EXPLORING THE ALLELOPATHIC AND HERMETIC EFFECT OF KHATAMI (*Altheae officinalis*) ON EMERGENCE AND SEEDLING GROWTH OF RADISH (*Raphanus sativus*)

Rizwan Maqbool[1], Bilal Ahmad Khan*[2], Sumbal Parvez[3], Muhammad Ather Nadeem[2], Azhar Hassan[1], Jamshaid Qamar[2], Ali Nawaz[1], Muhammad Adnan[2], Rafia Khalid[3], Muhammad Usman[2]

Abstract

Plant release biochemicals into its surrounding environment that act as bioherbicide at high concentration and plant growth promoter at low concentration. Therefore, this study was planned at the Weed Science laboratory, Department of Agronomy, University of Agriculture Faisalabad-Pakistan, during winter 2018 to evaluate the hermetic effects of *Althaea officinalis* L. (Khatami) on Radish (*Raphanus sativus*). The experiments were laid out under completely randomized design (CRD) having three replications. The aqueous extracts of *A. officinalis* L. were used on radish at different concentrations (2.5%, 5%, 10%, 20%, 40% and 80%). Data regarding seed germination and seedling growth (shoot length, root length, shoot fresh weight, root dry weigh) of radish were recorded using standard procedures. Results revealed that the aqueous extract of *A. officinalis* at higher concentration (80%) act as bioherbicide and produced inhibitory effects on *R. sativus* that resulted in maximum time to 50% germination (4.68 days) and mean germination time (9.05 days) while minimum germination index (3.60), germination percentage (85.00%), shoot length (42.4 cm), root length (25.90 cm), shoot fresh weight (53.30 g) and root fresh weight (13.30 g). While at lower concentration (10%) it showed hermetic effect and produced maximum germination percentage (100.00%), shoot length (58.2 cm), root length (52.0 cm), shoot fresh weight (90.00g), root fresh weight (26.30 g) minimum time to complete 50% germination (3.36 days), germination index (6.00%) and mean germination time (5.73 days) growth. In *A. officinalis* among different phenolic compound minimum Syringic acid (0.60%) and maximum Quercetion (12.3%) compound was found. Therefore, it was concluded from this study that aqueous extract of *officinalis* can be used as a growth promoter at lower concentration while as well as at higher concentration for environmentally friendly control of weeds.

Keywords: Allelopathy, Germination, Growth promoter, *Raphanus sativus* and Seedling growth.

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[1]Department of Agronomy, University of Agriculture, Faisalabad-3800, Pakistan.
[2]Department of Agronomy, College of Agriculture, University of Sargodha-40100, Pakistan.
[3]Department of Botany, University of Agriculture, Faisalabad-3800, Pakistan.
*Corresponding author: Bilal Ahmad Khan (bilalahmadkhan678@gmail.com)

Introduction

All crop plant release chemicals into surrounding environment that have the ability to either suppress or promote the growth of the target crop plant or weed. This phenomenon is very important biological control of weeds by releasing secondary metallic compounds. These secondary metabolic compounds are: Tannins, alkaloids, glycosides, cyanogen, sesquiterpenes, phenolic acids, flavonoids, and many others having allelopathic activity (King and Ambika, 2002). Weeds are undesirable plants in main crop that pose aggressive, competitive, troublesome, and multifaceted effect on crops and reduce the yield (Sodaeizadeh et al*.,* 2010). Herbicides are chemicals compounds that are used for managing weeds, but herbicide cause several environmental risks. (Hazrati et al*.,* 2017).

The biochemical compounds manufactured in plant as secondary compounds have a passive role in plants but act as a defense agent in plant. Phenolic as well as terpenoids usually characterized as allelochemical display chemical diversity and number of metabolic and physiological biochemical process. The phenomena of allelopathy in crop plant may increase the yield as well as growth of allelopathic plant via suppressing the weed growth, with the usage of allelochemicals as natural herbicides as well as growth promoter (Einhellig and souza, 1992). These allelochemical can pass into the atmosphere via diverse ways such as leakage, volatilization, root exudations, seed coat exudations by decaying of diverse part of plant (Putnam, 1974; Rice et al., 2007).

Allelochemicals formed in plant might be escaped out from the tissue of plant and different part of plant into soil atmosphere and into the environment through leaching exudation of root, decaying of plant remains and volatilization and effect the growth of neighboring plant (Golisz et al*.,* 2007).

Under stressful condition the concentration of allelochemical in plants increases and under normal conduction the concentration of allelopathic substance remain stable. The main purposes of allelochemical in plants to protect the plant form unpredictable environmental stress such as drought, mineral deficiency, temperature, herbivores grazing, water deficiency, etc. So, stress simply referred as any shortage or excess supply of plant essential compounds that hinder to complete the life cycle and retard the usual growth and development of plant. The aqueous extract of different part of allelopathic plant such as roots, stem, leaves and seed have been used valuable possessions to manage the weed through natural ways (Cheema et al*.,* 2000 a and b; Iqbal and Cheema, 2008; Jamil et al*.,* 2009). Ethanolic or methanolic aqueous extracts were used as possible herbicide in mixtures (Cheema et al*.,* 2012).

By these methods allelochemicals may be able to manage weeds via weaken weed-plant competition and enhance the crop growth and yield (Murrell et al*.,* 2011). Water extract application of allelopathy at lower concentrations stimulate development and growth of diverse crops (Anwar et al*.,* 2003; Cheema et al*.,* 2012: Nadeem et al., 2020a: Nadeem et al., 2020b). *Cinnamomum verum* are a normal-sized tree (10 to 15 m) native to Sri Lanka and tropical- Asia. The tree was cultivated in the Southern India due to strong scented leaf; bark and the aromatic oils take out from him through steam purification. Barks of *Cinnamomum verum* used in experiment to check the allelopathic potential, plane, light brown color and up to 10 mm thickness. The foremost compound gain from the bark of *C. verum* is Eugenol, Cinnamaldehyde, phenolic compounds such as chlorogenic acid, vanillic acid, caffeic acid and Linalool (Kubeczka, 2002). The presence of phenolic in bark exhibit inhibitor possession on the plant germination. *Althaea officinalis.* (Malvaceae), commonly known as khatami are a persistent herb, disseminated in the Himalayas from Kashmir to Himachal Pardesh (National Institute of Science Communication and

Information Resources, 2003). The seeds of these plants are smooth, diuretic and febrifugal (Mhaskar et al., 2000).

The biochemical investigation of methanolic extract of *Althaea officinalis* L. showing the presence of phytochemical compound such as glycosides, proteins and amino-acid, and numerous secondary metabolic compounds like phenolic compound such as gallic acid, P-coumaric acid, ferulic acid, saponin substance, tannin elements, flavonoids, oils, resin and mucilage compound. De Feo et al. (2003) examine the allelopathic consequence of *A. altissima* liquid extracts on sprouting and successive root growth of radish (*Raphanus sativus* L.) and garden cress (*Lepidium sativum*) that have raised on petri plates comprising filter paper and saturated by liquid abstract of *A. altissima*. *A. altissima* (Haven tree) abstract repressed the radical development of *Raphanus sativus* (radish), and garden cress. The vigorous elements were separated from the abstract of *A. altissima* are recognized as phenol and terpenoids (De Feo et al., 2003). De Feo et al. (2003) studies proposed that these compound act as natural herbicides. Therefore, the proposed research was conducted to study the allelopathic effect of *Althaea officinalis* on vegetable crop (*Raphanus sativus*).

Materials and Methods

Collection *of A. officinalis* plant *parts*

To make aqueous extract *A. officinalis* (seed) plant parts were purchased from Ayub Agricultural Research Institute of Faisalabad (AARI) Pakistan.

Preparation of *A. officinalis* plant *parts* aqueous extracts

Plant parts such as of seed of *A. officinalis* were soaked in distilled water at 1:80 ratio for 2 days (about 48 hours). The aqueous extracts of chopped samples were filter through watman filter paper. The concentrated solution was then diluted with distilled water (v/v%) to make different solutions. Almost seven concentrations (0%,2.5%,5%,10%,20%,40%and 80%) were prepared to check the allelopathic activity of the extract. Seven concentrations 0,2.5, 5, 10, 20, 40, and 80% were prepared by taking extract into 250 ml flask and adding 2.5, 5, 10, 20, 40 and 80ml stock solution of *A. officianlis.* Whereas controlled solution contained only 250 ml distilled water.

Laboratory Experiment

Each dilution of each extract was placed in separate bottles and tagged with the respective plant name. The experiment was conducted in 9 cm petri plate lined with watman filter no.10-filter paper.

To estimate the allelopathic effect 0%, 0.25%, 0.5%, 1%, 2%, 4% and 8% concentration of *A. officianlis* were applied. A 20 seeds of *R. sativus* were placed in each Petri plates containing filter paper. To minimize the excess of evaporation petri plates were covered and rapped with parafilm. The petri plates were kept at the temperature of 30°C and were again moistened with 3 mL after one week. The data regarding emergence of the seeds were noted every day for 14 days. After the 14 days, harvest the germinated seedlings of *R. sativus* and observed the different parameters like shoot length, root length, fresh and dry weight. Fresh weight was recorded instantly after harvesting while the dry weight of seedling was observed after oven drying for two days at 60 °C.

Experimental site

Laboratory experiments were conducted at weeds Science Laboratory, Department of Agronomy, University of Agriculture, Faisalabad to check the allelopathic effects of *A. officinalis* on radish (crop plant).

Laboratory Experiment

This study was carried out using water as extracting medium because allelochemicals are often water soluble and released into the environment through root exudation, leaching by dews and rains or decaying of plant tissue (Turk

and Tawaha, 2003). Ten vigorous seeds of radish and wild pea were placed in Petri plates and *C. verum* and *A. officinalis* prepared aqueous solution were functionally applied at every specific petri dish purified water also cast-off like control treatment. After applying solution petri plate were wrapped with paper tape and placed at room temperature. Petri dishes would keep moisture by applying solution whenever needed. Percentage of germination, mean germination, root length, shoot length and fresh weight of root and shoot were taken afterward the 12th day. Shoot length, root length was measured with measuring scale and fresh weight on weight machine. The diluted extracts of *Althaea officinalis* (0%, 2.5%, 5%, 10%, 20%, 40%, 80%) were applied separately on radish seed.

Data collection

Mean emergence time of *R. sativus*

Ellis and Reborts (1981) equation were used to examine the mean emergence time (MET).

$$MET = \frac{\sum(Dn)}{\sum n}$$

Emergence index of *R. sativus*

By using formula of association of the official seed analysis (1983) we record the emergence index

$$GI = \frac{No.of\ emerged\ seeds}{Days\ of\ first\ count} + - - - - - + \frac{No.of\ emerged\ seeds}{Days\ of\ final\ count}$$

Emergence percentage of *R. sativus*

No of emerged seeds were counted daily according to the method of the association of Official Seed Analysis (1990) and converted into emergence percentage by the following formula.

$$Emergence\ (\%) = \frac{No.of\ emerged\ seeds}{Total\ seeds} \times 100$$

Time taken to 50% emergence of *R. sativus*

The time to the 50% emergence (E_{50}) was recorded by using the formula purposed by Coolbear et al. (1984**)**

$$E\mathbf{50} = ti + \left[\frac{\frac{N}{2} - ni}{nj - ni}\right](tj - ti)$$

Growth attributes of *R. sativus*

All seedlings from each petri plate were separated 14 days after emergence. After that both shoot length and root length were calculated by using meter rod from base level to top of the plants. Seedlings fresh weight was examined by separating seedlings from petri dish and measuring by using digital weight balance.

Phenolic contents

Phenolic contents were determined by using HPLC (Gradient, Reverse Phase made from shimadzu japan detector SPD-10 Av Pump LC-10-AT). Made the (w/v) solution at 1.10 ratio (10g powdered of *A. officanlis* and 100ml methanol) Then wraped the beaker with aluminum foil and placed for 10 days. After 10 days the material was semidried. 5mg weight with electrical balance taken out for phenolic analysis. In *A. officanlis* Quercetin, Pcoumaric, ferulic acid, gallic acid and syringic acid were detected.

Statistical analysis

Statistics software (version, 8.1Statistix, Tallahassee, FL, USA) was used to analyze the collected data and least significant difference test (LSD) was used to compare the means of treatment at probability level of 5%.

Results and Discussions

Allelopathic effect of *Altheae officinalis* on emergence of *Raphanus sativus* L.

Time to 50% germination (T_{50})

The aqueous extract of *Althaea officinalis* had significant effect on T_{50} of *Raphanus sativus* L. (Table 1). Maximum T_{50} (4.68) of radish seed was observed at T_7 (80%) concentration. While minimum T_{50} (3.36) of radish seed was observed at T_5(20%). T_7 (80%) concentration showed non-significant relationship with T_1 (0%) concentration. T_6 (40%) concentration showed non- significant relationship with T_3 (5%) concentration which was statistically at par with T_4 (10%) concentration. The T_{50} of radish seed was significantly decreased by 22% at T_5 (20%) concentration as compared to controlled T_1 (0%). The time taken to 50% germination of radish seed was increased by 8% at T_7 (80%) concentration as compared to controlled T_1 (0%). Amongst all the treatments, T_5 (20%) and T_2 (2.5%) showed reduced germination of seedling as compared to all other treatments.

Herro and Callaway (2003) reported that allelochemical had negative effects on crops in the ecosystem causing reduction in germination and growth of seedlings. Aqueous extract of certain plants induced mortality and inhibited germination of plants. According to Nadeem et al. (2020a) who reported that all the concentrations of *C. tinctorius* enhance the time to complete 50% emergence of *O. punctata* with 8% concentration Similar inhibitory effects of aqueous extracts.

Germination index (GI)

The effects of *A. officinalis* seeds extracts had significant effect on germination time of radish seeds described in Table (1). Maximum seed germination (6.00) was observed at control T_1 (0%) concentration. The minimum germination (3.60) was observed at T_5 (10%) concentration. T_7 (80%) concentration showed significant relationship with T_4 (10%) concentration. T_6 (40%) concentration showed non-significant relationship with T_4 (10%) concentration. Germination time of radish seed significantly decreased by (3%) at T_7 (80%) concertation as compared to control T_1 (0%) concertation.

These results are parallel to the conclusion of Dongre and Singh (2007) and Yadav et al., (2007) described inhibitory effects posed by the water extract of different parts of *Alternanthera* species. Aqueous extract at high concentration were inhibitory. Nadeem et al. (2020b) reported that Water extracts of leaf of *C. tinctorius* at 8% concentration result in lowest *E. cruss-galli* emergence index.

Mean germination time (MGT)

The mean germination time of radish seed was significantly affected by the *A. officinalis* seed extract Table (1). Maximum MGT (9.05) of radish seed was observed at T_7 (80%) concentration of extract. While minimum MGT (5.73) of radish seed was noted at T_2 (2.5%) which was statistically at per with T_5 (20%) concentrations. T_7 (80%) concentration showed significant relationship with T_1 (0%) concentration. T_6 (40%) concentration showed non-significant relationship with T_1 (0%) concentration. The MGT of radish seed was statistically increased (14%) at T_7 (80%) concentration of extract as compared to control T_1 (0%) concentration. The MGT of radish seed was statistically decreased (25%) at T_5 (20%) concentration as compared to control T_1(0%) concentration.

Gao et al. (2009) reported that the germination inhibition is dependent on extract the concentration, it is might be to the entry of water solvable allelochemicals into the seed, which delays the growth and germination.

Germination percentage (GP)

The effect of *A. officinalis* seeds extract had significant effect on germination percentage of radish Table (1). Maximum germination percentage (100.0) of radish seed was observed at T_1 (0%) concentration of extract. While minimum germination percentage (85.0) of radish seed was noted at T_7 (80%) concentrations. T_7 (80%) concentration

showed significant relationship with T_1 (0%) concentration. T_5 (20%) concentration showed non-significant relationship with T_4 (10%), T_3 (5%) and T_2 (2.5%) concentration of extract. The germination percentage of radish seed was statistically decreased (15%) at T_7(80%) concentration of extract as compared to control T_1(0%) concentration.

From the findings of Zeng et al. (2008) it may be inferred that the allelopathic chemicals are distributed broadly among organs such as seeds, flowers, pollen, leaves, stems, and roots, however, just one or two of such organs inhibited germination and growth injury to certain food crops.

Allelopathic effect of aqueous seed extract of *Althea officinalis* on seedling growth of Radish

Shoot length (mm)

The effect of *Althaea officinalis* seeds aqueous extract on shoot length of *Raphanus sativus L*. was shown in Table (2). The aqueous extract of *A. officinalis* seeds had significant effect on shoot length of *Raphanus sativus* L. Maximum shoot length (58.2mm) were observed at T_7 (80%) concentration which were statistically at par with that of T_1 (0%) concentration. Minimum shoot length (42.4mm) were observed at T_2 (2.5%) concentration of extract. T_7 (80%) concentrations showed non-significant relationship with T_6 (40%) which was statistically at par with T_1 (0%) concentration. T_3 (5%) concentration showed non-significant relationship with T_4 (10%) and T_5 (20%) concentrations. T_2 (2.5%) concentrations showed significant relationship with T_3 (5%) and T_1 (0%) concentration. The shoot length of radish increased (3%) at T_7 (80%) concentration as compared to control T_1 (0%) concentration while shoot length of radish decreased (11%) at T_5 (20%) as compared to control T_1 (0%) concentration. The results are supported by Rice (1984) analysis that difference in extract concentration might be shown different results, (both inhibitory and stimulating results in certain cases).

Root length (mm)

The effect of *A. officinalis* (seeds) extract on root length of *R. sativus* L. were significant as describe in Table (2). Maximum root length (52.0 mm) of radish were observed at T_1 (0%) concentration of the *A. officinalis* seeds extracts. Minimum root length (25.9mm) of radish were observed at T_7 (80%) concentrations of the extracts. The root length of radish was significantly decreased (50%) at T_7 (80%) concentrations as compared to control T_1 (0%) concentration. The root length of radish increased (79%) at T_4 (10%) concentration as compared to T_7 (80%) concentration. T_5 (20%) concentration showed non-statistically significant relationship with T_6 (40%) concentration. T_3 (5%) concentration showed non-significant relationship with T_4 (10%). T_7 (80%) showed significant relationship with controlled T_1 (0%). T_2 (2.5%) showed significant relationship with control T_1 (0%) concentration. T_7 (80%) concentration showed significant relationship with controlled T_1 (0%) concentration. De Feo et al. (2003) examined the allelopathic effect of *A. altissima* liquid extracts on germination and successive root growth of radish (*Raphanus sativus* L.) and garden cress (*Lepidium sativum* L.) that have raised on petri plates comprising filter paper and saturated by liquid abstract of A. altissima. *A. altissima* extract suppressed the radical growth of *Raphanus sativus* (radish), and garden cress. Nadeem et al. (2020b) studied the effect of aqueous extracts of various parts of *C. tinctorius* on the root length of barnyard grass results revealed that minimum root length of barnyard grass was produced by produced by safflower leaves aqueous extract whereas, seedlings with lengthiest roots were noted by application of stem aqueous extracts of *C. tinctorius*.

Shoot fresh weight (mg)

The aqueous extract of *A. officinalis* seeds had nonsignificant effect

on fresh weight of shoot of *Raphanus sativus* L. Table (2). Maximum fresh weight (90.0mg) of *Raphanus sativus* shoot was observed at T_7 (80%) concentration. Minimum fresh weight (50.0mg) of *Raphanus sativus* shoot was observed at T_2 (2.5%) and T_5 (20%) concentration. T_7 (80%) concentration showed significant relationship with controlled T_1 (0%) concentration. T_2 (2.5%) concentration showed non-significant relationship with T_5 (20%) concentration while T_3(5%) concentration showed non-significant relationship with T_4 (10%) concentration. The fresh weight of shoot was significantly increased (22%) at T_7 (80%) concentration as compared to control T_1 (0%) while the fresh weight of shoot was significantly decreased (27%) at T_5 (20%) concentration as compared to control T_1 (0%). The increase in fresh weight of radish seed may be due to the stimulatory allelochemicals in these extracts and shoot of radish showed more resistance at higher concentration of extract. While lower concentrations of extract were suitable to reduce the fresh weight of radish. That results are not corresponding to Shahida et al. (2002) that water extracts of plants and inflorescences at higher concentration suppressed the germination seedling growth and reduce the fresh and dried mass of barley wheat, radish and pea. The results are supported by Rice (1984) analysis that difference in extract concentration might be shown different results, (both inhibitory and stimulating results).

Root fresh weight (mg)

The effect of aqueous extract of *Althaea officinalis* seeds were significant on fresh weight of radish root as described in Table (2). The fresh weight of radish root was mainly reduced as concentration of extract increase. Maximum fresh weight (26.3mg) of root was observed T_1 (0%) concentration. While minimum fresh weight (13.3mg) of root was observed at T_7 (80%) concentration of the extract. T_7 (80%) concentration showed non-significant relationship with T_5 (20%) concentration. T_2 (2.5%) concentration showed non-significant relationship with T_3 (5%) concentration. T_4 (10%) concentration showed significant relationship with T_7 (80%) concentration. The fresh weight of radish was significantly decreased (49%) at T_7 (80%) at par similar with T_5 (20%) concentration as compared to control T_1 (0%) concentration. The fresh weight of radish root was significantly reduced at different concentration of extract as compared to controlled treatment. These results were supported with the finding of Daniel (1999) and Uddin et al. (2000) that mass of root and shoot development were significantly reduced as increase the concentration of aqueous extract. These findings also in accordance with Sisodia and Siddiqui (2008) who stated that the inhibition effect was found to increase with increasing concentration at different aqueous extracts.

Phenolic compounds and their concentration in *C. verum* and *A. officinalis*

Phenolic compounds and their concentration in *A. officinalis* were presented in Table 3. In *A. officinalis* minimum Syringic acid (0.60%) and maximum Quercetion (12.3%) compound was found.

Conclusion:

The results of experiment directed that aqueous extract of *A. officinalis* showed inhibitory effect on radish germination and seedling growth at higher concentration (80%) and growth regulatory effect at lower concentration So, aqueous extract of *A. officinalis* can be use as potential bioherbicide to control weed at 80% concentration and growth regulator at lower concentration.

Table 1: Allelopathic effect of *A. officinalis* on emergence of seeds of *R. sativus*

Concentration (%)	Time to 50% germination	Germination Index	Mean germination Time	Germination %
0 %	4.32ab	5.21bc	7.93bc	91.6ab
2.5 %	3.54c	3.87d	7.43cd	93.3ab
5%	4.01b	4.87c	7.12d	93.3ab
10%	3.36c	6.00a	5.73e	100.0a
20%	4.25b	5.81ab	5.93e	93.3ab
40%	4.25b	5.22bc	8.13b	86.6b
80%	4.68a	3.60d	9.05a	85.0b
LSD:	**0.3826**	**0.6753**	**0.6764**	**8.5450**

Table 2: Allelopathic effect of *A. officinalis* on seedling growth of seeds of *R. sativus*

Concentration (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)
0 %	56.2a	46.4b	73.3c	20.0c
2.5 %	46.8b	37.3c	50.0e	23.3b
5%	49.7b	43.9b	60.0d	23.3b
10%	58.2a	52.0a	90.0a	26.3a
20%	56.6a	31.0d	83.3b	16.6d
40%	50.2b	31.6d	63.3d	13.3e
80%	42.4c	25.9e	53.3e	13.3e
LSD:	**3.8627**	**3.4431**	**4.8338**	**2.9601**

Table 3: Phenolic compounds and their concentration in *A. officinalis*

Phenolic compounds	Concentration (%)
Syringic acid	0.60
p-crumeic	0.94
Ferulic acid	7.48
Quercetion	12.3
Gallic acid	4.69

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