.8	3.6	3.5	3.0
	Leaf	3.5	3.8	3.8	3.8	3.6
96hrs	Root	5.4	6.1	6.1	6.2	5.7
	Stem	5.0	5.3	4.3	4.2	3.2
	Leaf	4.9	6.2	5.8	5.5	5.5
Biomass of fresh seedlings (gm)						
144hrs	Root	1.22	1.10	1.12	1.35	1.39
	Stem	1.22	1.22	1.17	1.10	1.10
	Leaf	1.22	1.42	1.34	1.11	1.10

Figure-1: Effect of *Argemone mexicana* L. root, stem and leaves leachates on seed germination of fresh wheat seedlings

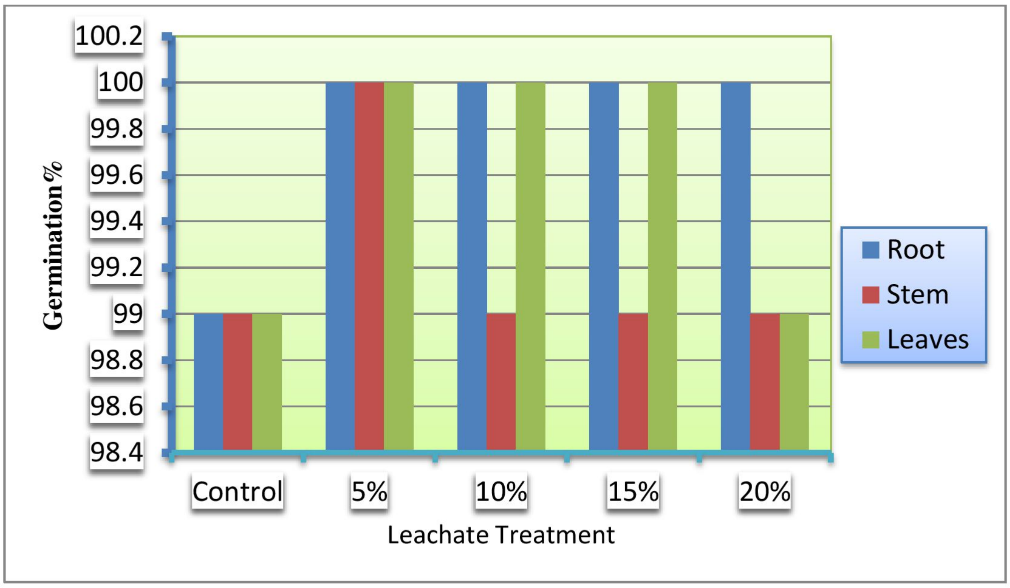


Figure-2: Effect of *Argemone mexicana* L. root, stem and leaves leachates on root length of fresh wheat seedlings

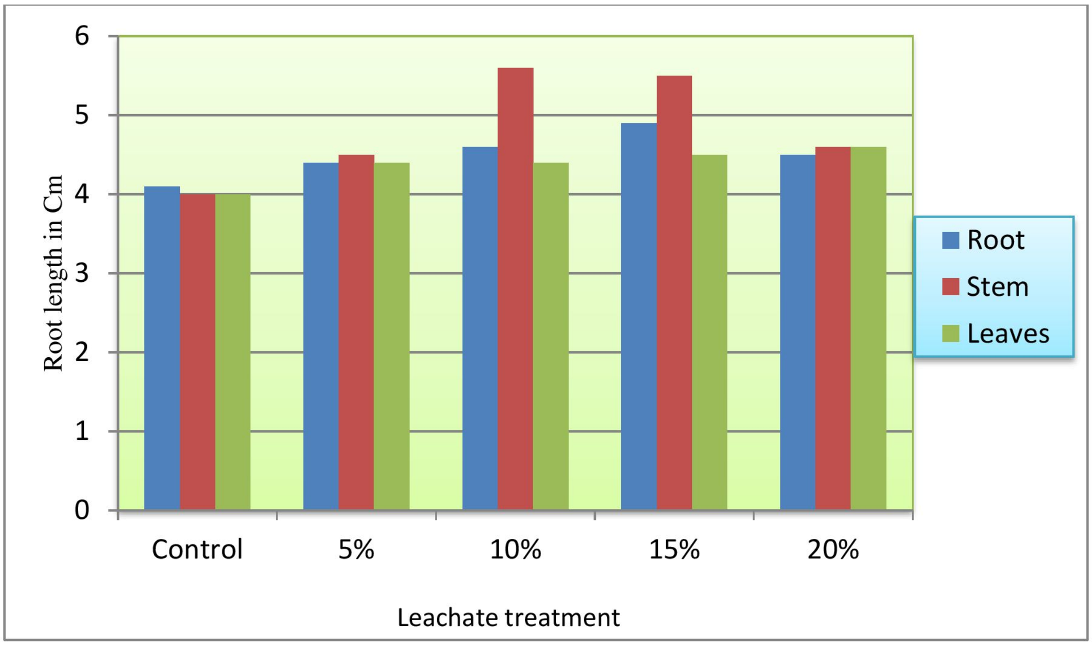


Figure-3: Effect of *Argemone mexicana* L. root, stem and leaves leachates on shoot length of fresh wheat seedlings

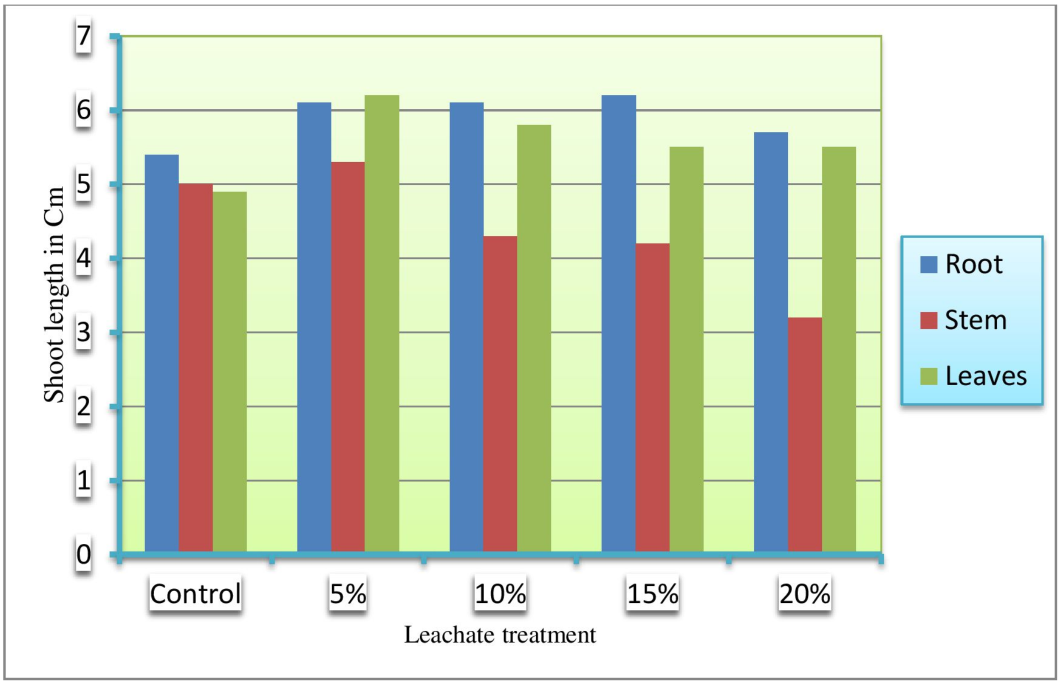


Figure-3: Effect of *Argemone mexicana* L. root, stem and leaves leachates on biomass of fresh wheat seedlings

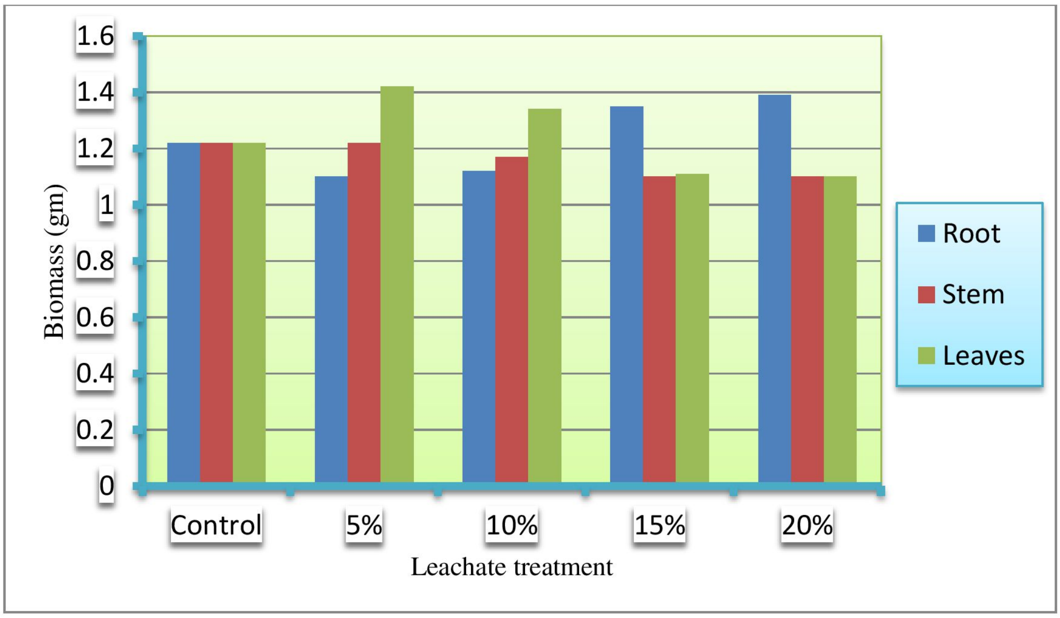


Table -2: Effect of *Argemone mexicana* L. root, stem and leaves leachates on chlorophyll content of wheat seedlings

Parts used	Chlorophyll content from 144hrs old seedlings of wheat (mg chl./g of fresh weight)					
	Parameter	**Control**	**5%**	**10%**	**15%**	**20%**
Root	Chl.a	3.1	3.7	4.7	3.3	3.0
	Chl.b	3.4	3.5	3.9	3.0	2.4
	Total Chl.	6.6	6.8	8.8	6.6	5.4
Stem	Chl.a	2.9	3.2	4.2	2.9	2.7
	Chl.b	3.8	4.0	4.8	3.3	3.0
	Total Chl.	6.7	7.2	9.8	6.2	5.7
Leaves	Chl.a	3.7	4.5	4.8	4.7	4.5
	Chl.b	3.8	4.1	4.1	4.1	3.8
	Total Chl.	7.5	8.5	8.8	8.8	8.4

Figure -5: Effect of *Argemone mexicana* L. root leachates on chlorophyll content of wheat seedlings

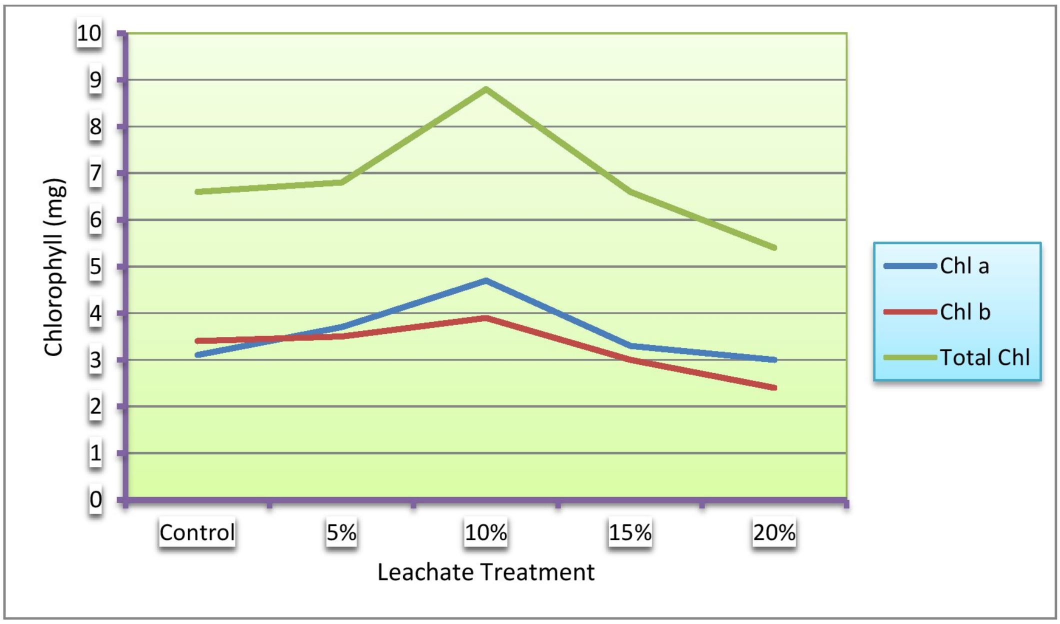


Figure-6: Effect of *Argemone mexicana* L. stem leachates on chlorophyll content of wheat seedlings

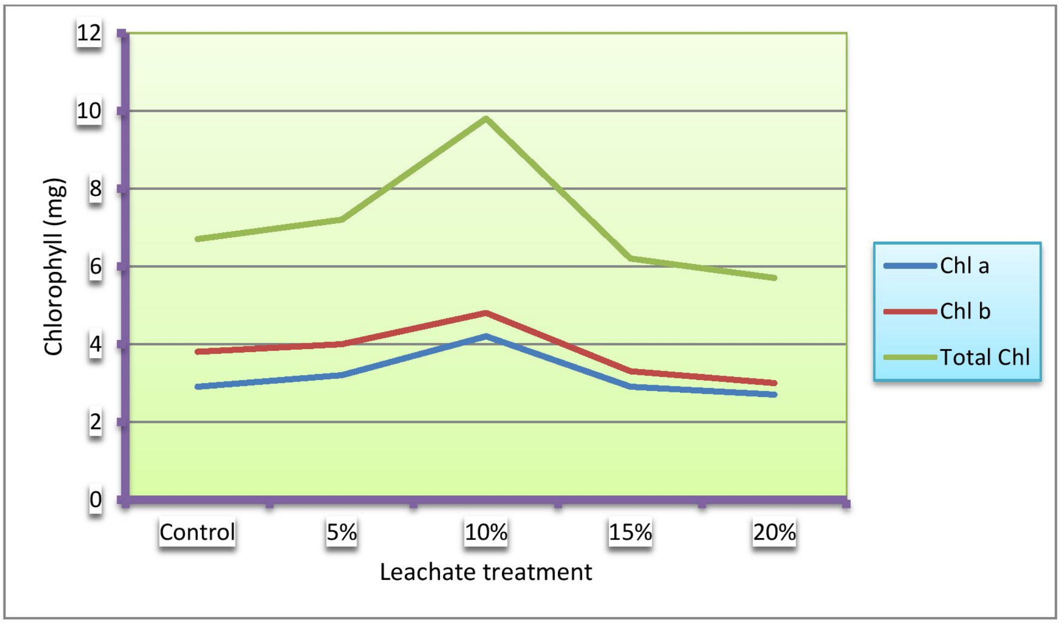


Figure -7: Effect of *Argemone mexicana* L. leaves leachates on chlorophyll content of wheat seedlings

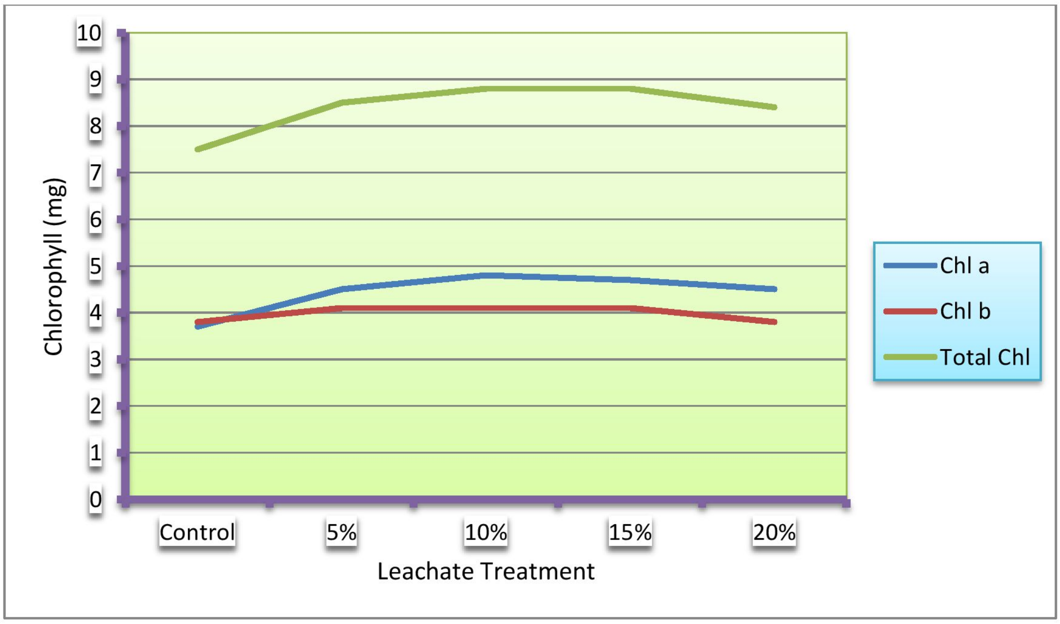
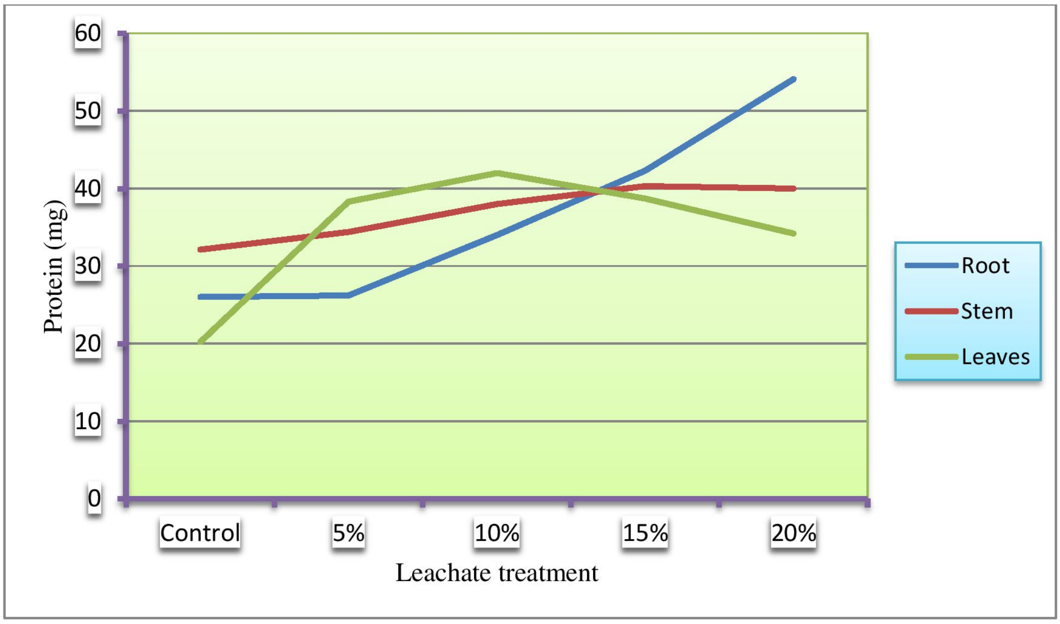


Table -3: Effect of *Argemone mexicana* L. root, stem and leaves leachates on protein content of wheat seedlings

Protein content from 192hrs old seedlings of wheat (mg protein/g fresh tissue)					
Root	26.0	26.2	34.0	42.3	54.1
Stem	32.1	34.4	38.0	40.3	40.0
Leaves	20.2	38.3	42.0	38.7	34.2

Figure -8: Effect of *Argemone mexicana* L. root, stem and leaves leachates on protein content of wheat seedlings



SUMMARY AND CONCLUSION

In the present work we also found favourable response of *A. mexicana* root, stem and leaf leachates on seed germination, root-shoot length, biomass, photosynthetic pigment and protein content of wheat at lower concentrations. This may be happened due to potential of allelochemicals present in *A.mexicana*.

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