

**IDENTIFYING THE HERMETIC POTENTIAL OF KHATAMI (*ALTHEAE OFFICINALIS*)
EMERGENCE AND SEEDLING GROWTH OF WILD PEA (*PISUM SATIVUM SUBSP.*
ELATIUS).**

Rizwan Maqbool¹, Bilal Ahmad Khan*², Sumbal Parvez³, Muhammad Ather Nadeem², Muhammad Mughees Ud Din⁴, Jamshaid Qamar², Muhammad Waqas¹, Muhammad Mohsin Amin², Burhan Khalid¹

Abstract

Plants release biochemicals into surrounding environment that act as bioherbicide at high concentration and plant growth hormone at low concentration. Therefore, this study was planned at the Department of Agronomy in Weed Science Laboratory, University of Agriculture Faisalabad during Winter 2018 to evaluate the allelopathic effects of *A. officinalis* on weed Wild Pea (*Pisum sativum subsp. elatius*). The experiments were laid out under completely randomized design (CRD) having three replications. The aqueous extracts of *C. verum*. were used on radish and wild pea seeds 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 weed were recorded following standard procedures. Results Reveals that the aqueous extract of *A. officinalis* at higher concentration (80%) act as bioherbicide and produced inhibitory effects on *R. sativus* result in Maximum time to 50% germination (6.70 days) and mean germination time (10.00 days) while minimum germination index (4.70), germination percentage (66.60%), shoot length (49.8 cm), root length (8.16 cm), shoot fresh weight (10.00g) and root fresh weight (10.361g). While at lower concentration (10%) it shows hermetic effect and produce maximum germination percentage (90.00%), shoot length (84.7 cm), root length (66.2 cm), shoot fresh weight (82.20 g), root fresh weight (58.30 g) and germination index (8.21%) minimum time to complete 50% germination (4.33 days) and mean germination time (5.49 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 growth promoter at lower concentration as well as at higher concentration for environmentally friendly control of weeds.

Keywords: Allelopathy, Bioherbicides, Germination, Root length and Seedling growth.

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¹ Department of Agronomy, University of Agriculture, Faisalabad-38000, Pakistan.

² Department of Agronomy, College of Agriculture, University of Sargodha-40100, Pakistan.

³ Department of Botany, University of Agriculture, Faisalabad-38000, Pakistan.

⁴ Institute of Soil and Environmental Sciences, Univerisity of Agriculture, Faisalabad-38040, Pakistan

*Corresponding author: Bilal Ahmad Khan (bilalahmadkhan678@gmail.com)

Introduction

All crop plant release chemicals into surrounding environment that have ability to either suppress or promote that growth of target crop plant or weed. This phenomenon is very important biological control of weeds by releasing secondary metabolic compounds. These secondary metabolic compounds such as Tannins, alkaloids, glycosides, cyanogen, sesquiterpenes, phenolic acids, flavonoids, and many others having allelopathic activity (Nadeem et al., 2020a; King and Ambika, 2002). Weeds are unwanted plants in main crop that pose aggressive, competitive, troublesome, and multifaceted effect on crops and reduce the yields of crop (Sodaeizadeh et al., 2010). Herbicides are chemicals compounds that are used for managing weeds, but herbicide cause several environmental risks. So, find the harmless and potent systems for controlling weeds were of main attention for maintaining the agricultural yields (Hazrati et al., 2017). Usual product escape out from the remains of medicinal plant might assist to decrease the usage of artificial made herbicides for weed management (Sodaeizadeh et al., 2010).

The biochemical compounds manufactured in plant as secondary compounds have been nonsignificant role in plants but act as defending 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, 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 (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 (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 (Nadeem et al., 2020a; Nadeem et al., 2020b). *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 methanol 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.) that have raised on petri plates comprising filter paper and saturated by liquid abstract of *A. altissima*. 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 wild Pea.

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).

Preparation of *A. officinalis* plant parts aqueous extracts

Plant parts such 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 filter paper. The centered 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 80-ml stock solution of *A. officinalis*. Whereas controlled solution contained only 250 ml distilled water.

Laboratory Experiment

Each dilution of each extract placed in separate bottles and then tagged these bottles by name of each dilution with its plant name too carefully for their easy utilization in next procedure. The experiment was conducted in each 9cm

petri plate lined with filter no.10-filter paper.

To estimate the allelopathic effect 0%, 0.25%, 0.5%, 1%, 2%, 4% and 8% concentration of *A. officinalis* were applied. 20 seeds of wild pea 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 wild pea.

Laboratory Experiment

This experiment was conducted to identify the hermetic effect of *A. officinalis* on wild pea. Ten vigorous seeds of wild pea were placed in Petri plates *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 *Pisum sativum subsp. elatius* (day)

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

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

Emergence index of *Pisum sativum subsp. elatius*

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

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

Emergence percentage of *Pisum sativum subsp. elatius* (%)

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.

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

Time taken to 50% emergence of *Pisum sativum subsp. elatius* (day)

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

$$E_{50} = t_i + \left[\frac{\frac{N}{2} - n_i}{n_j - n_i} \right] (t_j - t_i)$$

Growth attributes of *Pisum sativum subsp. elatius*

All seedlings from each petri plate were separate 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 *C. verum* and *A. officinalis* and 100ml methanol) Then wrapped 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. officinalis*

Quercetin, Pcoumaric, ferulic acid, gallic acid and syringic acid were detected.

Statistical analysis

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

Results and Discussion

Time to 50% germination (T_{50})

The aqueous extract of *A. officinalis* seeds had significant effect on T_{50} germination of wild pea seeds (Table 1). Maximum T_{50} (6.70) were observed at T_4 (10%) concentration of extract. While minimum T_{50} (3.46) of wild pea seed was observed at T_7 (80 %) concentration of extract.

T₇ showed statistically non-significant relationship with T₅(20%) which was statistically at par with T₁(0%) concentration. T₄(10%) concentration showed significant relationship with T₁(0%) concentration. The T₅₀ of radish seeds was significantly increased (87%) at T₄(10%) concentration as compared to controlled T₁(0%) concentration. The T₅₀ of radish seed was statically decreased (3%) at T₇(80%) concentration of extract as compared to control T₁(0%) concentration. The extract of different plant parts of *M. oleifera* affected the rate of germination of wild pea in laboratory condition. The degrees of inhibitory effects of different plant parts on germination were different. 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 effect *Athaea officinalis* seed aqueous extract had significant effect on germination time of wild pea seed were summarized in Table 1. *A. officinalis* seed extract significantly reduce the germination period of wild pea as compared to control treatment. Maximum germination time (8.21) of wild pea seed was observed at T₄(10%) concentration. while minimum germination time (4.70) of wild pea seed was observed at T₇(80%) concentration of extract. T₇(80%) concentration showed statistically non-significant relationship with T₅(20%) and T₃(5%) concertation of extract. T₂(2.5%) concentration showed non-significant relationship with controlled T₁(0%) concentration. T₄(10%) concentration showed significant relationship with controlled T₁(0%) and all other concentration of extract. The germination time of wild pea seed decreased (21%) at T₇(80%) concentration of extract as compared to controlled T₁(0%). The germination time of wild pea seeds increased (36%) at T₄(10%) concentration as compared to controlled T₁(0%). According to Nadeem et al.

(2020b) who reported that water extracts of leaf of *C. tinctorius* at 8% concentration result in lowest *E. cross-galli* emergence index.

Mean germination time (MGT)

The effect of aqueous extract of *Althaea officinalis* had significant effect on mean germination time of wild pea (Table 1). Maximum MGT (7.73) of wild pea was observed at controlled T₁(0%). Minimum MGT of seedling was noted at T₇(80%) concentration treatment. T₇(80%) concentration showed significant relationship with T₄(10%) concentration. T₆(40%) concentration showed significant relationship with T₇(80%) concentration. The MGT of wild pea was significantly increased (29%) at T₄(10%) concentration as compared to controlled T₁(0%). The MGT of wild pea was decreased (28%) at T₇(80%) concentration as compared to control.

The inhibition effect mainly due to present of natural allelochemicals in different part of plant. These allelochemical mainly phenolic interfere with other seeded pant and influence on germination rate of target seed. According to Rice (1984) phenolics are the most common and widely distributed water soluble allelochemicals. These chemicals were reported to have had allelopathic potential on various agronomic crops and weeds (Mersie and Singh, 1987) and vegetable crops (Mersie and Singh, 1988).

Germination percentage (GP)

The effect of aqueous extract of *Althaea officinalis* had significant effect germination percentage of wild pea (Table 1). Maximum percentage of germination (96.7) of wild pea was observed at controlled T₁(0%). Minimum percentage of germination (69.9) of seedling was noted at T₇(80%) concentration treatment. T₇(80%) concentration showed non-significant relationship with T₆(40%) concentration. T₇(80%) concentration showed significant relationship with T₁(0%) concentration. While T₃(5%) concentration showed non-

significant relationship with T₄ (10%) concentration. The percentage of germination of wild pea was significantly decreased (27%) at T₈ (80%) concentration as compared to controlled T₁ (0%). The extract of different plant parts of *M. oleifera* affected the rate of germination of wild pea in laboratory condition. The degrees of inhibitory effects of different plant parts on germination were different. Nadeem et al. (2020b) reported that emergence percentage of barnyard grass seeds was significantly affected by the different concentration of water extracts of *S. oleraceus*. maximum emergence percentage (100%) was achieved under control (0%) whereas minimum (46.67%) by 8% aqueous extract.

Shoot length (mm)

The extract of *A. officinalis* (seeds) had significant effect on the shoot length of *Pisum sativum sub species elatius* (wild pea) as described in Table 2. Minimum shoot length (49.8mm) of wild pea was observed at (80%) concentration extract. Maximum shoot length (84.7mm) of wild pea was recorded at (20%) concentration extract. T₆ (40%) concentration showed statistically non-significant relationship with controlled (0%). While T₇ (80%) concentrations showed statistically significant relationship with controlled T (0%). Similarly, T₅ (20%) concentration showed significant relationship with controlled (0%). Shoot length of wild pea was decreased (31%) at (80%) concentration as compared to control T₁ (0%). Shoot length of wild pea was increased (16%) at (20%) concentration as compared to control T₁ (0%). Nadeem et al. (2020b) studied the effect of aqueous extracts of various concentration of *C. tinctorius* on the shoot length of barnyard grass results revealed that minimum shoot length of barnyard grass was produced at higher concentration while maximum under control (0%).

Root length (mm)

The effect of *A. officinalis* (seeds) extract at different treatments has been

summarized in table 2. The root length of *Pisum sativum sub species elatius* (wild pea) were found to be repressed at higher concentration of extracts. The inhibition effect on root length was increased as increased the concentrations of the extracts. Maximum root length (66.2mm) of wild pea were observed at (2.5 %) concentration of the *Althaea officinalis* seeds extract. Minimum root length (8.16 mm) of wild pea were observed at 80% concentrations of the extracts. The root length of wild pea was significantly decreased (82%) at 80% concentration as compared to control (0%) concentration. The root length of wild pea was found significantly increased (41%) at T₂ (2.5%) concentration as compared to control T₁ (0%) concentration. While the root lengths of wild pea were recorded non-significant at T₄ (10%), T₅ (20%) and T₆ (40%) concentrations. T₃ (5%) concentration showed statistically non-significant relationship with controlled T₁ (0%). T₄ (10%) concentration showed statistically non-significant relationship with T₅ (20%) which was statistically at par with those of T₆ (40%) concentration. T₇ (80%) showed significant relationship with controlled T₁ (0%) concentration. Reduction in root length by application of *A. officinalis* aqueous extract may be due to inhibitory effects of allelopathic compounds present in the seed aqueous extracts of *A. officinalis*. 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 effect of *Althaea officinalis* (seeds) aqueous extract was significant on fresh weight of wild pea shoot presented in Tables 2. Maximum fresh weight (85.2mg) of wild pea shoot was observed at T₄ (10%) of extract. Minimum fresh weight (10.0mg) was observed at T₇

(80%) concentration of extract. T₅ (20%) concentrations showed non-significant relationship with T₂ (2.5%) concentration. T₆ (40%) concentrations showed non-significant relationship with T₃ (5%) concentration which was statistically at par with T₁ (0%) concentration. T₇ (80%) concentration showed significant relationship with T₁ (0%) concentrations. Fresh weight of shoot decreased (16%) at T₇ (80%) concentration as compared to control T₁ (0%) concentration and fresh weight of shoot (223%) increased at T₄ (10%) concentration as compared to control T₁ (0%) concentration. The reduction in fresh weight of seedling might be due to presence of allelochemicals in seeds extract. The reduction in plant root and shoot length and fresh weight with water extract of other plants has been reported by other researchers (Uremis et al., 2005; Yasmin et al., 1999). Allelochemicals can lower the levels of hormones like G.A. and IAA (Kamal and Bano, 2008).

Root fresh weight (mg)

The aqueous effect of *Althaea officinalis* of seeds extracts on the fresh weight of wild pea root were significant described in Tables 2. Highest fresh weight (58.3mg) of wild pea root was observed at T₁ (0%) and lowest fresh weight (10.6mmg) of root was noted with T₇ (80%) concentration of extract. The fresh weight of root decreased significantly (81%) at T₇ (80%) concentration as compared to control T₁ (0%) concentration. The fresh weight of root decreased (45%) at T₃ (5%) and T₆ (40%) concentration as compared to control T₁ (0%) concentration. T₃ (5%) concentration showed statistically non-significant relationship with T₄ (10%) and T₆ (40%) concentration. T₇ (80%) concentration showed significant relationship with T₁ (0%) concentration. Increase concentration of *A. officinalis* aqueous extract dramatically reduce the fresh weight of seedling as compared to control treatment that shown maximum fresh weight of root. The result was like to Rice show that extract of Jack bean on

safflower exhibit suppressing effect and chiefly decrease the leaf fresh weight and dry fresh weight at increase concentration of extract. The reduction in plant root and shoot length and fresh weight with water extract of other plants has been reported by other researchers (Uremis et al., 2005; Yasmin et al., 1999). Our results are in line with Nadeem et al., (2020b) that water extracts of fruit, leaves and stem of *C. tinctorius* at higher concentration (8%) import inhibitory and result in reduce emergence and seedlings growth of *E. cruss-galli* seedling as compare to low concentration. Herro and Callaway (2003) reported that allelochemical had negative effects on crops in the ecosystem causing reduction in germination and growth of seedlings. Pratley et al. (1996) reported that aqueous extract of certain plants induced mortality and inhibited germination of plants. Nadeem et al., (2020a) revealed that application of aqueous extract of *C. tinctorius* various parts at higher concentrations result in reduction in emergence and seedling growth of *O. punctata* as compare to low concentration.

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 produce hermetic effect at lower concentration 10% aqueous extract concentration of *A. officinalis*. 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 influence of *Althaea officinalis* on emergence and seedling growth of wild pea (*Pisum sativum sub species elatius*.)

Concentration (%)	Time to 50% germination	Germination Index	Mean germination Time	Germination %
0 %	3.58c	6.01b	7.73b	73.3c
2.5 %	4.00bc	5.99b	7.66bc	86.6ab
5%	3.41d	4.87cd	6.66cd	83.3ab
10%	4.33b	8.21a	5.49e	90.0a
20%	3.46cd	4.78cd	6.18d	88.3ab
40%	3.75cd	5.60bc	7.28bc	81.6b
80%	6.70a	4.70d	10.0a	66.6c
LSD:	0.5711	0.8301	1.0129	8.1065

Table: 2 Allelopathic influence of *Althaea officinalis* on seedling growth of wild pea (*Pisum sativum sub species elatius*.)

Concentration (%)	Shoot length (cm)	Root length (cm)	Shoot fresh weight (cm)	Root fresh weight (cm)
0 %	72.6c	46.7b	26.3b	29.0d
2.5 %	51.4d	25.8c	16.1c	44.3b
5%	79.0b	47.2b	24.6b	31.6cd
10%	84.7a	66.2a	85.2a	58.3a
20%	77.9b	28.0c	25.6b	33.3c
40%	74.7c	25.5c	16.3c	15.6e
80%	49.8d	8.16d	10.0d	10.6f
LSD	3.1010	4.8942	4.7465	3.8245

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

<i>Althaea officinalis</i>	
Phenolic compounds	Concentration
Syringic acid	0.60
p-cumeic	0.94
Ferulic acid	7.48
Quercetion	12.3
Gallic acid	4.69

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