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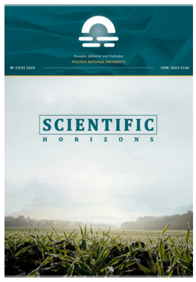

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Herbicidal Effects of *Chenopodium murale* and *Coronopus didymus* Sm. Residues Against Germination and Early Growth of *Hordeum vulgare*

Dinesh Kumar Gautam[1], Dushyant Kumar Singh[2*], Rohan John D' Souza[3], Rajneesh Kumar Agnihotri[1]

[1]Dr. B.R. Ambedkar University
282005, Khandari Campus, Agra, India

[2]Bundelkhand University Jhansi
284128, Kanpur Rd, Jhansi, India

[3]St. John's College
282005, Mahatma Gandhi Rd, Agra, India

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Abstract. This study reports the common problems of weed species that adversely affect the crop productivity of the agriculture crops at a large scale. The purpose of this study is to investigate the impact of dried application of *Chenopodium murale* and *Coronopus didymus* on seed germination and early growth of *Hordeum vulgare*. The experiment was performed in a randomised block design with three replications under the greenhouse condition in pot culture. Shoots and roots materials were separately dried in shade for 15-20 days, dry powdered shoot and root residues of *C. murale* and *C. didymus* were applied at 5 and 10 g kg^{-1} doses to barley seeds in 6 pots with control for three weeks. *C. didymus* (10 g) shoot residues were most inhibitive against germination (31.16%), GVI (0.85), SVI (4.90) and leaf area (3.94 cm^2) of barley while 5 g root residues of the weed had least pronounced effect. Root length, shoot length, and dry biomass were most inhibited by *C. murale* 10 g in both shoot and root residue treatments. Shoot residues were more inhibitive of germination and growth than root residues of both weeds. Chlorophyll accumulation patterns showed mixed results with some treatments even stimulating their concentrations. Root treatments were in general more inhibitive than shoot treatments. All treated seedlings exhibited higher levels of proline accumulation compared to control. At lower dose, *C. murale* treatments were more inhibitive than *C. didymus* treatments. There is a great scope of research on these species to isolate and identify the active factors and also to understand their implication in the biocontrol of weeds apart from their potential negative effects on agricultural crops, especially cereal crops, which can be useful to increasing crop production in northern India, namely in Uttar Pradesh

Keywords: agriculture activity, allelopathy, seed germination, proline estimation, biomass production, weed residues

*Corresponding author

INTRODUCTION

Weeds are undesirable as they compete with crops for moisture, nutrients, and light. Although these are non-native plants, but their spreading and fast-growing nature makes it as a huge threat to the growing crop species. They have persisted as a huge problem for farmer ever since the beginning of agriculture because they cause high economic loses of crop producers in crops yield, increase costs of crop production and reduced crop quality (Bhular *et al.*, 1998). Identification of distinct species of weed plants with allelopathic potential and characterisation of their adverse effects on associated crops are essential to understand weed-crop interactions in agro-ecosystems. The crop growth can also be influenced through exudates secreted from weeds commonly known as allelochemicals into the surrounding habitat (Kadioglue *et al.*, 2005). Farmers are thus compelled to use chemical weedicides which adversely affect the yield, consumer health, and the environment. Allelochemicals are secondary metabolites released from leaves, stem, roots, fruits, and seeds which may delay or completely inhibit seed germination of target plant and result in stunted root and shoot proliferation. They represent a wide pool of chemical compounds with an equally wide range of possible uses. Plants having allelopathic properties may be prospered as a cover crop or their residues incorporated to prevent other weeds/pests. They may increase fertility because it is organic matter being added. Some workers are exploring their use as bioherbicides as they are considered safer than synthetic chemicals. In such ways their negative properties can be used in positive ways (Zeng *et al.*, 2008).

Barley ranks 5^{th} among the world-wide produced crops (Soleymani & Shahrajabian, 2011). An annual crop, it is used for food, brewing malt beverages, and livestock feed. *Chenopodium murle* is an annual weed that can grow in most environmental and soil conditions (Guertin, 2003). It produces cyanogenic glycosides, saponins, tannins, naphthoquinones, alkaloids, and flavonoids (Verma & Agarwal, 1985). *Coronopus didymus* has some medicinal uses (Prabhakar *et al.*, 2002). It belongs to family Brassicaceae known to produce allelochemicals such as glucosinolates (Bones & Rossiter, 1996). Such compounds restrict their value as a feed or fodder but indicate their potential use as a natural weedicide.

The purpose of this study was to explore the potential of shoot and root residues with allelopathic properties of *C. murale* and *C. didymus* against germination, growth, and biochemical parameters of barley and to make comparisons based on plant, plant part, and dosage used.

MATERIALS AND METHODS

The greenhouse experiment was set up to assess the allelopathic potential of the two weeds selected for the study of seed germination and productive physiological growth of barley at the Department of Botany, School of Life Sciences, Dr. B.R. Ambedkar University, Khandari Campus, Agra during February–June 2015.

Two weeds (*Chenopodium murale* and *Coronopus didymus*) were collected in polythene bags, brought to laboratory, and air-dried in shade for about 15-20 days and then powdered and stored at 5°C till further use. Pots (6” diameter) were filled with soil (soil sand ratio 3:1) previously sterilised in an autoclave at 121°C and 12-14 psi for about 25 minutes. Shoot/root powder from each selected weed was applied separately to all treated pots at 5 g and 10 g soil. The control set up was maintained with no residual treatment.

A completely randomised block design (RBD) was laid out on the whole experiment with three replicates and control. Barley seeds were washed with distilled water and surface sterilised with 5% Bavistin (2.5 g/100ml distilled water) and 0.1% mercuric chloride (0.10 g/80 ml distilled water). Fifteen seeds were sown in each pot and observed for three weeks. All pots were watered with tap water. Number of seeds germinated was recorded every day. The measurement of the experiment started when the seedlings reached 2 mm in height. After three weeks of germination, shoot/root length and dry biomass were measured. To obtain the dry biomass, the samples were thoroughly washed with water, dried on blotting paper, and were then placed in an oven.

Germination percentage was computed according to AOSA (1990); Germination Velocity Index [GVI] according to AOSA (1983) and Seedling Vigour Index [SVI] according to A. Abdul-baki and J. Anderson (1973). Leaf area was determined by using graph paper method where the leaves were outlined on graph paper and the covered square area was measured (Taghipour & Salehi, 2008). The dry biomass was taken after thorough washing and drying at 60°C to constant weight. Chlorophyll (Chlorophyll a and Chlorophyll b) and proline estimation was done as per D. Arnon (1949) and L.R.P. Bates *et al.* (1973), respectively. Three ways of variance analysis were performed for the data and the mean differences were separated using Fisher’s LSD test at 5% probability level. Graphical representation of the data was made, and standard errors were computed using MS Excel.

RESULTS AND DISCUSSION

Treatment with *C. didymus* (10 g) shoot residue was most inhibitive against germination (31.16%), GVI (0.85), and SVI (4.90) of barley while 5 g root residues of the weed had the least pronounced effect. Maximum reduction in shoot length (15.44 cm) was observed in seedlings treated with 10 g shoot residues of *C. murale*, slightly more than in those treated with 10 g of *C. didymus* root residues (15.67 cm) (Table 1). Least inhibition was observed in 5 g root residue treatment of *C. didymus* (25.23 cm). Root length and dry biomass were most inhibited by *C. murale* 10 g treatments in both shoot and root residue treatments. In general, shoot residues were more inhibitive of growth parameters with least inhibition observed in 5 g root residue treatments of *C. didymus*. The effects were dose dependent.

***Table 1**. Germination and growth parameters of barley seedlings*

Weeds	Part used	Concentration	Germination %	GVI	SVI	Leaf Area (in cm²)	Shoot Length (in cm)	Root Length (in cm)	Dry Biomass (in g)
Control		–	95.6±3.81	10.4±1.03	26.3±2.59	12.06±0.75	27.47±1.7	20.24±1.8	36.54±1.16
C. murale	Shoot	5 g	75.6±15.40	4.92±0.17	14.97±3.07	8.8±0.46	19.8±0.5	12.27±1.5	28.04±0.87
		10 g	62.23±36.71	2.6±2.11	9.39±5.30	7.53±0.65	15.44±0.9	6.97±1.8	25.44±0.65
	Root	5 g	91.16±3.87	8.98±2.09	21.98±1.63	11.13±1.19	24.1±1.0	15.3±1.2	34.0±0.72
		10 g	88.93±4.0	6.86±2.22	19.89±1.33	10.7±0.56	22.36±0.6	14.8±0.3	29.84±2.22
C. didymus	Shoot	5 g	86.7±6.70	6.70±2.14	20.79±2.06	10.83±1.27	23.97±0.7	15.2±0.6	31.44±1.74
		10 g	31.16±20.37	0.85±0.65	4.90±3.20	3.94±0.64	15.67±0.7	8.6±1.3	29.9±4.52
	Root	5 g	93.36±6.65	10.29±0.39	23.55±2.77	11.93±1.15	25.23±2.4	19.17±2.3	35.4±3.46
		10 g	91.16±3.87	9.95±2.40	22.2±2.59	11.23±2.17	24.26±2.7	17.97±3.9	32.2±0.50

Thus, at the higher dose, *C. didymus* shoot residues in soil inhibited the germination of barley seeds the most (germination %, GVI, and SVI). At the same dose, *C. murale* affected growth parameters of growth in root length, shoot length, and dry biomass the most. Thus, the two weeds had different effects on different parameters of the test plant (Table 1). Allelochemicals can reduce cell division or interfere with auxin, the phytohormone which influences shoot and root growth (Gholami *et al.*, 2011). A. Enyew and R. Nagapan (2015) found that leaf powder of *Lantana camara* at higher dose (75 g) inhibits the germination percentage, root and shoot length, stem thickness and biomass of *Zea mays* and *Triticum aestivum*. *Ocimum basilicum* shoot residues in soil have been demonstrated to reduce plant height, leaf number, root length and total biomass of cereal crops (Dafaallah *et al.*, 2017).

Leaf area trends were similar to GVI and SVI. Least leaf area of barley was observed in. *C. didymus* (10 g) shoot residue treatments, while highest readings were observed in 5 g root residue treatment of the same weed. Shoot treatments were more inhibitive while the difference in leaf area patterns among the root residue treatments of both species was not very pronounced. Such reduction in leaf area of test plants in response to different allelopathic species has been reported earlier on *Convolvulus arvensis* due to powder treatments of *Ricinus communis*, *Nicotiana tabacum*, *Datura inoxia,* and *Sorghum vulgare* (Nekoman *et al.*, 2013).

Apart from maximum inhibition of growth parameters at a higher dose, it is interesting to note that at a lower dose, both shoot and root treatments of *C. murale* were more inhibitive than *C. didymus* treatments. Such effects of this weed on growth and photosynthesis of barley have been reported by N. Al-Johani *et al.* (2012). In other studies, *C. murale* extracts suppressed shoot length, shoot biomass, total root length, number of roots and root biomass of test plants (Shafique *et al.*, 2011; Gautam *et al.*, 2018).

Allelochemicals can stimulate chlorophyll degrading pathways and affect the photosynthetic potential and thus the growth of target plant. Chlorophyll accumulation trends in test seedlings under study were variable (Fig. 1 A-C). Root treatments were generally more inhibitive than shoot treatments. Root residue treatments at 10 g dose of *C. murale* affected chlorophyll 'a' content the most while those of *C. didymus* inhibited chlorophyll 'b' and total chlorophyll contents. Effect of higher doses of *C. didymus* root and *C. murale* shoot on total chlorophyll content was almost similar (0.245 and 0.246 μg g^{-1} fw, respectively). Despite their inhibitory effect on most test seedling parameters, most treatments of. *C. didymus* and some of *C. murale* showed a positive effect and enhanced chlorophyll content in test seedlings. Three out of eight treatments showed increased chlorophyll 'a' content over control; five showed increased chlorophyll 'b' content and four showed increased levels in total chlorophyll. This again indicates the complex nature of the interactions evaluated and their sensitivity to plant part, dose, and test plant used. Both inhibitory and stimulatory activities of *Mimosa pigra* leaf residues have been reported on *Ruellia tuberosa* and *Portulaca oleracea* (Koodkaew & Rottasa, 2017). W. Al-Taisan (2014) reported inhibition of total chlorophyll in leaves of *Oryza sativa* and *Teucrium polium* due to *Heliotropium bacciferum* leaf extract at higher dose but stimulation of same total chlorophyll at lower doses. T. Vaithiyanathan *et al.* (2014) recorded the highest inhibition of photosynthetic pigments of *Abelmoschus esculentus* due to root extracts of *Azadirachta indica*. Similar findings have also been reported in the case of *C. didymus* leaf extract on *Triticum aestivum* (Khaliq *et al.*, 2015) and *Azadirachta indica* leaf extracts on *Vigna radiata* seedlings (Shruthi *et al.*, 2015).

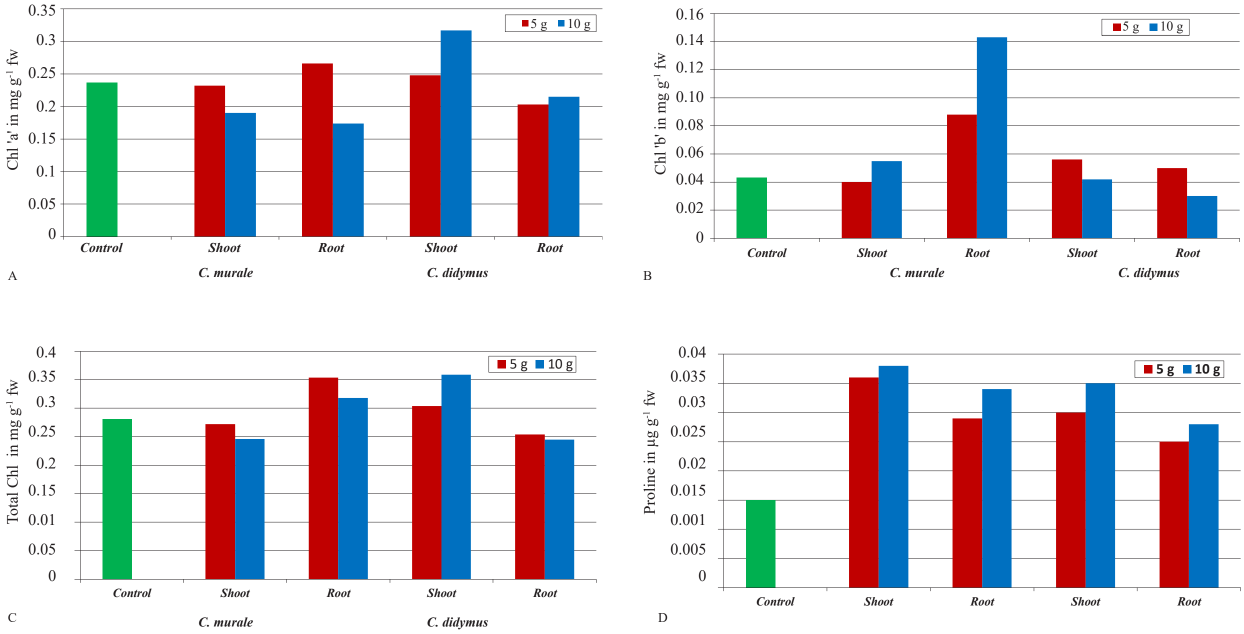

Figure 1. *Biochemical parameters of barley seedlings (A) chlorophyll 'a' (B) chlorophyll 'b' (C) total chlorophyll and (D) proline*

Proline is an osmoprotectant which increases tolerance of plants as an important part of structural proteins and enzymes. All treated seedlings showed increased proline accumulation in leaves, which was directly proportional to dose applied (Fig. 1 D). Shoot residues of *C. murale* (10 g and 5 g) promoted maximum proline content. Proline accumulation due to *C. didymus* 10 g shoot treatment was comparable to that of *C. murale* 5 g shoot treatment. Similar mode of effectiveness was inspected in case of root treatments too. In general, shoot residues caused greater proline accumulation indicative of greater stress as was clearly seen in germination and growth parameters. The effect was dose dependent. W. Al-Taisan (2014) reported dose dependent proline accumulation in leaves of *Oryza sativa* and *Teucrium polium* due to *Heliotropium bacciferum* leaf extracts. Similar increased proline levels have been documented in *Vigna unguiculata* (Oyeniyi *et al.*, 2016) and *Phaseolus aureus* (Christobel *et al.*, 2017) in response to allelochemicals.

Results show a statistically significant difference ($P \leqslant 0.01$) in the mean values of root length, shoot length and dry biomass of barley, based on the weed residue used. Similar findings were received for GVI, and dry biomass based on plant part (root/shoot) and concentrations used. The effect of different weed on mean chlorophyll 'a', chlorophyll 'b' and total chlorophyll concentrations depended on the plant part (shoot/root) used as indicated by the statistically significant interaction between weed and plant part. Significant interaction between weed and concentration was also seen for mean chlorophyll 'a' and 'b' concentrations; and between plant part and concentration for germination percentage, root length, shoot length, and chlorophyll 'a'. The interaction between weed × plant part × concentrations was statistically significant for mean values of SVI, leaf area, and proline.

Fisher's Least Significant Difference (LSD) test indicated significant difference between mean values of root length, shoot length, dry biomass, chlorophyll 'a', chlorophyll 'b' and proline based on weed (*C. murale* vs. *C. didymus*) used. Similar differences were obtained in values of germination percentage, GVI, SVI, root length, shoot length, dry biomass, leaf area, chlorophyll 'a' and 'b' based on plant part (root vs. shoot) used; and in values of germination percentage, GVI, SVI, root length, shoot length, dry biomass, leaf area, and proline based on concentration (5 g vs. 10 g) used.

CONCLUSIONS

In-vitro studies attempt to understand plant-soil-microbe interactions in a somewhat isolated microhabitat and the results are sometimes oversimplified. This study recommends the dry powder application of the selected these two weed species; *C. murale* and *C. didymus* had measurable inhibitory effect on most of the testing parameters of barley. The results obtained indicated that the higher applied dose had more negative effects. *C. murale* at 10 g dose was found most effective against shoot length, root length, dry biomass, chlorophyll 'a' and total chlorophyll content while germination percentage, GVI, SVI and leaf area were most affected under the application of *C. didymus* at 10 g dose. On the other hand, lower doses of both weeds considerably reduced the chlorophyll 'b' and proline content. Exceptionally, chlorophyll 'a' and total chlorophyll content were found to show positive effect at 5 g and 10 g doses of *C. murale* and *C. didymus,* respectively. Furthermore, the degree of inhibition of allelopathic plants depends not only on the dose applied but also on the plant part used due to different concentrations of allelochemicals. Thus, the scope for work on *C. murale* and *C. didymus* is much bigger to further identify and isolate the active factors responsible for their allelopathic properties to explore their possible use as biocontrol agents of weeds. Greenhouse and field studies are important to confirm such results to encompass the more subtle and complicated social environment of soil and plants.

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Гербіцидна дія *Chenopodium murale* та залишків *Coronopus didymus* Sm. проти проростання та раннього росту *Hordeum vulgare*

Дінеш Кумар Гаутам[1], Душ'янт Кумар Сінгх[2], Рохан Джон Д'Соуза[1], Ранджіш Кумар Агніхотрі[3]

[1]Dr. Університет Др. Б.Р. Амбедкара
282005, кампус Кхандарі, м. Агра, Індія

[2]Університет Бунделкханда Джхансі
284128, дор. Канпур, м. Джаньши, Індія

[3]Коледж св.Джона
282005, дор. Махатми Ганді, м. Агра, Індія

Анотація. У цьому дослідженні розглядаються загальні проблеми тих видів бур'янів, які негативно впливають на продуктивність сільськогосподарських культур у великих масштабах. Мета роботи – дослідити вплив висушеного коріння *Chenopodium murale* та *Coronopus didymus* на проростання насіння та ранній ріст *Hordeum vulgare*. Експеримент проводили у рандомізованому блочному плані з трьома повтореннями в умовах теплиці в горщиковій культурі. Пагони і коренеплоди окремо сушили в тіні протягом 15–20 днів, сухі порошкоподібні залишки пагонів і коренів *C. murale* і *C. didymus* вносили в дозах 5 і 10 г кг$^{-1}$ на насіння ячменю в 6 горщиках з контролем протягом трьох тижнів. Залишки пагонів *C. didymus* (10 г) найбільше пригнічували проростання (31,16 %), GVI (0,85), SVI (4,90) та площі листя (3,94 см^2) ячменю, а 5 г кореневих залишків бур'яну мали найменш виражену дію. Довжина кореня, довжина пагона та суха біомаса найбільше пригнічувалися *C. murale* 10 г при обробці як пагонів, так і кореневих залишків. Залишки пагонів пригнічували проростання та ріст, ніж залишки коренів обох бур'янів. Патерни накопичення хлорофілу показали неоднозначні результати, у деяких зразках їх концентрація навпаки посилювалася. Обробка коренів загалом була більш гальмівною, ніж обробка пагонів. Усі оброблені проростки демонстрували вищі рівні накопичення проліну порівняно з контролем. При меншій дозі застосування *C. murale* було більш інгібуючим, ніж застосування *C. didymus*. Існує великий обсяг досліджень цих видів, щоб виділити та ідентифікувати активні фактори, а також зрозуміти їх вплив на біоконтроль бур'янів, крім їх потенційного негативного впливу на сільськогосподарські культури, особливо зернові культури, які можуть бути корисними для збільшення виробництва сільськогосподарських культур. на півночі Індії, а саме в Уттар-Прадеші

Ключові слова: сільськогосподарська діяльність, алелопатія, схожість насіння, оцінка проліну, виробництво біомаси, залишки бур'янів