



Effect of Aboveground Aqueous Extracts of *Anthemis cotula* on the Growth and Development of Certain Important Crops

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Abstract

Anthemis cotula (Asteraceae) is a dominant invader in the agroecosystems in Kashmir Himalaya, India. However, the extent of its allelopathic effects on different crops is particularly unknown. The present study evaluated the allelopathic potential of aboveground extract (0.5 to 4% concentration) of *A. cotula* on the growth performance of four important crops (viz. *Avena fatua*, *Oryza sativa*, *Triticum aestivum* and *Vigna radiata*) in a laboratory bioassay. In addition, pH, electrical conductivity (EC) and total phenolic content (TPC) of the extracts were also measured. In this study, the extracts (0.5% to 4% concentration) of aboveground part of *A. cotula* significantly ($P < 0.05$) affected the growth characteristics of the test plants in a dose-dependent manner. Radicle length, coleoptile/plumule length, dry weight, and chlorophyll content of the test plants were found to be reduced by 30–77% with the increasing extract concentration. Moreover, the responses observed were species-specific, where *O. sativa* was found to be most sensitive whereas *T. aestivum* reflected relatively less reduction in different growth parameters in response to the aboveground extracts. Besides pH, EC and TPC contents also showed significant ($P < 0.05$) variation with the increasing concentration of the extract. Correlation analysis revealed a positive relationship between pH and the plant growth responses whereas negative relationships were observed between EC and TPC with growth parameters, particularly dry weight and chlorophyll content of different plant species. Based on the observations of the study, it can be inferred that *A. cotula* has substantial phytotoxic effects on the agricultural crops, especially *O. sativa* and *A. fatua*, by the leachate of potent allelochemicals.



Article History

Received: 31 August 2023

Accepted: 15 December 2023


Keywords

Allelopathy;
Chlorophyll Content;
Inhibition;
Kashmir Himalaya;
Leachate;
Water Soluble Phenolics.

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Doi: <https://dx.doi.org/10.12944/CARJ.11.3.03>

Introduction

Allelopathy involves secondary metabolites, known as allelochemicals that are released into the nearby environment and have direct or indirect effects on the growth of other plant species.¹ Allelochemicals are water-soluble phenolic compounds, sesquiterpene lactones, etc.^{2,3} that are released through multiple natural pathways such as leaching, root exudation, volatilization, and residue decomposition.⁴ Prior to release, allelochemicals are produced and stored in different plant tissues viz., rhizomes, roots, flowers, stems, leaves, etc.⁵ Live and dead tissues of plants, such as their leaves, roots, stems, flowers, and litter decompose and produce allelochemicals that have allelopathic effects,⁶ and suppress the growth of neighbouring plants.^{7,8} The inhibitory effect of released allelochemicals can be observed at different growth stages (e.g., seed germination and seedling stage) of co-occurring plant species.^{9,10} The allelopathic ability of a plant is determined by biomass, plant density, allelochemical solubility, and adsorption in the soil.^{11,12} The released allelochemicals can also alter soil properties, mainly physicochemical properties, and diversity of soil microbial communities.¹³ Build-up of these allelochemicals in the soil has a negative impact on the growth of accompanying plants and agricultural crops grown in the next-seasons¹⁴

The present study evaluated the allelopathic potential of *Anthemis cotula* L., an annual, invasive weed of family Asteraceae. Though a native of Eurasia, the weed also occurs in different parts of the world. In India, it is reported to be a serious invader of agroecosystems especially in Kashmir Himalaya.¹⁵ The plant was first reported in Kashmir Himalaya by Stewart,¹⁶ and is now common in forests, rangelands and agricultural lands throughout Kashmir region of India.¹⁷ The plant generally inhabits low drained areas, disturbed fields, margins of agricultural fields and road side trenches. *A. cotula* is an aggressive invader in croplands and pastures where it contaminates and degrades forage quality, and is both ecologically as well as economically harmful.¹⁸ The species alters the soil physicochemical properties for its invasion success.¹⁹ Earlier studies reported inhibitory effects of aqueous leaf extract of *A. cotula* on the germination and seedling growth of *Galinsoga parviflora*, *Conyza canadensis*, *Vigna radiata*, *Brassica campestris* and *Pisum sativum*.^{20,21,22} However, little is known regarding the

impact of *A. cotula* aboveground residues on the important crop plants grown in the agricultural fields of Kashmir Himalaya. In general, the aboveground parts of the plant are removed and/or piled up nearby to the agricultural lands during cultivation, and remain there for a longer period of time. During this period, the allelochemicals from these parts may leach out, accumulate in soil and interfere with the growth of the crops. We hypothesized that the allelopathic effects of the *A. cotula* aboveground extract will vary with the plant species and their functional groups.

Hence, this study was aimed to evaluate the allelopathic effects of *A. cotula* aboveground plant extract on the seedling growth responses of four important crop species, viz., *Avena fatua*, *Oryza sativa*, *Triticum aestivum* and *Vigna radiata* grown in the Kashmir region. The objectives of the study were to: (1) explore the growth responses of different crop species under varying doses of the aboveground extract of *A. cotula*, and (2) observe the variation in pH, electrical conductivity and total phenolic content of the extracts with the increasing concentration. The findings of the study will provide an insight into the allelopathic effects and interaction between different crop growth attributes and extract properties.

Materials and Methods

Collection of Plant Material

The plant material (harvested aboveground parts, referred to as aboveground residues) was taken from the invaded agricultural fields on the outskirts of district Bandipora, Jammu and Kashmir, India (30°53'47.82" N and 75°48'26.08" E; 1578 m above mean sea level). To remove soil and other adherent contaminants, the harvested plants were rinsed under running tap water. Following that, the aboveground parts were separated, shade-dried, and turned into powder. To get fine powder, the ground powder was sieved through a 2 mm sieve and stored in ziplock bags until further usage. *A. fatua*, *O. sativa*, *T. aestivum*, and *V. radiata* seeds were obtained from a local seller in Chandigarh, India. Before the bioassay studies, the seed surfaces were disinfected for 1 minute with 0.1% sodium hypochlorite, followed by at least four washes with distilled water. The bioassay experiments were carried out in the Department of Botany, Panjab University, Chandigarh, India.

Laboratory Bioassay

The extract of *A. cotula* aboveground residues was made by soaking 6 g of the powder in 150 mL of deionized water for 24 hours at room temperature. After that, the aqueous solution was filtered through a Whatman #1 filter paper to get a stock solution (4%; w/v). The stock solution (i.e., 4%) was serially diluted to obtain other treatment concentrations, viz., 0.5%, 1% and 2%. To include the possible effect of extract treatments on seed growth, the seeds of all the study plant species were immersed in different concentrations of extract (i.e., 4%, 2%, 1%, 0.5%) and deionized water (as control) for 24 hours and afterwards the seeds were planted in Petri plates (10 seeds per Petri). Petri plates ($\phi = 15$ cm) were prepared by lining them with a thin film of cotton and filter disk (Whatman #1) and pouring of 8 mL of the respective extract (for treatment) and distilled water (as control). All the treatments (including control) were applied in three Petri plates for each crop species, i.e., 60 plates (4 crop species x 5 treatment x 3 replicates). The Petri plates were then subjected to 25 ± 2 °C temperature, 16/8-h light/dark photoperiod, and $73 \pm 2\%$ relative humidity in a growth chamber. The plates were kept for 7 days in a completely randomized design (CRD), before terminating the experiment.

Parameters Studied

Estimation of Growth Characteristics

Radicle length (cm) and coleoptile / plumule length (cm) of each crop species growing under different treatment combinations were measured with a centimetre scale. Radicle by plumule length ratio was measured to observe the relative impact of different concentration of extract on various crop species

growth performance. Radicle and plumule were oven dried for 72 hours at 75 °C, and the dry weight (TDW; g) of the entire plant material was determined.

Estimation of Chlorophyll Content

Estimation of total chlorophyll content was done using the methods described by Hiscox and Israelstam,²³ using dimethyl sulphoxide (DMSO) as solvent.

Estimation of pH, Electrical Conductivity (EC) and total Phenolic Content (TPC)

The pH and EC of extracts were estimated using a digital pH meter and conductivity meter (EcoScan; Eutech Instruments, Singapore), respectively. Total water-soluble phenolic content was estimated using Swain and Hillis' method²⁴, and reported as ferulic acid equivalents in $\mu\text{g mL}^{-1}$.

Statistical Analyses

To examine the impact of extract concentration on different crop growth responses, the data was subjected to ANOVA, followed by Duncan's multiple range test (DMRT). Tukey's HSD was also used to analyse the species-level variation and considered as significant at $P \leq 0.05$. The plant growth responses to different extract concentrations were studied using regression analysis. Pearson's correlation analysis was used to investigate the interrelationships between various plant growth and extract parameters in order to observe overall and species-specific changes. SPSS (ver. 16.0), MS Excel (ver. 10) and Sigma Plot (ver. 11) statistical software packages were used for various statistical analyses and graphical representation.

Table 1: Analysis of variance (ANOVA) table showing the variations in plant growth parameters across the species, treatment and their interaction.

| Factors | df | Plant growth parameters | | | | |
|---------------------|--------|-------------------------|----------------|-----------------------|------------|-------------------|
| | | Radicle length | Plumule length | Radicle/Plumule ratio | Dry weight | Total chlorophyll |
| Species | 3, 40 | 429.44* | 654.37* | 221.86* | 158.29* | 308.49* |
| Treatment | 4, 40 | 75.20* | 84.32* | 8.37* | 193.86* | 84.45* |
| Species x Treatment | 12, 40 | 1.81 ^{NS} | 9.26* | 2.33* | 6.79* | 4.80* |

*represents significant variation at $P \leq 0.05$ level whereas NS represent non-significant variation

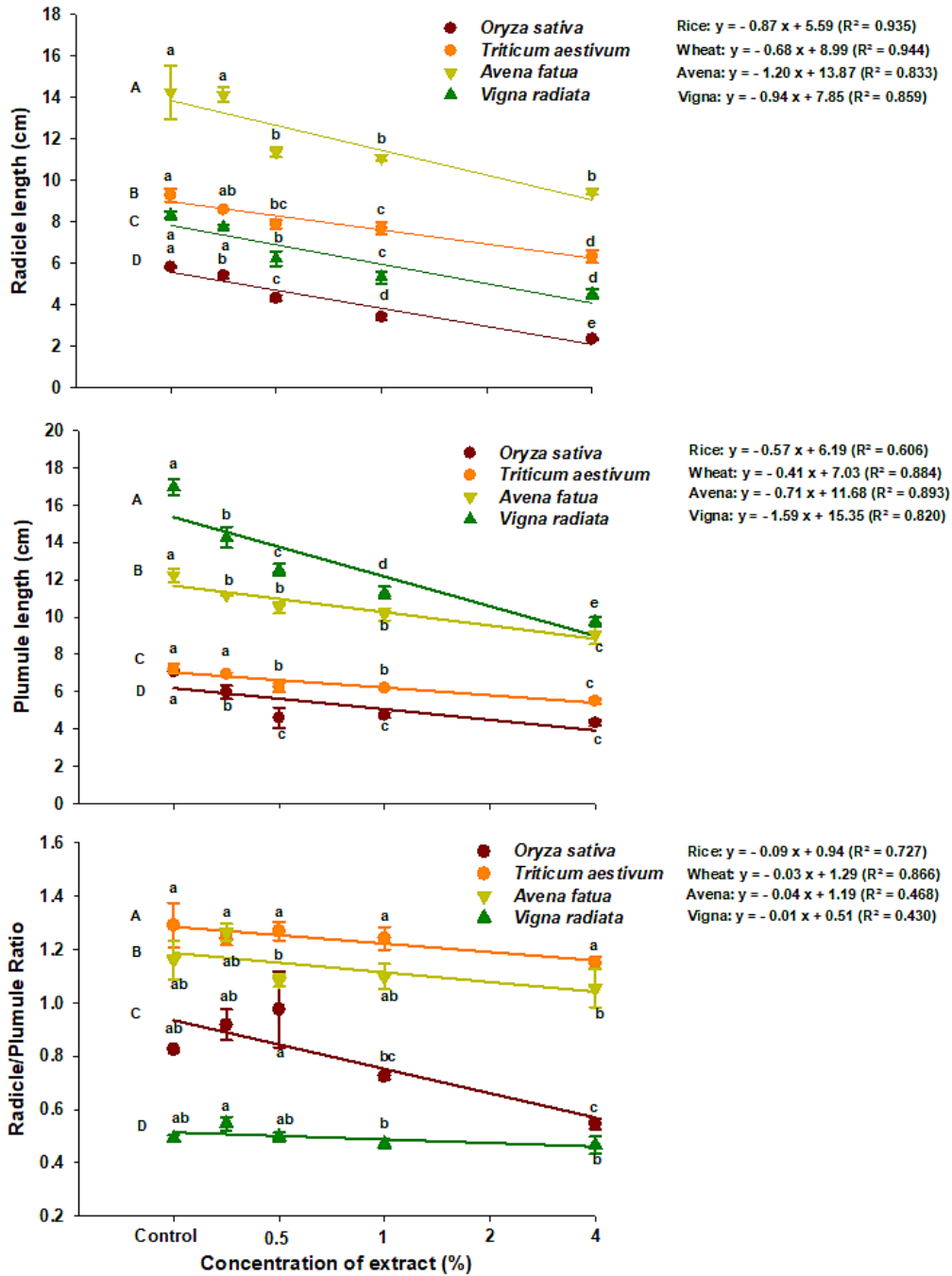


Fig. 1: Effect of *Anthemis cotula* extract (Control [0%], 0.5%, 1%, 2%, 4%) concentrations on crop growth parameters of *Avena fatua*, *Oryza sativa*, *Triticum aestivum*, and *Vigna radiata* after 7 days (DAS) in the laboratory conditions. Data are shown as regression lines, with vertical bars showing mean±SE and R^2 denoting the regression coefficient. Using Tukey's HSD, different capital letters show significant variation ($P \leq 0.05$) at the species level, whereas small letters above the dots represent significant variation ($P \leq 0.05$) in averages at different extract concentrations using DMRT.

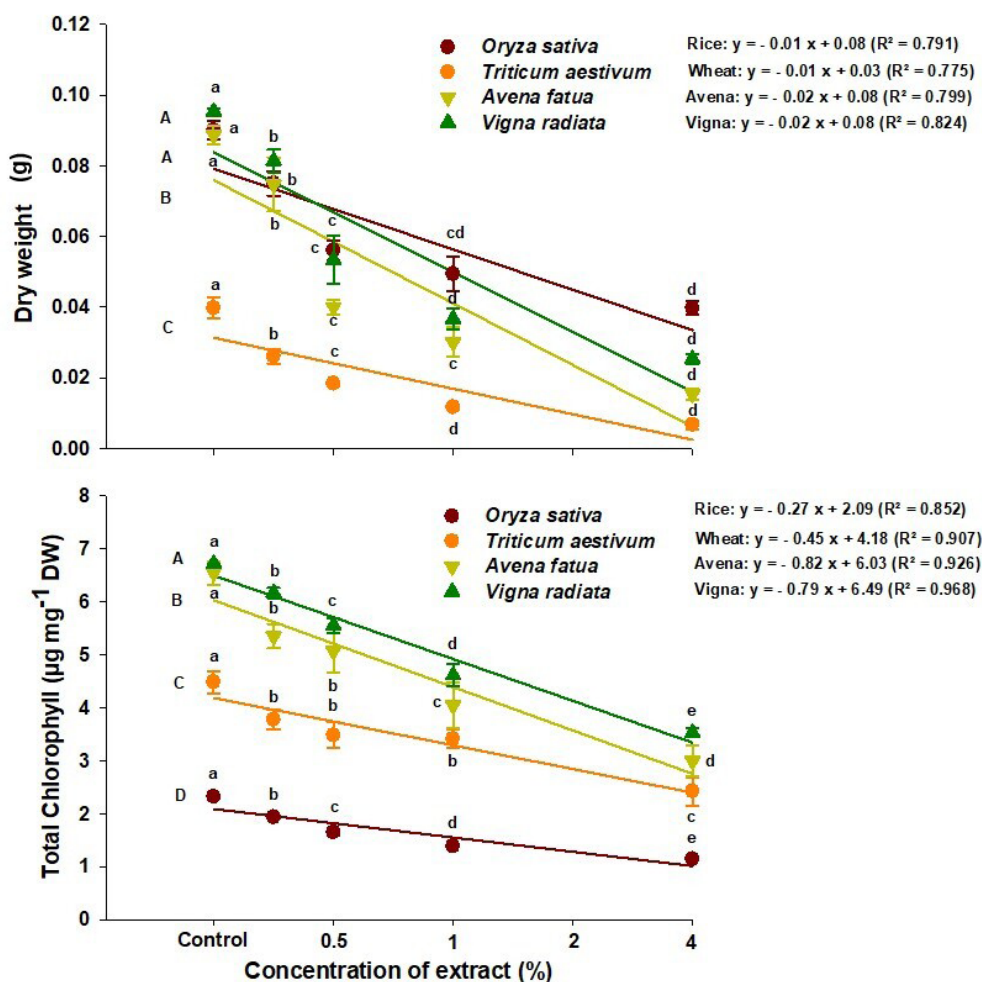


Fig. 2: The effect of different concentrations (Control [0%], 0.5%, 1%, 2%, 4%) of *Anthemis cotula* aboveground extracts on the dry weight accumulation and total chlorophyll content of four test crop species after 7 days (DAS) in the laboratory conditions. Data are shown as regression lines, with vertical bars showing mean±SE and R² denoting the regression coefficient. Using Tukey's HSD, different capital letters show significant variation (P≤0.05) at the species level, whereas small letters above the dots represent significant variation (P≤0.05) in averages at different extract concentrations using DMRT.

Results and Discussion

The current study investigated the allelopathic potential of an aqueous aboveground plant extract of *A. cotula* on the growth and development of four plant species, namely *A. fatua*, *O. sativa*, *T. aestivum*, and *V. radiata*, at different concentrations. Significant (P≤0.05) reductions in the development of test crop plants were noticed with increasing extract concentrations in the order of 0.5% < 1.0% < 2.0% < 4.0% in all analysed parameters and test plant

species (Table 1). DMRT and Tukey's analysis results revealed significant variation in the means of different extract concentrations and crop species, respectively (Figs. 1-2). In comparison to the control, the highest concentration (4%) led to the reduction of radicle length by ~60%, plumule length by ~39% (Fig. 1), dry weight by ~57%, and total chlorophyll content by ~51% (Fig. 2) in *O. sativa*. *T. aestivum* exhibited growth reductions by ~32%, ~24%, ~75%, and ~46% in radicle length, plumule length, dry

weight, and total chlorophyll content, respectively, as compared to the control. *A. fatua* showed reductions in radicle length, plumule length, dry weight, and total chlorophyll content by ~34%, ~26%, ~77%, and ~54%, respectively, when compared to the control. Furthermore, radicle length, plumule length, dry weight, and total chlorophyll content were reduced by ~45%, ~42%, ~77%, and ~47%, respectively, in *V. radiata* compared to the control (Figs. 1-2). Interestingly, the increasing concentration of extract resulted in an initial increase of up to 1% followed by a significant decrease in the radicle to plumule ratio of rice crop, whereas the variation was non-significant for wheat crop (Fig. 1). The detrimental impact was more obvious on radicle growth than plumule growth in the order of *O. sativa* > *V. radiata* > *A. fatua* > *T. aestivum*, whereas plumule length and dry weight were more affected in *V. radiata* with the increasing concentration of extract. Overall, *O. sativa* crop was found most sensitive to the aboveground extract of *A. cotula*, followed by *V. radiata*, *A. fatua* and *T. aestivum*.

Several studies have tested the allelopathic potential of different parts (particularly roots) of the *A. cotula* and reported its allelopathic potential for the adjacent herbaceous vegetation. Shahnawaz et al.²⁵ used the root extract of *A. cotula* for checking its allelopathic potential in-vitro, and reported significant inhibition on the growth of the tested crops *Zea mays*, *Brassica caulorapa* and *Raphanus raphanistrum*. Rashid and Reshi¹⁷ reported significant inhibition potential of *A. cotula* plant extract on the growth of the co-occurring weeds viz., *Conyza canadensis* and *Galinsoga parviflora*. Our findings further revealed that the impact of allelopathy is dose-dependent and species-specific. Similar observations have been reported for *Ageratina adenophora* by Yang et al.²⁶ and *Calyptocarpus vialis* by Lal et al.²⁷ The effect of aqueous extracts of *Chromolaena odorata* on *Salvadora persica* was also found concentration dependent, with the highest dose having the greatest inhibitory effect.²⁸ The root (radicle) and shoot (plumule) lengths, dry weight, and chlorophyll content were reported to decrease as the leachate dose was increased.²⁹ *Parthenium hysterophorus* reduced the growth of *R. raphanistrum* and *B. rapa* in a dose-dependent manner.¹³ Leachates made from the leaves, roots, and flowers of *Artemisia annua* and *Taraxicum officinalis* are reported to reduce the germination of maize and wheat crops

in the following order: leaf > root > flowers.³⁰ Similarly, *Chenopodium murale* has been shown to dramatically lower *Cicer arietinum* and *Pisum sativum* growth and total chlorophyll content.³¹

Different parameters such as pH ($F_{4,14} = 6877$, $P \leq 0.001$), EC ($F_{4,14} = 720800$, $P \leq 0.001$) and TPC ($F_{4,14} = 152.6$, $P \leq 0.001$) of the aboveground extracts varied significantly with the varying concentration levels (Fig. 3). The pH of the extracts was found to be decreasing with their increasing concentration i.e., 6.68 ± 0.01 (at 0.5%) and 5.81 ± 0.01 (at 4%). The EC of the leachates varied between $251.33 \pm 0.33 \mu\text{S cm}^{-1}$ (at 0.5%) and $1384.7 \pm 1.20 \mu\text{S cm}^{-1}$ (at 4%). The TPC of the leachate also increased with increasing concentration of the extract as $7.27 \pm 0.21 \mu\text{g g}^{-1}$ (at 0.5%) and $12.82 \pm 0.19 \mu\text{g g}^{-1}$ (at 4%). Lower pH values and higher EC and TPC values at the highest concentrations of the extract, for example, demonstrated that an increase in ions, particularly aromatic compounds or phenolics with acidic groups, may lead to the reduction in pH of the leachates.³² Moreover, the presence of phenolic compounds is indicated by concentration-dependent changes in pH, EC, and TPC.³³ Correlation analysis results revealed that pH had an overall positive effect whereas EC and TPC had negative effect on the different plant growth parameters, however, the extent of the effect varied with the plant species (Table 2).

We observed an initial increase in the radicle to plumule ratio with the increasing concentration of the leachate which further decrease in all the test crops, however, rice crop showed more sensitivity towards the decline. The findings of the study revealed that roots showed initial resilience to the extract concentration. However, after some extent (dose-dependent), a considerable reduction and overall mortality of the plants was observed due to the inhibitory allelopathic effects. Overall, the findings of the study suggest that with the increasing concentration of the leachates in the surrounding areas of *A. cotula*, considerable inhibitory effects can be seen on the major food crops such as rice and wheat. Since rice is a major crop grown in the rainy season, its sensitivity towards the allelopathic effects of *A. cotula* aboveground leachates is a major cause of concern and needs further attention of the scientific communities.

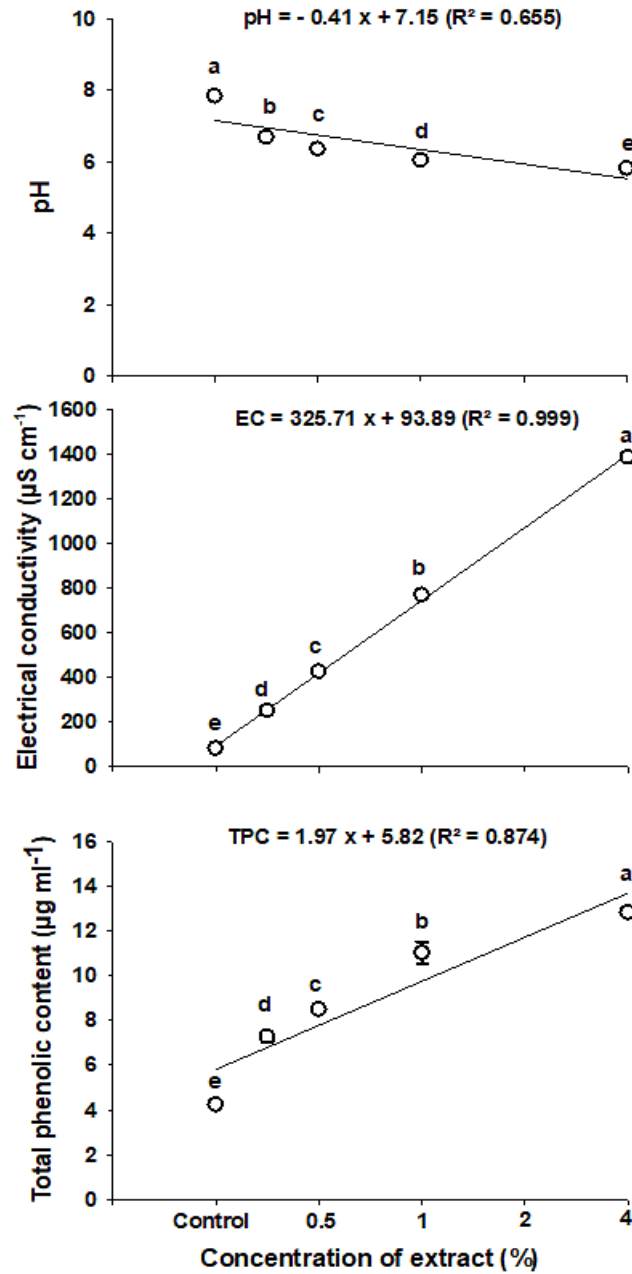


Fig. 3: The effect of *Anthemis cotula* aboveground extract (Control [0%], 0.5%, 1%, 2%, 4%) concentrations on pH, electrical conductivity, and total phenolic content. Data are shown as regression lines, with vertical bars showing mean±SE and R^2 denoting the regression coefficient. Significant variation ($P \leq 0.05$) in means at various extract concentrations using DMRT is represented by different small letters above the dots.

Table 2: Pearson's correlation analysis results for different extract and plant growth parameters for the overall dataset (N = 20), and for different crop plants (N = 5 for each).

| Parameters | pH | EC | TPC | RL | SL | R_S | DW |
|--------------------------|---------|---------|---------|--------|--------|-------|--------|
| Overall | | | | | | | |
| EC | -0.82** | | | | | | |
| TPC | -0.98** | 0.94** | | | | | |
| RL | 0.39 | -0.41 | -0.42 | | | | |
| SL | 0.36 | -0.34 | -0.37 | 0.48* | | | |
| R_S | 0.12 | -0.20 | -0.17 | 0.59** | -0.37 | | |
| DW | 0.72** | -0.69** | -0.75** | 0.17 | 0.48* | -0.31 | |
| TChl | 0.47* | -0.49* | -0.50* | 0.68** | 0.91** | -0.06 | 0.39 |
| Oryza sativa | | | | | | | |
| RL | 0.89* | -0.98** | -0.98** | | | | |
| SL | 0.96** | -0.79 | -0.91* | 0.89* | | | |
| R_S | 0.44 | -0.84 | -0.68 | 0.76 | 0.37 | | |
| DW | 0.97** | -0.89* | -0.98** | 0.97** | 0.98** | 0.57 | |
| TChl | 0.97** | -0.93* | -0.99** | 0.98** | 0.94* | 0.63 | 0.99** |
| Triticum aestivum | | | | | | | |
| RL | 0.89* | -0.98** | -0.96** | | | | |
| SL | 0.89* | -0.94* | -0.95* | 0.99** | | | |
| R_S | 0.74 | -0.92* | -0.84 | 0.89* | 0.81 | | |
| DW | 0.99** | -0.89* | -0.99** | 0.94* | 0.94* | 0.78 | |
| TChl | 0.91* | -0.96* | -0.96* | 0.99** | 0.97** | 0.91* | 0.93* |
| Avena fatua | | | | | | | |
| RL | 0.86 | -0.91* | -0.92* | | | | |
| SL | 0.96* | -0.96* | -0.99** | 0.94* | | | |
| R_S | 0.52 | -0.69 | -0.63 | 0.88 | 0.67 | | |
| DW | 0.92* | -0.90* | -0.97** | 0.99** | 0.97** | 0.79 | |
| TChl | 0.93* | -0.97** | -0.99** | 0.91* | 0.99** | 0.61 | 0.94* |
| Vigna radiata | | | | | | | |
| RL | 0.91* | -0.93* | -0.98** | | | | |
| SL | 0.98** | -0.91* | -0.99** | 0.98** | | | |
| R_S | 0.36 | -0.67 | -0.56 | 0.70 | 0.52 | | |
| DW | 0.93* | -0.91* | -0.98** | 0.99** | 0.98** | 0.67 | |
| TChl | 0.89* | -0.99** | -0.98** | 0.98** | 0.96* | 0.67 | 0.97** |

* and ** represent significance level at $P < 0.05$ and $P < 0.01$ levels, respectively. EC = electrical conductivity; TPC = water soluble phenolics; RL = radicle length; SL = plumule length; R_S = radicle to plumule ratio; DW = dry weight; TChl = total chlorophyll.

Conclusions

Based on the findings of this study, it can be concluded that *A. cotula* exhibits allelopathic behaviour via leachate. Allelopathic effects observed were both dose-dependent and species-specific. The aboveground extracts of *A. cotula* was found inhibitory to the seedling's growth of all the test plants viz., *A. fatua*, *O. sativa*, *T. aestivum* and *V. radiata*. All the treatments inhibited the seedling

growth parameters (viz., radicle length, plumule length and chlorophyll content) of the test plants, over the control. Rice plant was found to be most sensitive to the extract concentration whereas wheat crop showed comparatively better resilience. Further research is required to identify the allelochemical components and their amount in *A. cotula* causing the inhibitory effects on the test plants or the co-occurring plant species in the Kashmir Himalaya.

Acknowledgement

DZ and MAD are thankful to the Council of Scientific and Industrial Research (CSIR), India, for providing research fellowships.

Funding

"DZ and MAD are thankful to the Council of Scientific and Industrial Research (CSIR), India,

for providing financial support. RS is thankful to Science and Engineering Research Board (SERB), New Delhi, India for providing research funding as NPDF fellowship (PDF/2020/001607).

Conflict of Interest

The authors do not have any conflict of interest.

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