EXPLORING THE ALLELOPATHIC EFFECT OF *CINNAMOMUM VERUM*ON EMERGENCE AND SEEDLING GROWTH OF WILD PEA (*Pisumsativum subsp. elatius*)

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Abstract

It is important to identify plant species that have herbicidal potential so that their bio-extracts can be used as a safer and more effectual novel weed management factor. Therefore, proposed research was planned in Weed Science Laboratory at Department of Agronomy, University of Agriculture Faisalabad during Winter 2018 to check out the allelopathic effects of Cinnamomumverum (Dalchini) on weed Wild Pea (Pisumsativum subsp. elatius) and determination and quantification Phenolic compound present in aqueous extract of bark of C. verum. The experiment was arranged under completely randomized design (CRD) having 3 replications. The aqueous extract of C. verum was utilized on wild pea seeds at separate concentrations (2.5%, 5%, 10%, 20%, 40% and 80%). Data with regard to seed germination and seedling growth (shoot length, root length, shoot fresh weight, root dry weigh) of weed was noted following standard procedures. C. verum extract at greater concentration act as bioherbicide and cause inhibitory effects on Pisumsativum subsp. elatius. And at low concentration it showed hermetic effect and enhanced the emergence and seedling growth. Among different Phenolic compound (Syringic acid, p-crumeic, Ferulic acid, Quercetion and Gallic acid) determine in aqueous extract of barck of C. verum. Maximum Quercetion (12.3 %) and minimum Syringic acid (0.60%) was found. Therefore, it was summarized from this study that aqueous extract of C. verummay be used as bioherbicide for biocontrol of weed at higher concentration (80 %) while at lower concentration (10%) as growth promoter.

Keywords: Allelopathy, Growth regulator*, Pisumsativum,* Germination, Seedling growth.

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Introduction

Weeds are ubiquitous in the arable fields and hinder the germination, growth and yield of nearby grown crops. They reduce crop growth by competing for resources and lessen seedling growth by the release of different kind of allele chemicals (Aziz et al., 2021). Weeds are known as the global risk to agro and natural ecosystems (Maqbool et al., 2021a: Hussain et al., 2020). Weed invasion on large scale has been responsible for more than 1/3rd of the loss in crop productivity that grow across the world (Nadeem et al., 2020b). Weeds can cause crop yield losses up to 35%-69% in mung bean, 15%-40% in cotton, 58%-85% in soya bean, 10 to 100% in rice, 10%- 60% in wheat and 25%-93% in maize (Nadeem et al., 2021a; Ali et al., 2020). Currently, problematic weeds have been controlled by employing synthetic herbicides which is responsible for causing negative impact on environment by amassingin water and soil besides their effect on the biological diversity (Maqbool et al., 2021b).

The biochemical compounds manufactured in plant as secondary compounds have been nonsignificant role in plants but act as defensing 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; Maqbool et al., 2021c; Nadeem et al., 2021b). 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 2007; Shrestha, 2015).

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 (Goliszet *al.,* 2007; Nadeem et al., 2021a).

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 (Nadeem et al., 2020a).

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 (Nadeem et al*,* 2021a). Water extract application of allelopathy at lower concentrations stimulate development and growth of diverse crops (Cheema *et al.,* 2012: Nadeem *et al.*, 2020a: Nadeem *et al.*, 2020b). *Cinnamomumverum* 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 *Cinnamomumverum* 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. Therefore, the proposed research was

conducted to study the allelopathic effect of *Cinnamomumverum* on wild pea weed (*Paisumsativum sub species elatius*).

Materials and Methods

Collection *C. verum*plantparts

To make aqueous extract *C. verum* (bark) plant parts were purchased from Ayub Agricultural Research Institute of Faisalabad (AARI).

Preparation of *C. verum* parts aqueous extracts

Plant parts such as bark of *C. verum* were chopped in 2 to 3 cm parts. Then the chopped bark was 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 concentered 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 *C. verumm* 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 each plant part of *C. verum*were applied on *wild pea* seeds separately. Twenty (20) seeds of O. *punctate* were placed in each Petri plates containing filter paper. A 7mL of all *C. verum* extracts dilutions of each part (leaves, stem, flower and fruit) was added in respective petri plates having 3 replications of each dilution. One treatment was kept as control and moist with distilled water. 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 *wild pea*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 *Cinnamomunverum* on wild pea (weed) and 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 wild pea were placed in Petri plates and *C. verum* 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 *C. Verum*(0%, 2.5%, 5%, 10%, 20%, 40%, 80%) were applied separately on wild pea.

Data collection

Mean emergence time of *Pisumsativum subsp. elatius(*day)

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

$$MET = \sum(Dn) / \sum n$$

Emergence index of *Pisumsativum subsp. elatius*

By using formula of association of the official seed analysis (1990) 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 *Pisumsativum 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.

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

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

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 *Pisumsativum 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* 100ml methanol) Thenwraped 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.

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

Allelopathic effect of *C. verum* on emergence of seed of *Pisumsativumelatius*

Time to 50 % germination (T_{50})

The aqueous extract of *C.verum* had significant effect on T_{50} of *Pisumsativum sub species elatius* (Table 1). Maximum T_{50} (6.88) of wild pea seeds was observed at T_7 (80%) concentration of extract. while minimum T_{50} (4.23) of wild pea seed was observed at T_1 (0%) concentration. T_7 (80%) concentration showed statistically non-significant relationship with T_6 (40%) concentration.T_2 (2.5%) concentration showed significant relationship T_5 (20%) concentration. The time taken to 50% germination of wild pea seeds was increased by 62% at T_7 (80%) concentration as compared to controlled T_1 (0%). These results shows that aqueous extract of *C. verum*prduce hermetic effect at lower concentration

(10%) while allelopathic effect at higher concentration. The previous study revealed that extract of different plant parts of *M. oleifera* affected the rate of germination of V. radiata in laboratory condition. The degrees of inhibitory effects of different plant parts on germination were different. Same result reported Cheema *et al.* (1997) that sorghum water extracts inhibited germination of certain weed species. sorghum extract could significantly decrease germination percentage in some crops. 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 extract of *Cinnamomumverum* bark had significant effect on germination time of *Pisumsativum sub species elatius* (Table 1). Maximum germination time (1.86) of wild pea seed was observed at T_3 (5%) concentration of extract. while minimum germination time (1.21) of wild pea seed was observed at T_7 (80%) concentration. T_7 (80%) concentration showed statistically non-significant with T_4 (10%) concentration. T_6 (40%) on concentration showed significant relationship with T_7 (80%) concentration. The germination time of wild pea seeds was decreased by 32% at T_7 (80%) concentration as compared to controlled T_1 (0%).These results are parallel to the conclusion of Dongre and Singh, (2005)and Tanveer*et al.*(2003) who described inhibitory effects posed by the water extract of different parts of *Alternanthera* species. Aqueous extract at high concentration were inhibitory.

Mean germination time (MGT)

The extract of *Cinnamomumverum* bark had significant effect on mean germination time of *Pisumsativum sub species elatius* (Table 1). Minimum MGT (7.57) of wild pea seeds was observed at T_7 (80%) concentration of extract. while maximum MGT (9.08) of wild pea seed was observed at T_1 (0%) concentration. T_6(40%) concentration showed statistically non-significant relationship with T_5 (20%) concentration. T_7 (80%) concentration showed significant relationship with T_1 (0%) concentration. The MGT of wild pea seed was significantly increased (4%) at T_5 (20%) concentration as compared to controlled T_1 (0%). The MGT of wild pea seeds was decreased (16%) at T_7 (80%) concentration as compared to controlled T_1 (0%). These results are parallel to the conclusion of Dongre and Singh, (2005) and Tanveer *et al.*, (2003) who described inhibitory effects posed by the water extract of different parts of *Alternanthera* species. Aqueous extract at high concentration were inhibitory.

Germination percentage (GP)

The bark extract of *Cinnamomumverum* had significant effect germination percentage of *Pisumsativum sub species elatius* (Table 1). Maximum percentage of germination (93.3) of wild pea seeds was observed at T_1 (0%) concentration of extract. while minimum percentage of germination (43.3) of wild pea seed was observed at T_7 (80%) concentration. T_7 (80%) concentration showed statistically significant relationship with T_1 (0%) concentration. T_6 (40%) concentration showed non- significant relationship with T_5 (20%) concentration. The germination percentage of wild pea seed was significantly decreased 53% at T_7 (80%) concentration as compared to controlled T_1 (0%). Same result reported Cheema *et al.* (1997) that sorghum water extracts inhibited germination of certain weed species. Sorghum extract could significantly decrease germination percentage in some crops.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 effect of C. *verum* bark had significant effect on shoot length of

Pisumsativum subsp. elatius as in (Table 2). Maximum shoot length (91.1mm) of wild pea was observed T_4 (10%) concentration and minimum shoot length (21.6mm) of wild pea was observed T_7 (80%) concentration of extract. T_7 (80%) concentration showed significant relationship with controlled T_1 (0%) concentration. T_6 (40%) showed non-significant relationship with T_5 (20%) concentration. T_4 (10%) concertation showed significant relationship with controlled T_1 (0%) concentration. The shoot length of wild pea significantly decreased (64%) at T_7 (80%) concentration as compared to control T_1 (0%) while shoot length of wild pea significantly increased (51%) at T_4 (10%) concertation compared to control T_1 (0%). The result supported with previous studies that shoot length of *T. portulacastrum* was significantly reduced by application of sorghum water extract and sunflower water extract (Ashrafi *et al.*2009) over control. Similar result was shown Tworkoski, (2002) to determine the herbicidal effect plant extract oil of common spice mainly cinnamon.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 *Cinnamomumverum* bark extract had significant effect on root length of *Pisumsativum subsp. elatius* as in (Table2). Maximum root length (52.1mm) of wild pea was observed T_4 (10%) concentration of extract. Minimum root length (15.3mm) of wild pea was observed T_7 (80%) concentration of *C. verum* bark extract.T_7 (80%) concentration showed significant relationship with controlled T_1 (0%) concentration. T_6 (40%) showed non-significant relationship with T_5 (20%) concentration which was statically at par with T_3 (5%) and T_2 (2.5%) concentration. T_4 (10%) concentration showed significant relationship with controlled T_1 (0%) concentration. The root length of wild pea significantly decreased 64% at T_7 (80%) concentration as compared to control T_1 (0%) while shoot length of wild pea significantly increased 22% at T_4 (10%) concertation compared to control T_1 (0%). The results show that the inhibitory effect of aqueous bark extract of *C. verum* on the seed germination, root and shoot length were a concentration dependent phenomenon. The aqueous extracts of medicinal plants reserved the germination of Picao Preto (Cruze*et al.,* 1999). Cinnamon oil showed severe inhibiting influence, and in a semi-controlled state, higher concentration of cinnamon aqueous extract entirely repressed the seedling germination of *A. retroflexus* L. (Cavalieri and Caporali, 2010).Arooj et al., (2021) directed that the atrazine produces hermetic effect at dose of 10 g a.e. (lower dose) while showed herbicidal at higher dose (80 g a.e.) and produce maximum root length (9.99) and minimum root length (7.34 cm) respectively. Arooj et al. (2021) directed that the atrazine produces hermetic effect at dose of 10 g a.i. (lower dose) while showed herbicidal at higher dose (80 g a.i.) and produce maximum root length (9.99) and minimum root length (7.34 cm) respectively.

Shoot fresh weight (mg)

The aqueous extract of *Cinnamomumverum* (bark) had significant effect on shoot fresh weight of *Pisumsativum subsp. elatius* as described in table 2. Maximum fresh weight of shoot (293.0 mg) was noted at T_3 (5%) concentration. Minimum fresh weight of shoot (150.0 mg) was noted at T_1 (0%) concentration.T_7 (80%) concentration showed non-significant relationship with T_4 (10%) concentration. T_5 (20%) concentration showed significant relationship with control T_1 (0%) concentration. The fresh weight of shoot was significantly increased (95%) at T_3 (5%) concentration as compared to control T_1 (0%). The fresh weight of shoot significantly decreased (21%) at T_4 (10%) concentration as compared to control T_1

(0%) concentration. The fresh weight of shoot was statically similar at T_4 (10%) and T_7 (80%) concentration.These findings are not corresponding to Daniel (1999) and Uddin *et al.* (2000) studies that germination, root and shoot development was more sensitive and responded more strongly to the increasing concentration of the aqueous extract in comparison to control. (Arooj et al., 2021) reported that Atrazine at lower dose (10 g a.e.) produce hermetic effect and gave highest fresh weight while at higher dose (80 g a.e.) produce herbicidal effect and produce minimum fresh weight of *Tribulusterrestris*.Maqbool at el., (2021b) reported that the aqueous extract of *A. officinalis* (seeds) imports hermetic effect on the shoot length of wild pea. Minimum shoot length (49.8mm) of wild pea was observed at (80%) concentration extract and maximum (84.7mm) at (20%) concentration extract.

Root fresh weight (mg)

The effect of aqueous bark extracts of *C. verum*on fresh weight of wild pea root were significant summarized in (Table 2). Maximum fresh weight (166.0 mg) of wild pea root was observed at T_7 (80%) concentration and minimum fresh weight (23.3 mg) was recorded at T_4(10%) concentration of the extract. T_7 (80%) concentration showed significant relationship with T_6 (40%) concentration. T_6 (40%) showed significant relationship with controlled T_1(0%). The fresh weight of root was significantly increased (33%) at T_5 (20%) concentration as compared to control T_1 (0%). The fresh weight of root was significantly decreased 59% at T_4 (10%) concertation as compared to control T_1 (0%) concentration. Our results have shown that allelopathic activity of *C. verum* was partly depend upon the amount of allelochemicals released from bark extract as well as uptake of the compound by pant root. Results are not supported by the findings of Ashrafi *et al.* (2008) who reported significant reduction in root biomass of wild barley by application of sunflower water extract. This contradictory result might be attributed to differences in weed species under test.Maqbool et al., (2021a) revealed that the aqueous extract of *A. officinalis* seeds imports herbicidal effect on fresh weight of radish root. The fresh weight of radish root was mainly reduced as concentration of extract increase. Maximum fresh weight (26.3mg) of root was observed at 0% concentration. While minimum fresh weight (13.3mg) of root was observed at 80% concentration.

Phenolic compounds and their concentration in *C. verum*

Phenolic compounds and their concentration in *C. verum*were presented in table 3. In *C. verum*Quercetion, vanilic acid Pcoumeric acid, caffic acid and chlorogenic acid were detected.Among these phenolic compounds detected in *C. verum*maximum Quercetin (12.3 %) compound and minimum p-coumaric aid (0.89) was found.

Conclusion: The results of experiment directed that aqueous extract of bark of *C. verum*showed inhibitory effect on wild pea germination and seedling growth at higher concentration (80%) and growth regulatory effect at lower concentration. Among phenolic compounds detected in *C. verum*maximum Quercetin (12.80%) compound and minimum Syringic acid (0.60 %) was found. So, aqueous extract ofbark of *C. verum*can be use as potential bioherbicide to control weed at 80% concentration and growth regulator at lower concentration.

Table 1:Allelopathic effect of *Cinnamomumverum*on emergence of seed of *Pisumsativumelatius*

Concentration (%)	Time to 50% germination	Germination Index	Mean germination Time	Germination %
0 %	6.67a	1.79b	8.39ab	70.00ab
2.5 %	6.43ab	1.64b	8.69ab	66.67bc
5%	6.05c	1.85ab	7.99bc	70.00ab
10%	6.05c	1.86ab	7.80cd	73.33ab
20%	4.23e	2.07a	7.57d	80.00a
40%	6.86a	1.77b	8.72ab	66.67bc
80%	6.88a	1.21c	9.08a	60.00c
LSD:	**0.321**	**0.2663**	**1.171**	**11.464**

Table 2: Allelopathic effect of *Cinnamomumverum* seedling growth of seed of *Pisumsativumelatius.*

Concentration (%)	Shoot length (cm)	Root length (cm)	Shootfresh weight (cm)	Root fresh weight (cm)
0 %	60.1d	42.7b	15.0e	57.7c
2.5 %	71.4c	33.6c	16.5cd	49.3d
5%	82.8b	37.9bc	17.0c	48.3d
10%	78.6b	39.1bc	27.6b	77.3b
20%	91.1a	52.1a	29.3a	83.4a
40%	69.2c	35.6c	16.3d	30.0e
80%	21.6e	15.3d	16.2d	23.3f
LSD:	**4.8548**	**6.1069**	**5.4848**	**6.3447**

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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