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Evaluation of the Physicochemical Characteristics of Residue/Powder of Above-ground Parts (Leaf and Stem), Bio-efficiency on the Morphology and the Extraction of Phytochemicals from the Leaves of *Amaranthus viridis* L.

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Abstract

Weeds compete with the crops grown by taking their necessities of growth and germination and affecting crop productivity and suppress the growth of crops by releasing allelochemicals present in them and thus, this ability of weeds could be exploited technically to suppress the growth of other weeds. Thus, reducing the use of synthetic weedicides and pressure on the ecosystem creating a green-globe. The purpose of the study was to closely examine the phytotoxic effects of various soils amended with various residue extracts of stem and leaf powder of *Amaranthus viridis* {(a) Residue amended soil (RAS) (b) Residue extract amended soil (RAES) and (c) Residue extract (RE)} on the morphology of the selected plants, their physicochemical features of soils amended with various extracts and to understand the dynamics of the release of allelochemicals (phenolics) and to extract them when exposed to aqueous and organic solvents.

***Key words*:** *Amaranthus viridis*, Amended soils, Morphology, Physicochemical, Phenolics

Amaranthus viridis L. is known by its common name, Chowlai, and is a cosmopolitan species found in the tropical regions [1]. Amaranthus viridis is valued for its edible leaves and young shoots, which are consumed as a leafy vegetable in many cultures. According to Reyad-ul-Ferdous *et al*. [2], it is a short-lived perennial herb that can grow up to 1 m tall or an erect annual/glabrous plant with angular branches and a slender to sparsely hairy upper stem. The leaves are ovoid, 3-6 cm long, 2-4 cm wide, and have extended leaflets up to a point of 5 cm, when several pedestal-like offshoots emerge. According to Parveen *et al*. [1], the plant produces terminal panicles with few branches and little green flowers with three stamens.

The allelopathic potentiality of above-ground plant material, often known as residue, was to be examined for which the following criteria were taken into account -Assessment of the residue/unit area, Phytotoxic potential of the residue-amended soil (RAS), residue-extract-amended soil (REAS) and the residue-extract (RE) on *Cicer arietinum* L., *Triticum aestivum* L., *Phalaris minor* Retz. *Vicia villosa* Roth, dynamics of phytochemicals in RAS, REAS and RE and identification of physico-chemical properties such as pH, osmotic potential, total water-soluble phenolics in RAS, REAS and RE and to extract phytochemicals from the leaf of *Amaranthus viridis* so, to be used for exploration of their bio-efficiency against plant species such as *Phalaris minor*, *Vicia villosa*, *Amaranthus spinosus*, and *Oryza sativa* (in the form of germination percentage, seed vigour, and mean seedling growth).

Fig 1 Photograph of *A. viridis* L. at the flowering stage was taken from mobile. It was captured in the month of November, 2019 during the collection period of material from the Aligarh Muslim University Campus to carry out the current experiment

MATERIALS AND METHODS

Compilation of the material

AMU campus's periphery was the scene of *A. viridis* invasion. After the plants were utterly dehydrated at the end of their life cycle, 20 quadrats (1 m^2/quadrat) of a square meter

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were set up to measure plant density and biomass. The naturally dehydrated plant residue (above ground) was obtained, smashed into a powder, and stored in polyethene bags for later use. The dirt that came from a public space was removed from *A. viridis*. After air drying, it was separated into 1 kg chunks and sieved through a 2 mm filter. Weed and crop seeds were bought from NRCWS in Jabalpur, India, and IARI in New Delhi (India).

Preparation of residue-amended soil (RAS) and residue extract-amended soil (REAS)

In the natural world, *A. viridis* dies and falls to the ground, mingling with the soil. To replicate these conditions, 1 kilogram of soil was mixed with 40 g, 20, 10 and 5 gm of residue, blending the mixture to yield 4%, 2%, 1%, and 0.5% RAS, respectively. In order to frame REAS, we first made RE, which involved immersing 40 g of residue in 1000 ml double distilled water (DDW) for a full day at room temperature. To obtain 4% extract, it was sieved through two layers of muslin cloth and then Whatman No. 1 filter paper. DDW was used to dilute the extracts further to provide 2%, 1% and 0.5%. We referred to these solutions as RE. A portion of the RE was utilized to create REAS, and the remainder was set aside in a different experiment (growth studies using RE in a lab setting). One kilogram of soil was placed discretely, 27 by 15 cm plastic trays, and 500 millilitres of 0.5%, 1%, 2%, and 4% residue extract was added. The trays were then permitted to dry in the shade for 30 hours. Subsequently, 200 g of RAS or REAS were added to 15 cm diameter Petri dishes. As a control, untreated/unaltered soil, denoted as "US," was also obtained and put in Petri dishes.

Growth studies in amended soil

In 15 cm diameter Petri plates filled with RAS and RAES, ten uniform seeds of each chosen crop and weed seed were placed beside the US, which acted as the control. Every treatment was maintained in five repetitions using a completely randomized block design (CRBD). The replicates were kept in a growth room with a 16/8 hours light/dark photoperiod, 25±2°C, and 75±2% relative humidity. Every day for eight days, 25 millilitres of pure water were added to each Petri dish. After that, the saplings were carefully removed not to damage the roots. After 24 hours of oven drying at 80°C, the biomass of the seedlings was calculated, and their root and shoot lengths were measured using a meter scale.

Growth studies in residue extract (RE)

The RE's total phenolic content, pH, and osmotic potential were measured. Test plant seeds were subjected to various extract concentrations (0.5%, 1%, 2%, and 4%), individually, in vitro. The control was double distilled water (DDW). Ten seeds of each test plant were equally distributed throughout 15 cm diameter Petri plates lined with Whatman Grade 1 filter paper, sanitized absorbent cotton, and sprinkled with 15 cc of the appropriate treatment solution. For every treatment, five replicates were kept in a fully randomized setup. The entire setup was maintained at 25±2°C and 75±2% relative humidity in a seed germinator. Following eight days in each Petri dish, the length of the seedling's roots and shoots and the dry biomass was calculated using the previously stated approach.

Estimation of phenolic allelochemicals in A. viridis residue extracts (RE) and amended soils (RAS)

Four separate components were used to evaluate the total amount of phenolics. 500 ml of 4% residue extract was applied to 1 kilogram of soil (RAS) for the first section. In the second section, 1 kg of dirt was used as REAS, and 40 g of residue and 500 ml of pure water were added. After 0, 5, 10, 15, 20, 30, 40, 50, 80, 90, 100, 110, 120, 130, 140, 160, 170, 180 and 200 hours in RAS and 3, 6, 9, 12, 20, 28, 38, 52, 70, 90 and 100 in REAS, five grams of soil were removed from each section separately. The soil was then air-dried and phenolic acids were extracted using the Folin-Ciocalteu reagent method, as Swain and Hillis [3] described. In the third section, 1 kilogram of soil and 500 ml of pure water were combined and mixed well (control), following which the phenolic content was determined. The fourth section involved adding 40 g of residue to 1000 ml of pure water and blending it well (RE). After 2, 5, 7, 10, 14, 18, 24, 28, 34, 42, 62 and up to 74 hours, 5 ml of the residue was extracted, and the amount of phenolics in each of these extracts was then calculated. During the whole trial, five replicates of each treatment were kept.

Evaluation of physicochemical properties of amended soils

An array of characteristics was examined in amended soils, such as residue amended (RAS), residue extract amended (REAS) and unamended soils (US or Control), including pH (1:5 soil/water, w/v), osmotic potential, and OP (1:5 soil/water, w/v). A pH meter (Eutech Instruments Pvt. Limited, Singapore) was used to measure the pH. Using an EcoScan digital conductivity, the OP was calculated using the following formula:

Osmotic Potential = 0.36 × Conductivity (mS) and EC

Growth attributes

Various biomarkers of growth like root length and shoot length with seed vigor for *O. sativa* seeds did not show any growth or germination because aqueous leachates (AL), petroleum fraction (PF), the methanolic fraction (MF) and chloroform fraction (CF) proved to be most detrimental for its development but it showed very little germination with meagre per cent of 5.33 in water fraction (WF) with 3.08 and 3.10 per cent of root length observed with the help of the hand lens. Moreover, the germination per cent at control displayed to be 80.63, with seed vigor of 65.56, along with the root length and shoot length of 5.62 and 9.47 per cent was generated on the second day in control (Table 2). For *P. minor*, all the growth markers, germination, and seed vigor failed when the seeds were treated with aqueous leachates, petroleum fraction, chloroform fraction, water fraction, and methanol fraction extracted allelochemicals. Seeds of *Phalaris* presented pronounced germination of 84.85 with seed vigor of 71.19, root length and shoot length of 3.79 and 8.43 per cent at control (Table 2), respectively. Maximum values of germination per cent (76.52 and 65.86) and seed vigor (71.58 and 71.66) were attained by both *A. spinosus* and *V. villosa* on the third day at control which were almost similar in case of seed vigor (Table 3) and were statistically significant. The seeds of *A. spinosus* failed to germinate in AL, PF and CF while *V. villosa* in AL, PF and MF. Slight germination was achieved by *A. spinosus* in response to MF (2.08%) and CF (6.49%) solvents with root and shoot lengths (Table 3). Nonetheless, CF and WF solutions proved to be highly detrimental for *Vicia* as a consequence of which exhibited 3.12% and 9.20% germination respectively (Table 3).

RESULTS AND DISCUSSION

Growth studies in residue-amended soil (RAS)

All test plant seeds were reported to germinate 100e control and in various amended soil treatments. Consequently, the data needed to be prepared or presented. The most significant root length (RL) of the radicle was reported for *C. arietinum* (12.48 cm), then *T. aestivum* (11.67 cm) walked

behind, followed by *V. villosa* (7.00 cm) and *P. minor* (6.00 cm) in unamended soil (control). However, a considerable decrease in their RL was noticed in RAS. The RL devaluated along the conc. gradient, i.e. from 0.5% to 4%. The most considerable reduction perceived by *P. minor* (90%) at the highest conc. of 4%. *V. villosa* (83.78%), *T. aestivum* (78.44%) and the smallest RL in *C. arietinum* (75.78%) followed the trend against control (Fig 2a).

The shoot length (SL) of targeted plants also varied considerably in all the treatments and control. SL reported values were almost the same in crops in *T. aestivum* (14.26 cm) and *C. arietinum* (14.10 cm). In weed plants, the maximum SL was calculated for *V. villosa* (8.43 cm) and the least for *P. minor* (7.03 cm) in control. In the group of test plants treated with RAS, it was noticed that when concentration was increased, SL started reducing. 4% showed the most damaging effects in the group of all treatments. The maximum reduction was perceived by *P. minor* (92.46%) and the least by *C. arietinum* (75.88%) at 4% compared to the control. The SL of all the plants tested significantly showed a decrease in increasing concentration (Fig 2b).

T. aestivum showed luxuriant growth in control with the highest dry biomass (DB) (15.23 mg/seedling), *C. arietinum* (14.13 mg), and *V. villosa* (9.13 mg) were the next. The least DB was reported for *P. minor* (7.40 mg). Surrounded by an ambience of treatments, the plants witnessed the most enormous inhibitory effect at 4%. *V. villosa* (92.00%) displayed the highest reduction, and *C. arietinum* (61.07%) was the least. The reduction along all the concentrations was statistically significant for DB (Fig 2c).

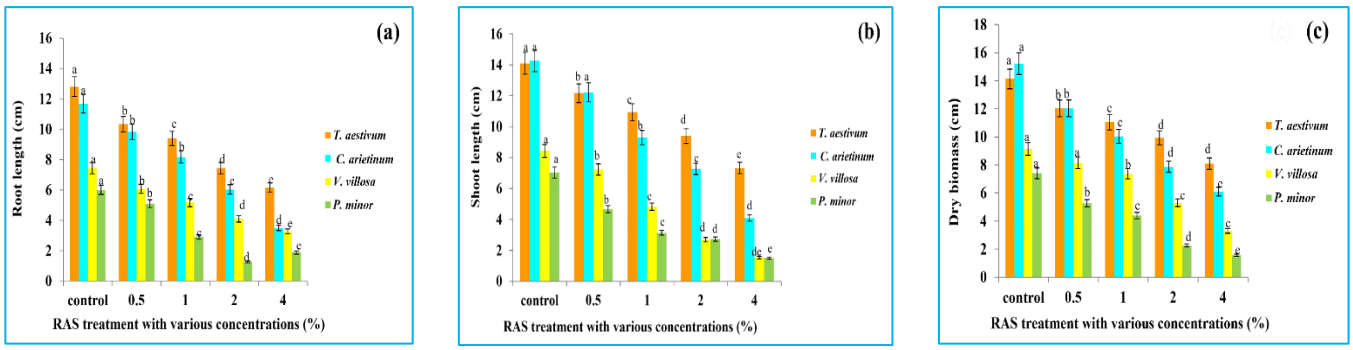

Fig 2 Allelotoxic potentiality of residue amended soil (RAS) on (a) root length (RL) (b) shoot length (SL) and (c) dry biomass (DB) of preferred plant species. Bars showing the letter (s) are significantly different at $p < 0.05$ as determined by Duncan's multiple range test of ANOVA. Error bars (┬) show standard errors (SE)

Growth studies in residue extract (RE)

The seeds of the selected plants were kept under the phytotoxic investigation in Petri dishes. The current study has affected the growth of all the plants antagonistically. Still, the maximum depletion was 70% for *C. arietinum* while the slightest impact of RE was 51.80% for *T. aestivum* at 4%. However, at control, *T. aestivum* grew better than RE-treated seeds, which exhibited an RL of 12.80 cm. The order of RL was *T. aestivum* (12.80 cm)>*C. arietinum* (11.67 cm) >*V. villosa* (7.43 cm) >*P. minor* (6.00 cm) for control (Fig 3a).

The seeds fed in control drew better results for the development of shoot length (SL), but a reduction was detected in the seeds treated with RE. Each chosen plant to be sown in control reported the maximum lengths. The most extensive length among all was filed for *C. arietinum.* The order was maintained as *C. arietinum*, *T. aestivum*, *V. villosa* and *P. minor* with14.27, 14.10, 8.43 and 7.03 cm. Besides the RL, SL also exhibited retardation at 4% concerning control. *V. villosa* was impacted the most and showed the highest degree of reduction in SL at 81.85% and the least in *T. aestivum* at 48.22% (Fig 3b).

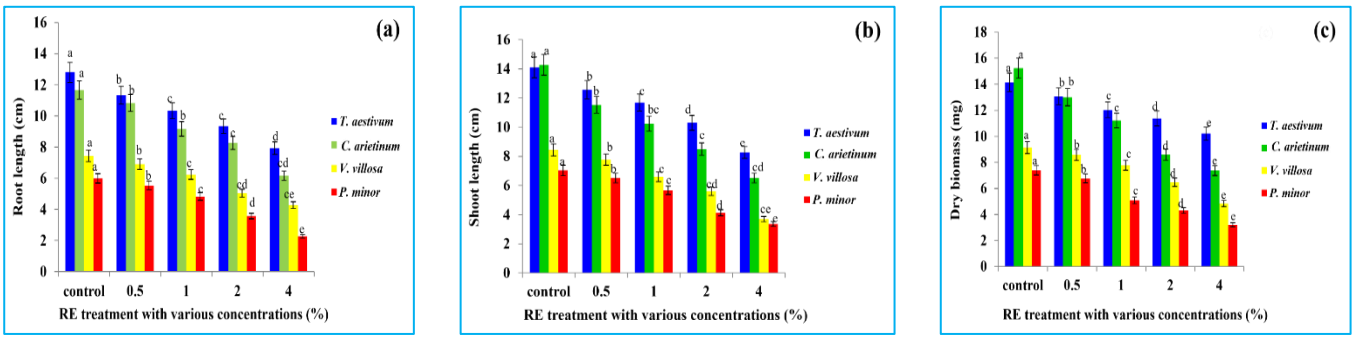

Fig 3 Allelotoxic potentiality of residue extract (RE) on (a) root length (RL) (b) shoot length (SL) and (c) dry biomass (DB) of preferred plant species. Bars showing the letter (s) are significantly different at $p < 0.05$ as determined by Duncan's multiple range test of ANOVA. Error bars (┬) show standard errors (SE)

Since the values of RL and SL showed depletion when exposed to RE, a scaling down was observed in the values of dry biomass (DB) of the seedlings. The DB of the selected plants catered to in control showed better results, such as RL and SL. For control, the order of values was as follows: *C. arietinum*>*T. aestivum* >*V. villosa* >*P. minor* (15.30, 14.13, 9.13, 7.40 mg/seedling). The reduction in DB was statistically significant. It was concentration-dependent. The higher the concentration (RE at 4%), the more the reduction. 4% extract was highly effective, so it reduced *P. minor* (78.80%) to the maximum extent, while *T. aestivum* was impacted the most petite (42.67%) accordingly (Fig 3c).

Growth studies in extract (residue) amended soil (REAS)

Residue extract amended soil (REAS) impacted the seedlings negatively and reduced their root length (RL). RL was

significantly reduced by increasing the treatment to 4%. The maximum reduction was noted at maximum conc. (4%). Hence, the depletion is reported in REAS-treated seeds rather than in control nurtured. The order of RL values for control was mentioned as the largest in *Triticum aestivum* (12.80), *Cicer arietinum* (11.67), *V. villosa* (7.43) and *P. minor* (6.00) cm. The root lengths of the treated samples were as follows: *P. minor* (62.17%) and *T. aestivum* (61.41%) with almost similar reductions to *Cicer arietinum* (47.13%) and a minor depletion in *V. villosa* (42.53%) was calculated (Fig 4a).

The shoot length (SL) increased in control while it decreased in REAS, which varied substantially in both cases. The maximum SL was measured for *Cicer arietinum*, the seedlings followed an order as *Triticum aestivum*, *V. villosa* and *P.* minor with 14.27, 14.10, 8.43 and 7.03 cm in control. Among all the concentrations, 4% has affected the SL of the plants chosen for survey the most and hence, showed maximum retardation in growth. The maximum reduction was yielded by *V. villosa* (56.10%), followed by *Cicer arietinum* (54.44%) and *Phalaris minor* (52.06%). The least was noted for *Triticum aestivum* (41.84%). The SL of selected plants decreased significantly in comparison to the control. The reduction was concentration-dependent (Fig 4b).

The maximum dry biomass (DB) was measured in control fed crops, so the order was as follows: *Cicer arietinum*, *Triticum aestivum*, *Vicia villosa* and *Phalaris minor* (15.23 mg/seedling), (14.13), (9.13) and (7.40) mg. Among all surveyed plants, 4% had the most prominent deleterious effects. The reduction order was *Vicia villosa*, *Phalaris minor*, *Cicer arietinum* and *Triticum aestivum*, with 89.02%, 56.76%, 51.60% and 38.53%. The DB reduction was along concentrations and was statistically significant (Fig 4c).

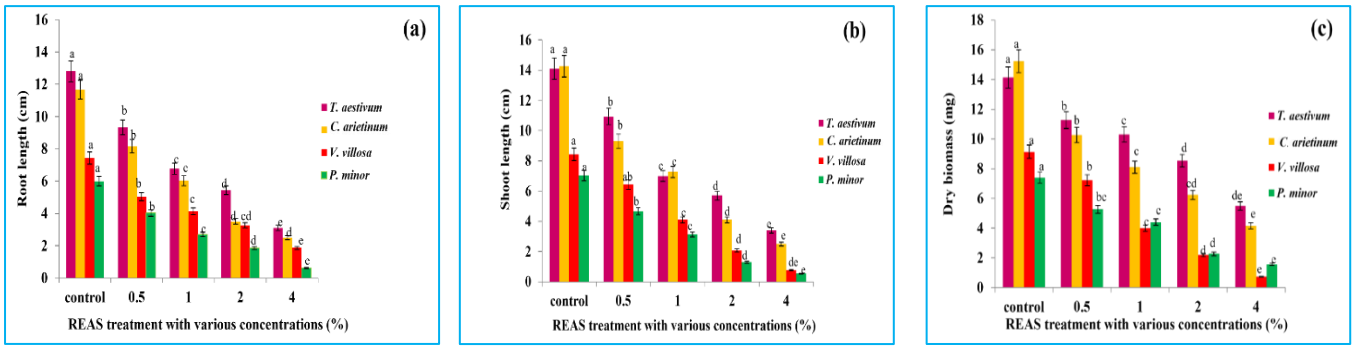

Fig 4 Allelopathic potentiality of residue extract amended soil (REAS) on (a) root length (RL) (b) shoot length (SL) and (c) dry biomass (DB) of preferred plant species. Bars showing the letter (s) are significantly different at $p < 0.05$ as determined by Duncan's multiple range test of ANOVA. Error bars (┬┴) show standard errors (SE)

Dynamics of phenolic allelochemicals in residue-amended soils and residue extracts

Residue-amended soil (RAS)

Control/unaltered soil (US) had the lowest phenolic content (51.6µg/g) among all treatments. (Fig 5a) shows that the overall value of phenolics is less than that of the US. After 120 hours of amendments, the greatest phenolic concentration of 116µg/g was found. Over 0–120 hours, the material showed a consistent increase. After that, there was a steady decrease, with the lowest value of 41.33µg/g recorded after 200 hours.

Residue extract amended soil (REAS)

Similar to REAS, (Fig 5b) shows the amount of phenolic compound lower than in control soil. Following the administration of the extracts, the phenolics steadily decreased until they reached 34.0µg/g after 100 hours, with the most tremendous volume calculated at 191.0µg/g (Fig 5b).

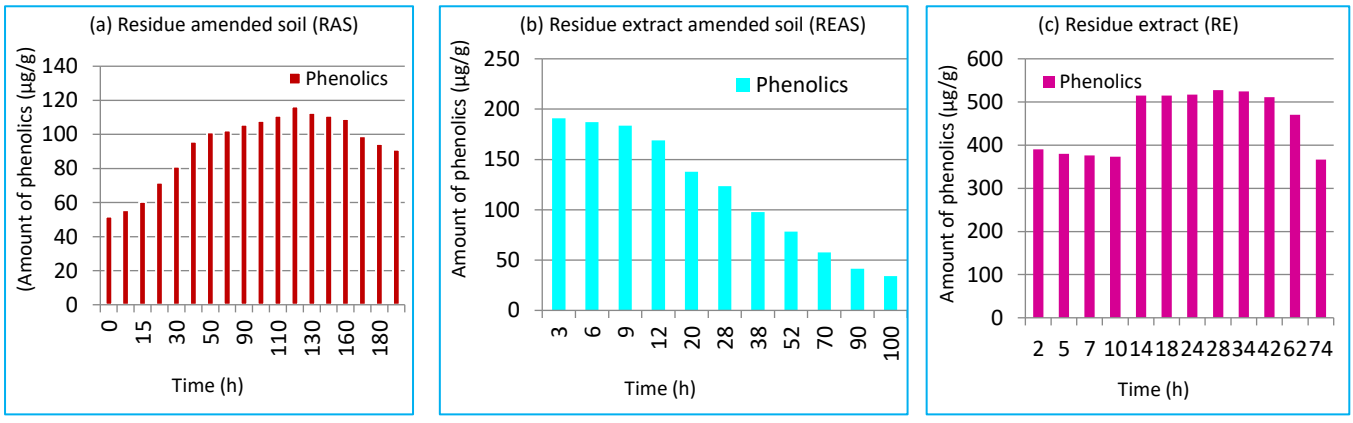

Fig 5 Dynamics of water soluble phenolics (allelochemicals) in (a) Residue amended soil (RAS) (b) Residue extract amended soil (RAES) and (c) Residue extract (RE)

Residue extract (RE)

The phenolic content of above-ground residue was 514.66 µg/ml after adding pure water to it for two hours. The amount of phenolics varied very little between 2 and 13 hours. However, after a sudden increase at 14 hours (373.33 µg/ml), the amount reached its maximum at 28 hours (527.33 µg/ml), gradually decreasing for the next 72 hours. At 74 hours, the slightest phenolic concentration (366.67 µg/ml) was noted (Fig 5c).

This pattern suggests an initial release and equilibrium phase of phenolic compounds, followed by a sudden release and peak, and finally a gradual decline in phenolic content over time. These fluctuations could be related to the breakdown of cellular structures

in the residue, changes in solubility, or microbial activity affecting the phenolic compounds' availability in the water.

Characteristics of residues extracts (RE)

pH

It was discovered that the extract's pH ranged from 5.39±0.004 to 6.47±0.002, which is almost neutral. With the concentration rising from 0.5% to 4%, it is clear that a significant portion of the shift was not recorded (Table 1).

Electrical conductivity (EC)

As the concentration of extracts rose, the electrical conductivity increased as well, rising from 1.63±0.07 µS to 5.71±0.16 µS (Table 1). A linear increase was seen.

Phenolic content (PC)

The 0.5% extract concentration contained 355.67±0.72 µg/ml of phenolics. As extract concentration increased, so did the value, and at 4% concentration, it was almost twice as high as at 0.5% (672.67±1.18 µg/ml) (Table 1). The rise was linear as well, following the pattern of electrical conductivity.

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Table 1 Values of pH, electrical conductivity (EC) and phenolic content (PC) in residue extract of *A. viridis*. Results obtained by Duncan's multiple range test of ANOVA. The letter (s) are significantly different at $p < 0.05$. ± denotes standard errors (SE)

	Residue extract (RE)		
Treatments	pH	Electrical conductivity (µS)	Phenolic Content (µg/ml)
0.5	6.47±0.002[a]	1.63±0.07[a]	355.67±0.72[a]
1	6.19±0.004[b]	3.11±0.10[b]	392.00±3.29[b]
2	5.89±0.002[c]	4.28±0.14[c]	407.00±0.47[c]
4	5.39±0.004[d]	5.71±0.16[d]	672.67±1.18[d]

Table 2 Response of germination behavior of (a) *O. sativa* and (b) *P. minor* seeds upon exposure to aqueous leachates and organic fractions extracted from leaves of *A. viridis* L. Results obtained by Duncan's multiple range test of ANOVA. The letter (s) are significantly different at $p < 0.05$. ± denotes standard errors (SE)

Treatment solutions	(a) *Oryza sativa*				(b) *Phalaris minor*			
	Germination (%)	Root length (mm)	Shoot length (mm)	Seed vigour (%)	Germination (%)	Root length (mm)	Shoot length (mm)	Seed vigour (%)
Control	80.63±0.17[b]	5.62±0.16[b]	9.47±0.08[b]	65.56±0.14[b]	84.85±0.21[b]	3.79±0.07[b]	8.43±0.13[b]	71.19±0.08[b]
Aqueous leachates (1% g/ml fresh wt.)	0	0	0	0	0	0	0	0
Petroleum ether fraction (0.09%w/v)	0	0	0	0	0	0	0	0
Methanolic fraction (0.09%w/v)	0	0	0	0	0	0	0	0
Chloroform fraction (0.09%w/v)	0	0	0	0	0	0	0	0
Water fraction (0.09%w/v)	5.33±0.07	3.08±0.06	3.10±0.56	0	0	0	0	0
LSD at 5%	0.31	0.40	0.32	0.29	0.38	0.48	0.32	0.29

Despite the allelopathic action of *A. viridis* residue, this weed is predicted to damage any crop or weed plant that comes in its touch. This was tested through diverse inspections on seedling development in net houses, including examining the impacts of changed soils (soil amended with weed residue and its aqueous extract). Certain tests looking for phenolics were performed for the determination of the phytochemicals by means of extracts and amended soil. Numerous investigations have shown that phenolics are a standard and readily soluble class of allelochemicals in plants that can be released through root exudation and breakdown [4]. A substantial high concentration of phenolics was detected in our study's aqueous residue extracts. As per the findings of Batish *et al.* [5], there were three to four times as many phenolics in RES and RS soils, respectively than in US soils. There may be fewer phenolics in RES than in RS due to the modification of phenolics when they enter the complex and heterogeneous soil environment. Phenolics can change in some ways, including sorption, detoxification, transformation into simpler forms, and even acting as a source of carbon for microorganisms [6]. Furthermore, the phytotoxicity of phenolics is determined by their influx from the donor plant, quality, and accessible bioactive concentration [7]. In the majority of allelopathic research, the addition of residue or plant material to the soil alters its pH, electrical conductivity, and nutritional status at the same time as a growth decline is detected [8]. Consequently, the residue's phytotoxic activity at different concentrations in comparison to the control showed that dry biomass and seedling growth decreased. The test plants' seedling growth was slower than the soil's, which had yet to be changed. The target plants showed varying degrees of restriction, with *C.*

sophera showing the most stringent effects. The extract exhibited the strongest delaying impression on opted plants at a concentration of 4%. Consequently, this could be due to the allelochemicals accumulating in the soil in bioactive amounts and leaving an inhibitory impression on the plants picked out for the survey. It was implied that *Amaranthus viridis* residue includes several phytotoxic chemicals that, when released, build up in high concentrations in the soil and inhibit the growth of neighbouring weeds and crops [9]. The contents of residue slowly mix and accumulate in the soil by the putrefaction process it goes through when introduced to or mixed with soil. This may also be the reason for inhibiting other preferred plant species. The chosen plants' germination was kept to compare to their exposure to soil treated with residual soil containing *A. viridis* to investigate this. In our instance, the experimental arrangement significantly and visibly impacted the germination of every target plant. Previous research by Gulzar and Siddiqui [10], Ding *et al*., [11], Szwed *et al*., [12], and Bi *et al*. [13] discovered that integrating residues from donor plants leaves a negative impression on the target plants also supports our findings. Remains of a nosy weed (*Ageratum conyzoides* L.) had been discovered to significantly inhibit the target species' growth, nodule number and weight, and leg-haemoglobin quantity [9]. This evaluates how distinct plants react differently based on seed size and genetic variations.

Table 3 Response of germination behavior of (a) *V. villosa* and (b) *A. spinosus* seeds upon exposure to aqueous leachates and organic fractions extracted from leaves of *A. viridis* L. Results obtained by Duncan's multiple range test of ANOVA. The letter (s) are significantly different at $p < 0.05$. ± denotes standard errors (SE)

Treatment solutions	(a) *Oryza sativa*				(b) *Phalaris minor*			
	Germination (%)	Root length (mm)	Shoot length (mm)	Seed vigour (%)	Germination (%)	Root length (mm)	Shoot length (mm)	Seed vigour (%)
Control	65.86±0.04[a]	3.66±0.18[a]	6.40±0.08[a]	71.66±0.17[a]	76.52±0.12[a]	4.98±0.06[a]	9.81±0.08[a]	71.58±0.10[a]
Aqueous leachates (1% g/ml fresh wt.)	0	0	0	0	0	0	0	0
Petroleum ether fraction (0.09%w/v)	0	0	0	0	0	0	0	0
Methanolic fraction (0.09%w/v)	0	0	0	0	2.08±0.05	0.88±0.03	1.65±0.07	0
Chloroform fraction (0.09%w/v)	3.12±0.05	1.46±0.09	3.46±0.07	0	0	0	0	0
Water fraction (0.09%w/v)	9.20±0.07	3.13±0.07	5.13±0.03	0	6.49±0.05	1.90±0.04	6.16±0.07	0
LSD at 5%	0.59	0.51	0.48	0.72	0.63	0.42	0.51	0.41

Decomposing plant waste from crops, weeds, and even trees can generate environmental inhibitors that are harmful to other plants, according to several studies on the allelopathicity of residue extracts (RE) [14-15]. This study includes the residue that remains after the plant life cycle is finished, which provides for dried stems, leaves, and roots. Because of the phytotoxic characteristics of weed, it was seen that the harmful effects are caused by the allelochemical release through various mechanisms, such as leaching, death, deterioration, and even discharge [16-17]. The final conclusions of this investigation demonstrate the biological activity and release of phytotoxins during leaching. Allelochemicals, like phenolics, naturally seep from plants; this was previously described [18]. Bio-efficiency reports as thriving experiments were conducted in extract-amended soil (REAS) to ascertain whether the inhibitory impact on the targeted species is for phytochemicals or antagonists to remain in the soil in biologically operative concentration after being released by leaching. Reactive oxygen species (ROS) are typically produced in small amounts by plants through respiration, photorespiration, and photosynthesis in their peroxisomes, mitochondria, and chloroplasts. Stressed plants produce more enzymatic (SOD, CAT, and POX) and non-enzymatic (proline) antioxidant defence systems to counteract the stress, preserving a balance between the production and quenching of these oxidants [19-20]. Allelochemicals harm proteins, DNA, RNA, membranes, and the plant's metabolism as a whole. They also cause an increase in ROS production, which damages these components further [21]. According to some research, plants cultivated in RAS accumulate ROS-scavenging enzymes non-significantly more than plants nourished in unaltered soil, which do not. RAS-grown plants are more susceptible to allelochemical-induced oxidative damage because they have less buildup of antioxidants. El-Katony *et al.* [22] obtained similar rice results. Proline also aids in plants' ability to scavenge free radicals. It is a common, multifunctional osmolyte that plants have and is thought to be their first line of defence in trying circumstances [23].

High or low, both the concentrations of the treatment (aqueous and organic solvents extracted from *A. viridis*) affect the growth and development of the chosen plant species. Higher concentrations affect the physiology and metabolism of the plant negatively, while low concentrations are vice-versa. So, there is a need to feed the plant with the required treatment doses to ensure the crop's correct development. The experiment was performed to check the effect of the extracted solvents on the selected plant species. After the investigations, elucidations of the results were done. The plant physiology and metabolism were remarkably affected by the aqueous/organic solvents. Substantial influence and verifications in germination and seedling growth were noticed [24]. The following research supports our study, too. The aqueous/organic fractions were extracted from the leaves of *A. viridis*, which is phytotoxic. When the seeds of the selected plants were given a treatment of extracted solvents, they exhibited a more comprehensive range of variation towards phytotoxicity. Variations may also have taken place as a consequence of the chemical properties possessed by the compounds used in the extraction process of aqueous and organic solvents [25]. The genuine study regarding allelopathy foreshadows the scheme of how the plant's secondary metabolites are extracted, purified and identified, which is a part of this study, given by Mushtaq and Siddiqui [26]. The retardatory effects which the selected plants showed may have taken place because of the capable chemicals present in *Amaranthus* [27]. This study is supported by previous studies which showed retardatory effects on the evolution of the plants [28]. Germination and development of plants (weeds and crops) repressed by the leachates from various parts of *N. plumbaginifolia* [29] have also supported our study. There is reduction in the germination of the seedlings because

phytotoxic substances affect the hormones which play role in their growth [30-31] and may be because of water-soluble allelochemicals in *N. plumbaginifolia* [32].

Many recent discoveries have reported inhibition in the germination of the chosen plants by allelopathy, supporting our experiment [33]. Sometimes, the enzymes are adapted to new changes in the environment, which possibly become responsible for the decline in germination and are confined to the transformation of nutrition [34]. In the current study, reduced root length and shoot length may be attributed to the decrease in cell division, hence, responsible for the modifications in the ultrastructure of the cells as a consequence of the exposure towards allelochemicals in the form of aqueous extracts/organic fractions [35]. The fall in seed germination (RL, SL and DB) may be caused by the potential presence of water-soluble allelochemicals extract of *A. viridis* [36] and is consistent with the findings of Bojovice *et al*. [27]. This study is consistent with earlier studies, which found that allelopathic plants depleted the seedling germination of the preferred plant species [28]. Allelopathic plants affect seed germination and seedling growth by affecting hormones [31]. The fall in germination during the germination period constrains the modifications of nutrients and inhibits seedling length, which is likewise caused by shifts in enzymatic activities [37].

According to our research, allelochemical exposure to water extracts and organic fractions decreased cell division and brought changes to the structural integrity of the cells [35]. Allelochemicals enter the environment through various processes like volatilization, exudation, and leaching [38] and are liberated as glycosides [39]. Additionally, facilitating the passage of allelochemicals among the plants encountered, glycosidic linkages also modify the danger chemicals pose to the donor plant. Either the enzymatic activity or acid hydrolysis could extract the glycosides from an organic component [40]. The massive reduction in germination showcased by *A. viridis* aqueous extract in the current study demonstrates that water is an optimal solvent for the extraction of allelochemicals. Phenolics constitute a significant class of allelochemicals that can be extracted most efficiently in water [41-42]. De Mastro *et al*. [43] discovered the highest allelopathic potential while testing organic and aqueous solvents against weeds and crops principally. In particular, the agricultural fields that are frequently infested with weeds, irrigated with water or receive water in the form of rain or dew are of incredible biological importance in allelopathic intrusion with respect to water-soluble allelochemicals [26]. It is confirmed by Zhu *et al*. [44] that the differential suppression by allelopathic substances is consistent with our findings. Furthermore, different techniques of sample preparation and extraction are anticipated to result in different levels of allelopathic infection.

In the current case, water is used as a solvent which is polar in nature, to extract the non-polar chloroform, petroleum ether, and methanol, because of which they might have shown different behaviour in their efficiencies of extraction. This may have led to the variable phytotoxicity towards aqueous and organic fractions. This is the basis of the quantitative and qualitative adjustments in the extracted allelochemicals in different aqueous and organic fractions. Phenolics are a principal group of allelopathic compounds and can be best extracted using water. The results of this study are supported by the findings of Tanveer *et al*. [25]. Abdul Khaliq *et al*. [45] confirmed that the different levels of suppression by allelopathic compounds are based on our results.

Indeed, negative effects brought about by aqueous leachates were of a lot more prominence than those of the organic fraction. More than that, organic solvents were avoided for the extract preparation, which can pave the way for allelopathic interactions to intrude in the fields. Additionally, to study allelopathic effects, it was suggested that water be used because it reflects more intently what might occur under regular conditions [46]. Despite everything, as soon as the allelopathic actions for some species been embellished, the dynamics of solubility in the organic solvents might be attractive according to the fact which assists in deriving the steps of isolation and identification of the compounds [47].

CONCLUSION

The present study highlights the allelopathic effects of residue-amended soil (RAS), residue extract (RE), and residue extract-amended soil (REAS) on the growth parameters of selected plant species. The results demonstrated that both RAS and REAS negatively impacted root length (RL), shoot length (SL), and dry biomass (DB) across all tested plants, with the effects intensifying at higher residue concentrations. Notably, the greatest reductions in growth metrics were observed at the highest concentration of 4%, particularly in P. minor and V. villosa, which exhibited significant declines in RL, SL, and DB. Phenolic content dynamics further revealed that the concentration of allelochemicals in the soil and extracts played a crucial role in these inhibitory effects. Phenolic compounds, known for their allelopathic properties, were found to be present in significant amounts in the amended soils and extracts, corroborating the observed growth suppression in the target plants. The study underscores the potential of these allelochemicals to accumulate in the soil, leading to sustained inhibitory effects on plant growth. Moreover, the study suggests that the allelopathic potential of residue amendments is a key factor in determining plant interactions within an ecosystem. The findings are consistent with previous research, indicating that the presence of phytotoxic chemicals in soil, particularly from decomposing plant residues, can substantially affect the growth of neighboring plants. These results provide valuable insights into the allelopathic interactions within agroecosystems and suggest that the careful management of residue amendments is essential to mitigate their potentially adverse effects on crop and weed growth.

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