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Unveiling the bioherbicidal potential of *Eupatorium capillifolium* (Lam.) Small for selective management of agricultural weeds

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Abstract

The global rise of herbicide-resistant weeds underscores the urgent need for sustainable weed management strategies. *Eupatorium capillifolium* (Lam.) Small, a perennial invasive weed native to North America and widespread in the Southeastern United States, presents untapped potential as a bioherbicide. This study evaluated the effects of its aqueous extract on seed germination and early seedling growth of thirteen weed species (nine broadleaf and four grasses) and four major crops (*Arachis hypogaea, Zea mays, Glycine max, and Gossypium hirsutum*). The extract significantly inhibited seed germination (92.62–100%) of four Amaranthus species (*A. palmeri*, *A. tuberculatus*, *A. retroflexus,* and *A. hybridus)* with minimal effects on *Zea mays* and *Arachis hypogaea* (6.12–6.25%). Other weeds showed a limited response. Inhibition of shoot and root growth confirmed the extract's allelopathic activity. Principal component analysis indicated inhibition of seed germination as the primary mode of action. The order of pigweeds' sensitivity to the aqueous extract was *A. hybridus* > *A. retroflexus* > *A. palmeri* > *A. tuberculatus*. Phytochemical screening identified 36 allelopathic compounds with gallic acid and hydroxy-1,4-benzoquinone as the dominant components. This is the first report demonstrating the bioherbicidal potential of *E. capillifolium* aqueous extract against *Amaranthus* spp. under laboratory conditions, highlighting its promise as a sustainable alternative to synthetic herbicides and a candidate for further field-based evaluation in integrated weed management systems.

Keywords: allelopathy, bioherbicide, *Eupatorium capillifolium,* seed germination, weed suppression

Introduction

Annual crop losses due to weeds have been estimated at USD 33 billion in the USA[1], AUD 3.3 billion in Australia[2], and USD 11 billion in India[3]. Since the 1970s, herbicides have emerged as one of the best practices for weed control along with substantial economic benefits, and the introduction of various herbicide-tolerant (HT) crop varieties since the mid-1990s has resulted in overreliance on chemical weed control strategies. Over 90, 93, and 96% of the corn, cotton, and soybean acreage in the USA are covered by various HT varieties. The over-reliance on limited herbicide chemistries has resulted in 534 unique reported cases (species x site of action) of herbicide-resistant (HR) weeds covering 273 species, including 156 dicots and 117 monocots, in 102 crops in 75 countries[4]. Therefore, the application of bioherbicides to control HR weeds has emerged as an important ecologically based weed control strategy and they play a significant role in regenerative agriculture by minimizing ecosystem disturbance while selectively targeting weeds and invasive species.

Bioherbicides offer potential avenues for effective weed management while promoting ecological sustainability and biodiversity, and could be effectively integrated into non-crop landscapes, including grasslands and woodlots, thereby supporting sustainable land management practices[5]. Integration of bioherbicides along with other chemical[6] and cultural[7,8] strategies could lead to sustainable management of weeds[9,10]. According to a market report[11], growing awareness of organic farming and the popularity of organic food products has been a driving force for the global bioherbicide market worth nearly USD 2.2 billion in 2024 and has been projected to reach USD 5.86 billion by 2031 with a CAGR of 14.24%.

The concept of bioherbicides has evolved to include a wide range of products derived from microorganisms, such as natural metabolites produced during microbial growth, plant viruses, and natural products like allelochemicals and essential oils from specific plant species[12,13]. Since plant-based bioherbicides offer simpler formulations and storage solutions compared to mycoherbicides, research on natural product-based bioherbicides is ongoing, offering the potential to discover herbicides with novel modes of action. Plants from the genus Eupatorium (family: Asteraceae), comprising around 1200 species distributed across America, Europe, Africa, and Asia, have been known for over 300 bioactive natural compounds including terpenoids, flavonoids, phenylpropanoids, quinonoids, pyrrolizidine alkaloids, thiophenes, furans, steroids, organic acids, depsidones, thymols and essential oils[26]. These bioactive molecules from various species of Eupatorium have shown a diverse range of bioactivity including cytotoxicity against cancer and tumor cell lines, antifungal[27], insecticidal[28], antibacterial, antioxidant[29], anti-inflammatory, and antiallergic activities[26], however, a systematic investigation on the bioherbicidal property of Eupatorium remains unrecognized

Among the *Eupatorium* species, *Eupatorium capillifolium* (Lam.) Small, or dogfennel (Fig. 1), is a perennial invasive weed native to North America, primarily found in pastures and rangelands of the Southeastern United States[30,31]. *E. capillifolium* is avoided by cattle and other grazing animals due to toxic alkaloids[32] and emits a distinctive odor from its essential oils[28]. Various compounds isolated

from different species of *Eupatorium* have demonstrated antibacterial[33], insecticidal[34,35], fungicidal[27,28,29], nematocidal[36], and plant growth-controlling activities[37,38] (Supplementary Table 1), except bioherbicidal activity. Though few studies observed reduction in germination of *Pinus elliottii* and *P. taeda* with the foliar extracts from *E. capillifolium*[37] or decreased growth and foliar developments of *Medicago sativa* and *Lolium multiflorum* with soil application of leaf tissues[38], but to date, no studies have reported the bioherbicidal potential of *E. capillifolium*.

This study aimed to evaluate the bioherbicidal potential of *E. capillifolium* aqueous extract on seed germination and early seedling growth of thirteen economically important weed species [nine broadleaf weeds namely, *Amaranthus palmeri*, *A. tuberculatus*, *A. retroflexus*, *A. hybridus*, *Erigeron canadensis*, *Sida spinosa*, *Rumex crispus*, *Ipomoea lacunose,* and *Chenopodium album* , and four grasses namely, *Dactyloctenium aegyptium*, *L. multiflorum*, *Echinochloa crus-galli* and *Digitaria sanguinalis*] commonly found weeds in agricultural fields of four major crops (*Z. mays, A. hypogaea, G. hirsutum,* and *G. max*) in the Southeastern United States[1,39]. In addition, present research sought to identify potential allelopathic compounds present in the *E. capillifolium* aqueous extract and explore possible mechanisms underlying weed suppression.

Results

Effect of *E. capillifolium* aqueous extract on seed germination and seedling growth parameters

The seed germination of weed and crop species in response to *E. capillifolium* extract varied significantly (Fig. 2) and showed a negative trend with increasing concentrations of *E. capillifolium* extract (Table 1). The relative inhibited germination (RIG) of broadleaf weeds, namely, *A. palmeri*, *A. retroflexus*, *A. tuberculatus, A. hybridus,* and *C. album* reduced significantly ($p<0.05$) by 85.1-97.87, 97.22-100, 73.27-92.68, 94.57-100, and 62.96-88.52%, respectively, with *E. capillifolium* extracts (5 and 10%) as compared to the respective control treatments (Fig. 2a and 2b). *E. canadensis* and *S. spinosa* showed moderate inhibition with RIG values ranging from 26.91-60.86 and 52.96-60.01%, respectively, with *E. capillifolium* extracts (5 and 10%) as compared to the respective control treatments. Whereas *I. lacunose* and *R. crispus* showed minimum inhibition of seed germination with RIG values ranging from 28.13-40.63 and 11.53-15.38%, respectively, with *E. capillifolium* extract (5 and 10%) as compared to the respective control treatments.

The seed germination of three out of four grass weeds studied, namely *L. multiflorum*, *D. sanguinalis*, and *E. crus-galli*, remained less affected with RIG values ranging from 7.40-14.87, 14.28-28.57, and 25%, respectively, with *E. capillifolium* extracts (5 and 10%) as compared to the respective control treatments. In contrast, *D. aegyptium* showed a significant ($p<0.05$) reduction in seed germination with RIG values of 32.31-75.64% with *E. capillifolium* extracts (5 and 10%) as compared to the control treatment (Fig. 2c). The 5% *E. capillifolium* extract did not inhibit seed germination in *A. hypogaea* and *Z. mays*, while the 10% extract exhibited 6.12 and 6.25% inhibition, respectively. The seed germination of *G. hirsutum* and *G. max* was more affected, with RIG values ranging from 16.66-25 and 21.43-28.57%, respectively, with *E. capillifolium* extracts (5 and 10%) as compared to the

respective control treatments (Fig. 2d). Overall, the RIG values showed negative correlations (r^2 ranging from 0.651-1) with the increase in *E. capillifolium* concentration across the studied plant species.

Table 2 indicated allelopathic impacts of *E. capillifolium* aqueous extracts on various seed germination parameters (G%, SG, and MGT) and seedling growth measures (R and S) of studied weed species, which varied significantly within a weed species and among the weed species. The allelopathic response index (RI) was calculated for each parameter following the methods of Dai et al.[40] and Williamson and Richardson[41] (Table 2). The RI typically ranges from -1 to +1, where positive values indicate a stimulatory effect of the treatment, and negative values reflect inhibition relative to control. The absolute value of the RI denotes the strength of the allelopathic effect, with values near zero suggesting little to no impact from the treatment. The RI values of all parameters studied increased with an increase in *E. capillifolium* concentration from 5 to 10%, indicating higher inhibition at higher concentrations. The order for germination inhibition [RI(G)] with 10% extract was *A. hybridus* (-1) = *A. retroflexus* (-1) > *A. palmeri* (-0.979) > *A. tuberculatus* (-0.923) > *C. album* (-0.856) > *D. aegyptium* (-0.756)> *E. canadensis* (-0.48) with moderate inhibition on *I. lacunose* (-0.408), *S. spinosa* (-0.367), and *E. crus-galli* (-0.278), and no/little inhibition on *L. multiflorum* (-0.137), *R. crispus* (-0.115) and *D. sanguinalis* (0). The order for inhibition of speed of germination [RI(SG)] with 10% extract was *A. hybridus* (-1) = *A. retroflexus* (-1) > *A. palmeri* (-0.975) > *A. tuberculatus* (-0.951) > *C. album* (-0.901) > *D. aegyptium* (-0.827)> *D. sanguinalis* (-0.639)> *S. spinosa* (-0.584)> *E. canadensis* (-0.533) with moderate inhibition on *E. crus-galli* (-0.478), *I. lacunose* (-0.447), and *R. crispus* (-0.283), and no/little inhibition on *L. multiflorum* (-0.105). The order for inhibition of mean germination time [RI(MGT)] was *A. hybridus* (-1) = *A. retroflexus* (-1) > *A. palmeri* (-0.979) > *A. tuberculatus* (-0.931) > *C. album* (-0.867) > *D. aegyptium* (-0.738) with moderate inhibition on *E. canadensis* (-0.49), *I. lacunose* (-0.414), *S. spinosa* (-0.376), *E. crus-galli* (-0.334), and *D. sanguinalis* (-0.224), and no/little inhibition on *R. crispus* (-0.153) and *L. multiflorum* (-0.015). The order for inhibition of root [RI(R)] was *A. hybridus* (-1) = *A. retroflexus* (-1) > *A. palmeri* (-0.928) > *A. tuberculatus* (-0.915) > *E. crus-galli* (-0.696)> *I. lacunose* (-0.502), with moderate inhibition on *D. aegyptium* (-0.491), *E. canadensis* (-0.468), *C. album* (-0.384), *L. multiflorum* (-0.372), and *R. crispus* (-0.26), and no/little inhibition on *S. spinosa* (-0.063), and *D. sanguinalis* (-0.002). The order for inhibition of shoot [RI(S)] *A. hybridus* (-1) = *A. retroflexus* (-1) = *A. palmeri* (-1) > *A. tuberculatus* (-0.784) > with moderate inhibition on *E. canadensis* (-0.452), *D. aegyptium* (-0.448), *I. lacunose* (-0.439), *E. crus-galli* (-0.387), *L. multiflorum* (-0.372), and *S. spinosa* (-0.206), and no/little inhibition on *C. album* (-0.19), *R. crispus* (-0.037), and *D. sanguinalis* (-0.055).

A PCA analysis was carried out to understand the effect of *E. capillifolium* extracts (5 and 10%) on different RI values for seed germination (G%, SG, and MGT) and seedling growth (R and S) parameters of studied weed species (Fig. 3.). PC1 and PC2 explained 96.59% variability in data, with the major contribution of PC-1 by 86.7%. The major contributing factors of PC1 with high correlation value were RI(G) [0.839], RI(SG) [0.884], and RI(MGT) [0.844]. Whereas RI(R) and RI(S) with correlation values of 0.917 and 0.812 were major contributory factors for PC2. The cluster analysis indicated that RI(G) was the major factor (r^2 = 0.937) describing the variation in data.

Dose-response assay on pigweeds

Germination

The phytotoxic effect of varying concentrations (0-20%) of *E. capillifolium* aqueous extract on four different species of pigweeds, namely, *A. hybridus*, *A. retroflexus*, *A. palmeri*, and *A. tuberculatus*, was systematically investigated (Fig. 4a-j; Table 3). Among these, seed germination (%G) of *A. hybridus* was most affected by the *E. capillifolium* extract with 91.3-95.65% RIG at 1-5% extract concentrations, followed by complete inhibition of germination at 10-20% concentrations. *A. retroflexus* showed up to 97.22% RIG at 5% extract, followed by complete inhibition at higher concentrations. *A. palmeri* and *A. tuberculatus* showed 97.87 and 92.68% RIG, respectively, with 10% extract, followed by complete inhibition of germination at 20% *E. capillifolium* extract. The SG and MGT of the respective pigweed species exhibited a decreasing trend with increasing concentrations of the *E. capillifolium* extract from 0.5 to 20%, indicating a dose-dependent inhibitory effect. To quantify this response, dose-response analysis was carried out (Fig. 5), and GI_{50} values, representing the concentration of *E. capillifolium* extract to inhibit 50% germination, were calculated. The GI_{50} values were 0.2687, 0.5572, 1.048, and 1.811% of *E. capillifolium* extract for *A. hybridus*, *A. retroflexus*, *A. palmeri*, and *A. tuberculatus*, respectively. These results revealed species-specific sensitivity of *E. capillifolium* extract, highlighting its potential as a selective bioherbicide agent, especially for pigweed management.

Early seedling growth

The impact of different concentrations of *E. capillifolium* extract on early seedling growth of various pigweeds was evaluated in terms of root (R) and shoot length (S) (Fig. 4 (i)h-(iv)h; Table 3). In *A. hybridus,* shoot length decreased significantly ($p<0.05$) by 44.74% at 1% *E. capillifolium* extract compared to the control, with complete inhibition of shoot development at concentrations of ≥2%. Root length of *A. hybridus* seedlings decreased significantly ($p<0.05$) by 17.57% at 1% extract, followed by the formation of only roots (85.14-86.49% relative decrease over control) at 2-5% extracts, and complete inhibition at higher concentrations of *E. capillifolium* extract. Whereas in *A. retroflexus* seedlings, shoot length showed a non-significant reduction (3.70-11.11%) at concentrations up to 2% compared to the control, followed by complete inhibition of shoot development at higher concentrations. However, the root length of *A. retroflexus* seedlings decreased significantly ($p<0.05$) by 34.04-57.45% at concentrations up to 2% extracts compared to the control, followed by complete inhibition of germination at higher concentrations of *E. capillifolium* extract. The shoot and root lengths of *A. palmeri* seedlings exhibited a relative decrease of 14.47-35.33 and 22.09-62.79%, respectively, at extract concentrations of up to 5% compared to the control. It was followed by the formation of roots only (a 93.02% relative decrease) at 10% extract, and no germination occurred at higher concentrations. *A. tuberculatus* seedlings showed 32.97-37.84 and 11.82-42.73% relative decrease in shoot and root lengths compared to the control at extract concentrations up to 2%, followed by a 50% relative decrease in shoot and root lengths at 5% extract. Further, formation of deformed *A. tuberculatus* seedlings at 10% extract was observed with 78.38 and 87.27% relative reductions in

shoot and root lengths, respectively, and complete inhibition occurred at 20% *E. capillifolium* extract.

Synthetical allelopathic effects

The allelopathic potential of *E. capillifolium* aqueous extract on seed germination parameters (G%, SG, and MGT) as well as early seedling growth (R and S) of various pigweeds was estimated by calculating the allelopathic response index (RI) for each parameter (Table 3). The results revealed that *E. capillifolium* extract exerted inhibitory effects across all measured parameters, as evidenced by negative RI values (RI<0). The synthetical allelopathic effects (SE), which integrate the overall inhibitory impact of the *E. capillifolium* extract concentrations, were also negative (SE<0) for all species and concentrations tested (Fig. 6), confirming the suppressive potential of the extract. Within each species, SE values showed a concentration-dependent increase in inhibition. For *A.* hybridus, SE values were -0.486, -0.68, -0.947, -0.951, -1, and -1 at 0.5, 1, 2, 5, 10, and 20% extract concentrations, respectively. Similarly, *A. retroflexus* exhibited SE values of -0.411, -0.445, -0.598, -0.987, -1, and -1 across the same concentration range. SE values for *A. palmeri* were -0.156, -0.448, -0.566, -0.715, and -0.962, while *A. tuberculatus* showed SE values of -0.197, -0.396, -0.550, -0.654, -0.894, -1, and -1. Notably, *A. hybridus* and *A. retroflexus* showed higher inhibitory responses at lower *E. capillifolium* extract concentrations (0.5-2%) compared to *A. palmeri* and *A. tuberculatus*. Overall, the allelopathic effect of *E. capillifolium* extract followed the order: *A. hybridus*> *A. retroflexus*> *A. palmeri*> *A. tuberculatus,* indicating species-specific sensitivity of *E. capillifolium* extract.

Identification of allelopathic compounds in *E. capillifolium* aqueous extract

The LC-MS analysis indicated the presence of numerous compounds in the *E. capillifolium* aqueous extract and Table 4 represented the top 36 compounds, while some of them have earlier been reported for allelopathy elsewhere. Based on the % area of a peak in the TIC of LC-MS analysis, gallic acid, a phenolic compound, with a 4.50% contribution, was among the major components of the extract. Other important allelopathic compounds of the extract were hydroxy-1,4-benzoquinone (4.18%), (-)-alpha-Cedrene (3.25%), acetophenone (2.99%), gentisic acid (1.59%), caryophyllene oxide (1.24%), along with minor proportions of zedoarondiol (0.62%), capsidiol (0.62%), caffeic acid (0.57%), pyrogallol (0.34%), p-cymene (0.32%), trans-carveol (0.32%), 3,4-dihydroxy-L-phenylalanine/ L-DOPA (0.29%), quercetin (0.26%) etc.

Discussion

Allelochemicals released from a plant influence the ecology of neighboring plants by affecting various physiological processes and governing the successional processes. Since 2007, over 1500 articles have been published on allelochemicals released by various plants, and their effect on local ecology along with special reports on weed suppression[42,43]. Though the persistence of these allelochemicals in the soil is of short duration, the effective level to affect other plants' succession depends on constant supply[44]. *E. capillifolium* is a native and invasive weed species in the

Southeastern United States, and its allelopathic effect on other weed species is less known.

This study demonstrated the allelopathic potential of *E. capillifolium* aqueous extract by inhibiting seed germination and early seedling growth of several common weed species associated with four major row crops, *A. hypogaea, Z. mays, G. max, and G. hirsutum,* cultivated in the southeastern United States. The order of synthetical allelopathic effects (SE) for 10% DF extract was *A. hybridus* (-1)= *A. retroflexus* (-1)> *A. palmeri* (-0.972)> *A. tuberculatus* (-0.901)> *D. aegyptium* (-0.652)> *C. album* (-0.64)> *E. canadensis* (-0.485)> *I. lacunose* (-0.442)> *E. crus-galli* (-0.435)> *S. spinosa* (-0.319)> *L. multiflorum* (-0.194)> *D. sanguinalis* (-0.184)> *R. crispus* (-0.17), indicating differential sensitivity among species. These findings are consistent with previous reports on species-specific allelopathic responses. Liu et al.[45] reported different SE values for *M. sativa* (-0.35), *Elymus dahuricus* (-0.42), and *Agropyron cristatum* (-0.24) in response to a 12.5% aqueous extract of *Sophora chamaejasme*. Similarly, Dai et al.[40] observed various SE values for *Brassica rapa* (-0.70), *Triticum aestivum* (-0.40), and *E. crus-galli* (-0.65) in response to a 5% aqueous extract of *Flaveria bidentis*, indicating species-specific allelopathic sensitivity of that extract. Some earlier studies reported a reduction in germination of *P. elliottii* and *P. taeda* following exposure to *E. capillifolium* foliar extract[37] or soil incorporation of *E. capillifolium* leaf at a dose of 0.25% negatively impacted the growth and foliar development of *L. multiflorum* [38]. Though, few reported on allelopathic potential of essential oils extracted from *E. adenophorum* and its phytotoxicity on weeds like *Phalaris minor*[29] or *Polygonum plebejum*,[27], until this report, allelopathic effect of *E. capillifolium* aqueous extract on various weed species was unknown.

The present study reported 36 allelopathic compounds, including gallic acid and hydroxybenzoquinone as major components, in *E. capillifolium* aqueous extract, which were earlier reported in other plants with allelopathic potential[46-53]. Secondary metabolites such as phenolic acids, aromatic diketones, and flavonoids are well-documented for their phytotoxic properties. However, the dynamics of allelopathy are influenced by complex interactions between donor and receiver plant species. For instance, gallic acid-rich root exudates from *Phragmites australis* have been shown to inhibit seedling growth in *Nicotiana tabacum*, *Lactuca sativa*, *B. rapa*, and *Spartina alterniflora*[54]. Similarly, aqueous extracts of *Ricinus communis* containing gallic acid and other phenolic acids suppressed germination and growth of *Bidens bipinnata*[50]. Gentisic acid, identified in extract of *Buchloe dactyloides*, was reported to inhibit the growth of *E. crus-galli* and *Poa annua*[49]. Seed extracts of *Iris sanguinea*, rich in allelopathic benzoquinones such as 3-[10(Z)-heptadecenyl]-2-hydroxy-5-methoxy-1,4-benzoquinone, significantly inhibited the growth of *M. sativa*, *E. crus-galli*, *L. sativa*, and *B. rapa*[47]. Although previous studies have reported the allelopathic effects of *E. capillifolium* leaf biomass in soil[38] and foliar extract[37], no specific allelochemicals had been identified until now. This study is the first to report the presence of multiple allelochemicals in the aqueous extract of *E. capillifolium* with demonstrated weed-suppressing activity. The observed phytotoxicity is likely not attributable to a single compound, but rather to the synergistic action of multiple constituents present in the extract, underscoring the complexity and potential of plant-derived allelopathic interactions in natural weed management.

The allelopathic chemicals released by a plant could affect physiological processes including reduction in germination, poor seedling growth, low photosynthetic efficiency, decreased water and nutrient uptake in neighboring plants[42,44,47], and growth retardation has been reported as the most common response[10,43,55]. The inhibitory effects of *E. capillifolium* extract on seed germination and early seedling growth of various weed species are likely mediated through multiple, compound-specific mechanisms. The extract contains a diverse suite of allelochemicals, including phenolic acids (gallic acid, caffeic acid, gentisic acid), aromatic diketones (e.g., hydroxybenzoquinone), and flavonoids (e.g., quercetin), each known to interfere with plant physiological and cellular processes. Gallic acid has been reported to inhibit plant growth by inducing reactive oxygen species (ROS)-mediated cell death, which is associated with the disruption of root microtubule organization, thereby impairing root development[54]. Aqueous extracts of *Acacia melanoxylon* containing gallic acid were also shown to reduce protein content in *L. sativa*[56]. Similarly, the leaf extract of *Calotropis procera*, which contains caffeic acid and other phenolic compounds, inhibited the growth of *Cassia sophera* and *Allium cepa* by reducing the mitotic index and inducing chromosomal abnormalities[57]. Allelopathic benzoquinones, such as 3-[10(Z)-heptadecenyl]-2-hydroxy-5-methoxy-1,4-benzoquinone, found in seed extracts of *I. sanguinea*, have been shown to interfere with metabolic pathways related to aromatic amino acid biosynthesis and respiration, and to induce oxidative stress in the root tissues of *M. sativa*, *E. crus-galli*, *L. sativa*, and *B. rapa*[47]. These findings suggest that the mechanism underlying the phytotoxicity of *E. capillifolium* aqueous extract is not driven by a single compound but rather involves multiple overlapping pathways associated with its diverse chemical constituents. The presence of various phenolic acids (e.g., gallic acid, caffeic acid, gentisic acid), aromatic diketones (e.g., hydroxybenzoquinone), and flavonoids (e.g., quercetin) indicates that the *E. capillifolium* extract might have exerted its inhibitory effects through a combination of mechanisms, including the induction of oxidative stress, disruption of cell division, and interference with key metabolic processes[58], instead of a single dominant mechanism as found in case of synthetic herbicide.

Principal component analysis (PCA) analysis of selected response indexes (RI) related to seed germination and early seedling growth, also revealed that weed species from the genus Amaranthus (*A. hybridus*, *A. retroflexus*, *A. palmeri*, and *A. tuberculatus*), were selectively inhibited by *E. capillifolium* extracts as compared to other weed species. The selective inhibition might have been attributed to species-specific differences in the uptake and transformation of allelochemicals[59]. The differential sensitivity of weed species to *E. capillifolium* aqueous extracts might have been attributed to species-specific variation in the uptake of allelochemicals during seed imbibition. Since water absorption precedes germination and is governed by seed traits such as size, seed coat thickness, permeability, and dormancy status[60], it is likely that allelochemicals were co-absorbed with water, thereby influencing the extent of phytotoxic effects observed across different species. It was observed that highly sensitive weeds were small seeded (0.7-1.2 mm) species with thin and permeable seed coats representing weeds from the genus Amaranthus[61]. Further, the inhibitory effect of *E. capillifolium* extract among the weeds within the genus Amaranthus varied in following order: *A. hybridus*> *A. retroflexus*> *A. palmeri*> *A. tuberculatus,* indicating species-specific sensitivity of the extract and involvement of selective uptake or metabolic detoxification of

allelochemicals. Conversely, less sensitive weed species including *I. lacunose*, *E. crus-galli*, *S. spinosa*, *L. multiflorum, D. sanguinalis*, and *R. crispus* had larger seed size (2-4 mm) with thick and hard seed coats with lower permeability[62]. Notably, *E. canadensis,* despite its very small seed size (~0.5 mm) and highly permeable seed coat, exhibited greater tolerance to *E. capillifolium* extract than the *A.* spp., suggesting the involvement of additional mechanisms such as selective uptake or metabolic detoxification of allelochemicals. Selectivity of some allelochemicals towards different plant species has been reported earlier. *E. crus-galli* has been reported to be tolerant against Biochanin A, a major allelochemical present in *Trifolium pratense* and *T. repens*, as compared to broadleaf weeds (*Geranium molle* and *Silene noctiflora*) due to lack of uptake[59]. Within broadleaf weeds, *G. molle* was less susceptible to Biochanin A than *S. noctiflora*, owing to its ability to biotransform the compound into non-toxic derivatives. Similarly, root exudates of *P. australis*, containing gallic acid, inhibited seedling growth of *N. tabacum*, *L. sativa*, *B. rapa* and *S. alterniflora*, but had no effect on *B. juncea*, *Oryza sativa*, and *Triticum aestivum*[54]. These findings underscore the importance of seed morphological and physiological traits in mediating the sensitivity of weed species to allelopathic compounds and suggest that allelochemical selectivity is governed by a complex interplay of uptake dynamics and metabolic responses.

In conclusion, among nine broadleaf and four grass weed species, members from the Amaranthus genus (*A. hybridus, A. retroflexus, A. palmeri,* and *A. tuberculatus*) exhibited the highest (92.68-100%) inhibition of germination and early seedling growth with *E. capillifolium* aqueous extract. Dose-response analysis revealed *A. hybridus* as the most sensitive species (GI_{50} = 0.2687% extract), followed by *A. retroflexus* (GI_{50} = 0.5572% extract), *A. palmeri* (GI_{50} = 1.048% extract), and *A. tuberculatus* (GI_{50} = 1.811% extract). Seed germination of *Z. mays* and *A. hypogaea* were minimally impacted, while *G. hirsutum* and *G. max* showed some inhibition (RIG 25-28.57%) at 10% *E. capillifolium* aqueous extract. This study is the first to report demonstrating the bioherbicidal effects of *E. capillifolium* aqueous extract, particularly against *Amaranthus* spp. While the results are promising, they are based on controlled laboratory conditions. Therefore, field-based evaluations are necessary to validate the efficacy, selectivity, and environmental safety of *E. capillifolium* aqueous extract under agronomic conditions. This study provides a foundation for the development of *E. capillifolium*-based bioherbicides as a sustainable weed management strategy in *Z. mays* and *A. hypogaea* cropping systems.

Materials and methods

Collection of biomass

Above ground parts of mature plants of *E. capillifolium* were collected from natural areas in Auburn, Alabama, USA (32.6442^{0}N, 85.52265^{0}W) (Fig. 1). Fresh leaves were separated from the stems, washed under tap water to remove adhered dirt, and excess water absorbed by blotting them with tissue paper. A total of 200 g of freshly cleaned leaves (moisture content – 79.83%) were used for preparing aqueous extract and the remaining leaf materials was stored at -80 ^{0}C for use in subsequent experiments as required.

Preparation of aqueous extract

200 g of *E. capillifolium* leaf was weighed and macerated using a mortar and pestle[63]. The resulting paste was mixed with 800 ml double distilled water in a 2000 ml Erlenmeyer flask and agitated on an orbital shaker (Innova 4000, New Brunswick Scientific Co., USA) at 150 rpm for 48 h under 25±1 ^{0}C. The primary extract was collected by filtering the mixture through a double-layered cheese cloth, followed by centrifugation (Megafuge ST4R Plus-MD, Thermo Fisher Scientific GmbH, Ettlingen, Germany) at 3000 rpm for 30 min at 25±1 ^{0}C. The supernatant was collected in a glass bottle, marked as 25% w/v basis (200 g fresh leaves in 800 ml water), stored at 4±1 ^{0}C for use in various experiments. The primary stock solution was subsequently diluted with double-distilled water to obtain concentrations ranging from 0.5% to 20%, as required for the experiments.

Seed germination assay

Screening studies were conducted to understand the effect of *E. capillifolium* extracts (5 and 10%) on seed germination of thirteen common weeds and four crop species. The weeds included were nine broadleaves namely, *A. palmeri*, *A. tuberculatus*, *A. retroflexus*, *A. hybridus*, *E. canadensis*, *S. spinosa*, *R. crispus*, *I. lacunose*, and *C. album*, and four grasses namely, *L. multiflorum*, *D. aegyptium*, *D. sanguinalis*, and *E. crus-galli*. The seeds procured from the Azlin Seed Service, Leland, MS, were collected in 2022 and placed in permeable paper bags for storing under laboratory conditions at 20 ± 2 °C in the dark until commencement of the experiment. The crop seeds namely, *G. max* (NK65-26XFS), *A. hypogaea* (Georgia-12Y), *G. hirsutum* (DP 2038), and *Z. mays* (DKC117-27), were collected from Alabama Seed Technology Center, Auburn, Alabama. A preliminary viability test was conducted to ensure adequate seed viability for both weed and crop seeds before the experiment.

A total of twenty and twenty-five seeds for crops and weed species per population, respectively, were placed on three layers of Whatman No.1 filter paper (pre-soaked with distilled water) in a series of 9-cm diameter petri dishes and all experiments had three replications per run. Around 12 ml of *E. capillifolium* extracts (5 and 10%) were added to petri plates as per experimental requirements. Preliminary studies indicated that 12 ml volume of either water or *E. capillifolium* extract was sufficient for conducting 21 days germination studies and did not submerge studied seeds under present incubation conditions. Petri dishes were incubated at 25±1 ºC constant temperature, 60% relative humidity, and 12-h photoperiod with *E. capillifolium* aqueous extracts[64]. Another sets of control treatments with only double-distilled water were set up for all experiments under the same experimental conditions. A total of three runs were conducted for all studies. Seed germination was recorded at 0, 2, 4, 6, 8, 10, 14 and 21 days[64]. At the end of the germination test (21 days), seedling shoot, and root lengths were measured. At the conclusion of the germination test, seeds that exhibited blackened, decayed tissues or were empty were classified as dead. The viability of non-germinated seeds that appeared intact was assessed by gently tapping the seeds with forceps to check for the presence of a turgid embryo. The healthy non-germinated seeds were longitudinally dissected and immersed in a 1% solution of 2,3,5-Triphenyl Tetrazolium Chloride (TTC)[43] for 24 hours at 25±1 ^{o}C. Seeds with

stained embryos were considered viable. All viable but non-germinated seeds were categorized as dormant. Based on the results of the screen study, four broadleaf weeds which were affected most by *E. capillifolium* extracts were selected for dose response study.

Dose response study

The degree of tolerance to *E. capillifolium* extract on seed germination of four broadleaf weeds namely, *A. palmeri*, *A. tuberculatus*, *A. retroflexus*, and *A. hybridus*, were determined using a classical dose-response experiment. The assay consisted of seven concentrations (0, 0.5, 1, 2, 5, 10 and 20%) of *E. capillifolium* extract for the selected weed species. Germination studies were carried out following the procedure described earlier. Germination associate parameters, such as gemination percentage (G%), inhibited germination (IG%), relative inhibited germination (RIG%), speed of germination (SG), and mean germination time (MGT) were calculated by following equations.

$$G\% = (\text{Number of normal seedling/Number of seed}) \times 100 \quad (1)$$

$$IG\% = 100\text{-}G \quad (2)$$

$$RIG\% = [(IG\% \text{ at treatment} - IG\% \text{ at control}) / (100\text{-}IG\% \text{ at control})] \times 100 \quad (3)$$

$$SG = n_1/d_1+n_2/d_2+n_3/d_3+\text{----------} \quad (4)$$

$$MGT = (n_1 \times d_1 + n_2 \times d_2 + n_3 \times d_3 + \text{--------})/ \text{ Total number of days} \quad (5)$$

where, n represented the number of germinated seeds on d^{th} days

At the end of the germination test (21 days), seedling shoot, and root lengths were measured which served as an indicator of seed vigor. The allelopathic effects of extracts were measured by calculating the allelopathic response index (RI) as described by Williamson and Richardson[41].

$$RI = 1\text{-}C/T \ (T>C) \text{ or } RI = T/C\text{-}1 \ (T<C) \quad (6)$$

Where, C and T represent the corresponding index values for control and treatment. If RI > 0, it represented that there was a promoting effect, otherwise RI < 0 was meant for an inhibiting effect, and the absolute value of RI depicted the strength of the allelopathy. The synthetical allelopathic effects (SE) were assessed based on the average Relative Index (RI) value of five parameters: gemination percentage (G%), speed of germination (SG), mean germination time (MGT), shoot height (S), root length (R)[40,43]. All measurements were taken from the same receptor seeds subjected to the same treatment.

Identification of compounds in *E. capillifolium* extract with reverse phase liquid chromatography–mass spectrometry (LC–MS)

For reverse phase analysis, 100 µL of sample was mixed with 500 µL ice cold ethanol with 15 minutes of freezing time followed by centrifugation for 5 minutes to precipitate protein. The supernatant was concentrated on a Thermo Savant DNA 120 vacuum centrifuge on medium heat for 2 hours. The sample was re-dissolved with 100 µL water and analyzed. Analysis was performed on a Vanquish UHPLC system (Thermo Fisher Scientific, Waltham, Massachusetts, USA) coupled with a quadrupole orbitrap mass spectrometer (Orbitrap Exploris 120, Thermo Fisher Scientific, Waltham, Massachusetts, USA) with electrospray ionization (H-ESI) switching between positive or negative modes using Xcalibur software (V4.4.16.14). Injection of 10 uL of the sample was made on a C18 column (Accucore RP-MS 100 x 2.1 mm with 2.6 µm particles, Thermo Fisher Scientific, Waltham, Massachusetts, USA) held at 40 °C with a 200 µL/min flow rate of mobile phase solution A (99.9% water with 0.1% formic acid) and solution B (100% acetonitrile). The gradient began at 0% B, held for 2 minutes followed by a linear ramp to 95% B in 11 min, held at 95% B for 1 min, and decreased to 0%B in one min, then held for 5 min for a total analysis time of 20 minutes. The flow was diverted to waste for the first minute and a half of analysis and after 15 minutes. The MS scan range was 50-500 m/z with resolution of 120,000, 70% RF lens, maximum injection time auto, with EASY-IC run-start on. The spray voltage was 3300 V in positive and 2100 V in negative mode, the ion transfer tube temperature was 320 °C, and the vaporizer temperature was 275 °C. Data dependent acquisition on singly charged precursors only was used with dynamic exclusion on auto, with intensity threshold of 50,000, the window was 2 Da, the HCD collision energy was set to 40% normalized, the MSMS resolution was 15,000 and the AGC was set to standard for the 4 dependent scans. A targeted mass exclusion list was created based on a blank injection and apex detection was set to 30%.

The LC-MS results were used in Compound Discoverer v3.2 to align retention times, detect compounds, merge features, group compounds, search mzCloud, search ChemSpider with BioCyc, ChEBI, and ChEMBL databases with tolerance of 5 ppm, search mass lists including the Arita Lab Flavinoid Structure Database, EFS HRAM compound Database, and the Endogenous Metabolites database and predict compositions automatically.

Data analysis

For all germination and seedling growth data, deviations from normality and the homogeneity of the variances were evaluated in RStudio (v3.0.1) by using Shapiro–Wilk's test and Bartlett's test, respectively[65]. Differences in the values of various parameters of seed germination and seedling growth for all studied weed species were measured using an analysis of variance (one way ANOVA) with Tukey's honest significant difference (HSD) at a significance level of $\alpha = 0.05$ using JMP PRO v.18. (SAS Institute Inc., Cary, NC, 1989–2023). Principal component analysis (PCA) was performed to understand the primary effects of *E. capillifolium* aqueous extract on various inhibition parameters of seed germination and seedling growth across the studied weed species using JMP PRO v.18. Data presented in this manuscript indicated mean values ± standard error (SE) of various parameters for different weed species. Three-parameter sigmoidal curves (equation 7) fit on the seed germination data for RR, SPW, PA and WH from the dose-response assay, with log

concentration of extracts using the R Statistical Software (V4.3.2, R Core Team 2023) and the drc R package (v3.0.1)[65].

$$Y = d/[1 + \exp[b\{\log(x) - \log(e)\}] \quad (7)$$

where Y = germination inhibition (%), d = upper limit, x = concentration of *E. capillifolium* extract (%), b = relative slope around e, and e = GI_{50} (inflection point, mid-point or estimated dose when Y = 50%).

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Rakesh Kumar Ghosh: Writing – original draft, Investigation, Data curation, Formal analysis, Conceptualization; Andrew J. Price: Writing – review & editing; Melissa Boersma: Methodology, Data curation; Aniruddha Maity: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Data availability statement

All data generated in this experiment are presented including a supplementary file.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary Information

Supplemetary tabel is included for additional data.

Fig. 1. A representative fully grown *Eupatorium capillifolium* plant in Auburn, Alabama, used in the study

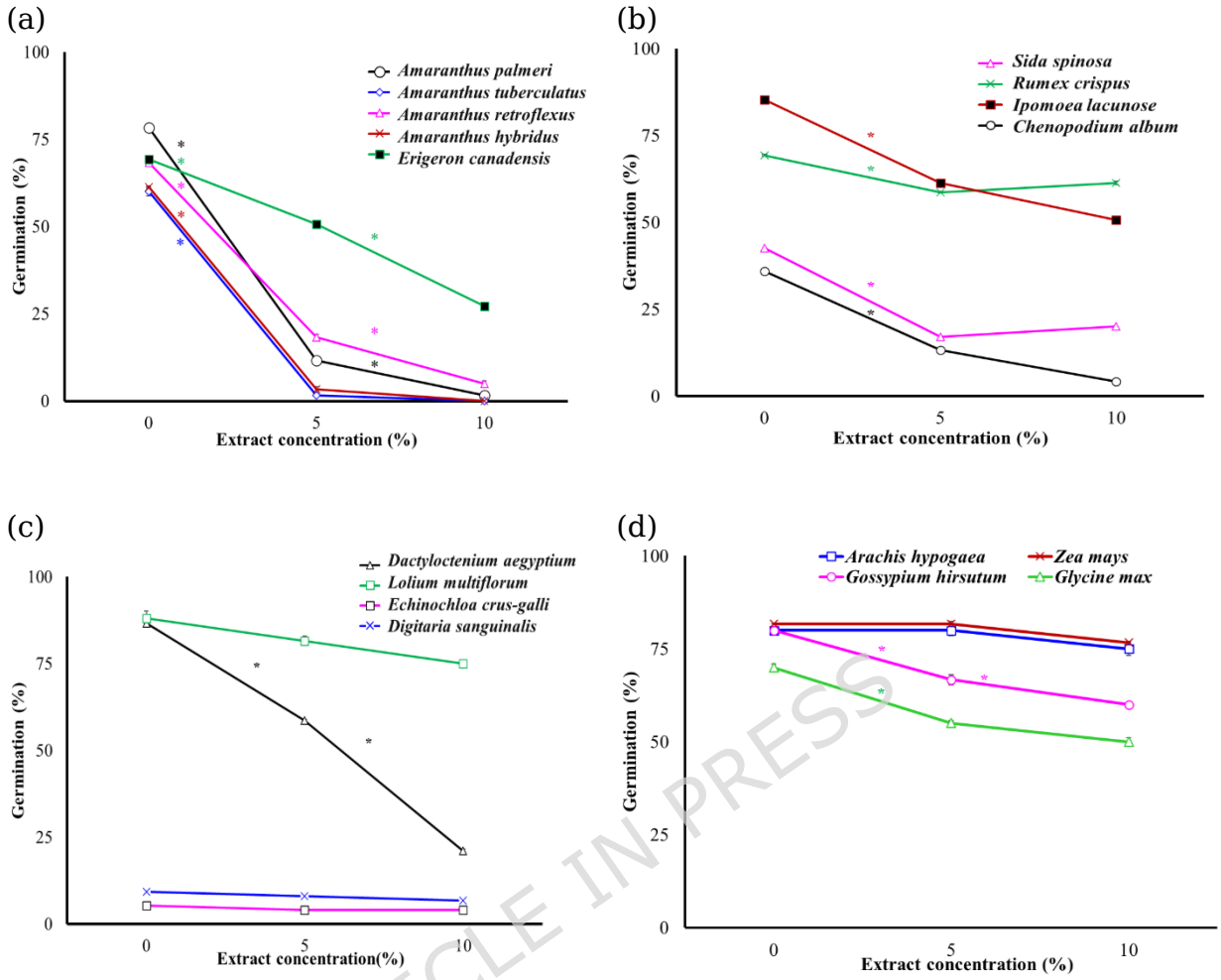


Fig. 2. Effect of *Eupatorium capillifolium* aqueous extracts (5 and 10%) on seed germination (%) of selected weed species (a) *Amaranthus palmeri*, *Amaranthus tuberculatus*, *Amaranthus retroflexus*, *Amaranthus hybridus*, and *Erigeron canadensis*, (b) *Sida spinosa*, *Rumex crispus*, *Ipomoea lacunose*, and *Chenopodium album*, (c) *Dactyloctenium aegyptium*, *Lolium multiflorum*, *Echinochloa crus-galli*, and *Digitaria sanguinalis*, and crop species (d) *Arachis hypogaea, Zea mays, Glycine max, and Gossypium hirsutum* at the end of a 21-day germination test. Asterisks (*) indicate significant difference ($p<0.05$) between among three DF treatments (0, 5 & 10%) for a given weed species.

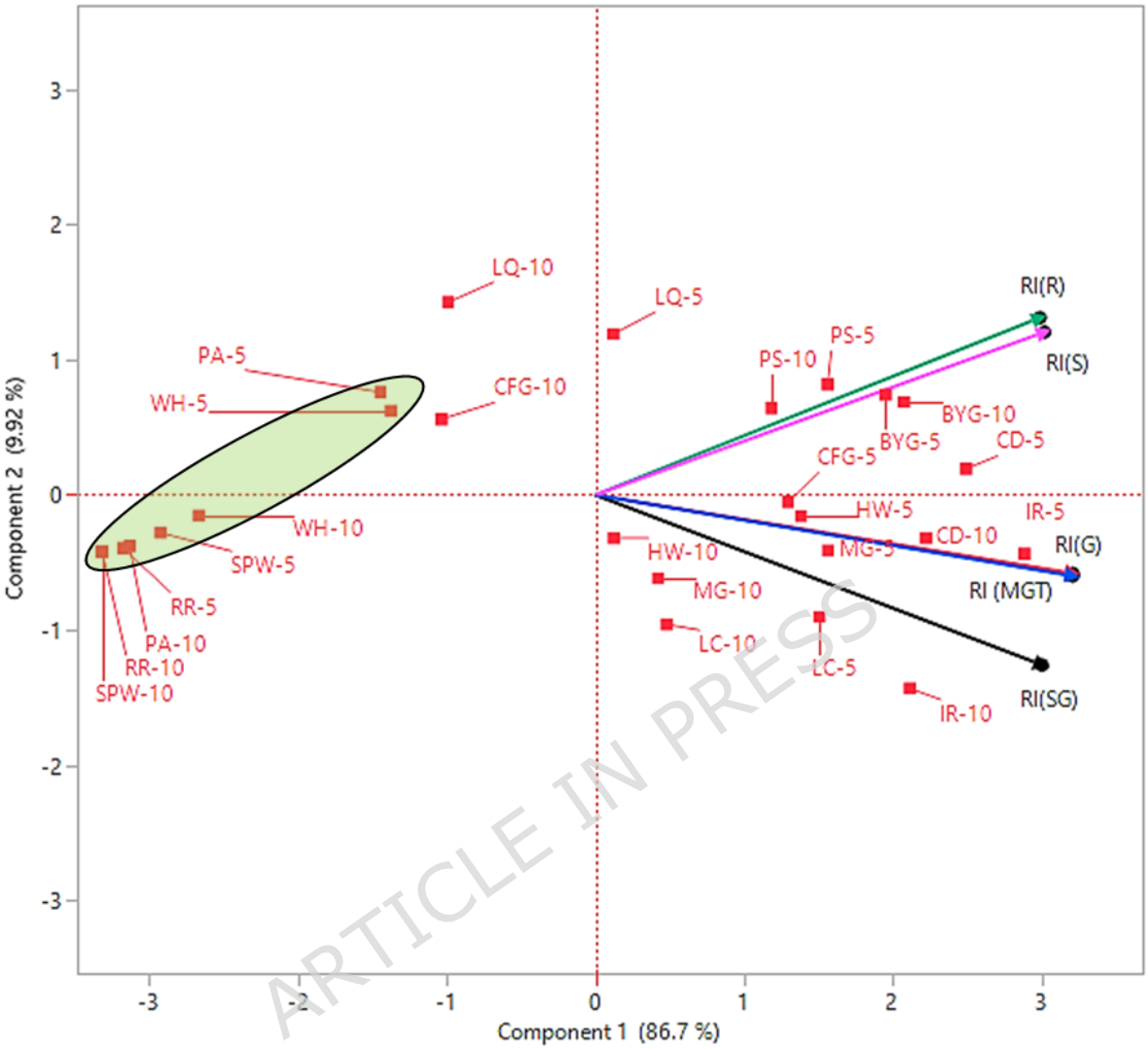
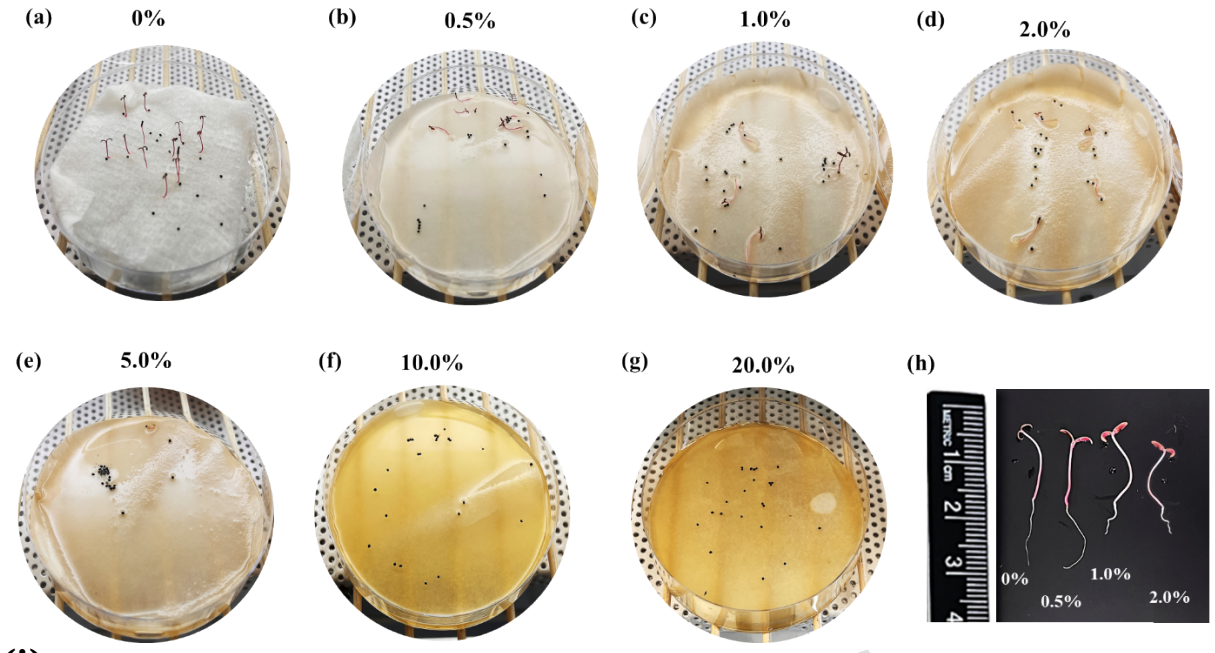
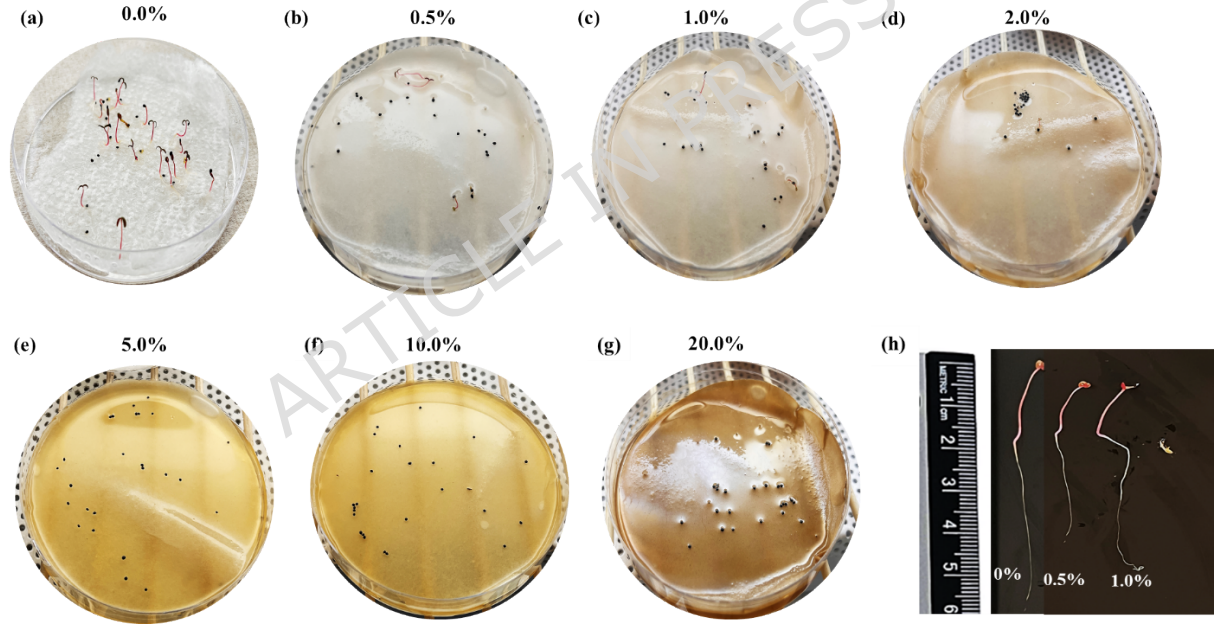


Fig. 3. Principal component analysis on the effect of *Eupatorium capillifolium aqueous* extracts (5 and 10%) on different response indexes of various weed species. *RI(G) Response index for germination(%), RI(SG) Response index for Speed of germination, RI(MGT) Response index for mean germination time, RI(R) Response index for root length, RI(S) Response index for shoot length, Dogfennel (DF), Amaranthus palmeri* (PA), *Amaranthus tuberculatus* (WH), *Amaranthus retroflexus* (RR), *Amaranthus hybridus* (SPW), *Erigeron canadensis* (HW), *Sida spinosa* (PS), *Rumex crispus* (CD), *Ipomoea lacunose* (MG), and *Chenopodium album* (LQ), and four grasses namely, *Lolium multiflorum* (IR), *Dactyloctenium aegyptium* (CFG), *Digitaria sanguinalis* (LCG) and *Echinochloa crus-galli* (BYG)



(i)



(ii)

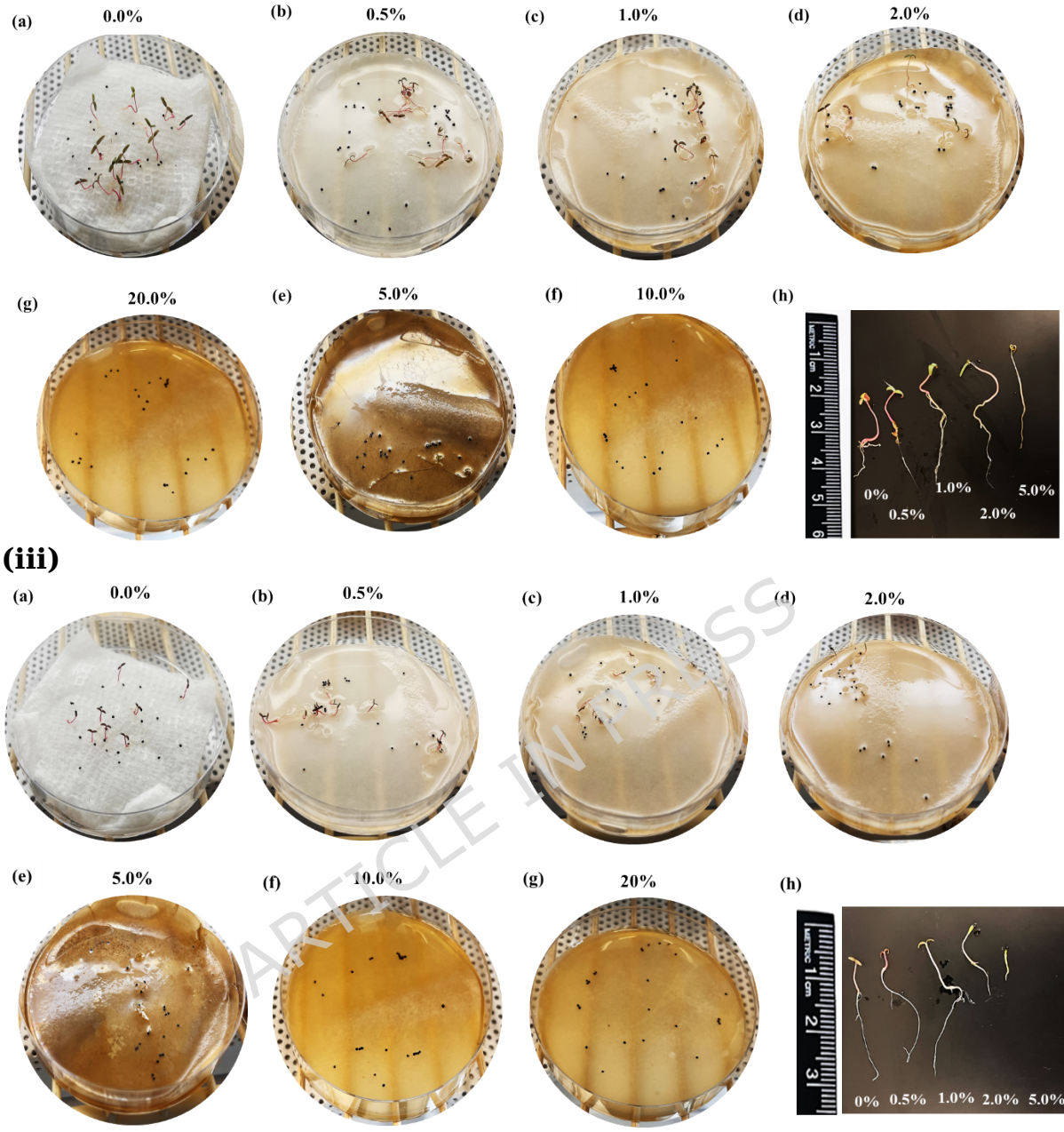


Fig. 4. (a→g) Germinating seeds and (h) seedling length of (i) *Amaranthus retroflexus*, (ii) *Amaranthus hybridus*, (iii) *Amaranthus palmeri*, and (iv) *Amaranthus tuberculatus* in response to increasing concentrations (0-20%) of *Eupatorium capillifolium* aqueous extracts at the end of a 21-day germination test.

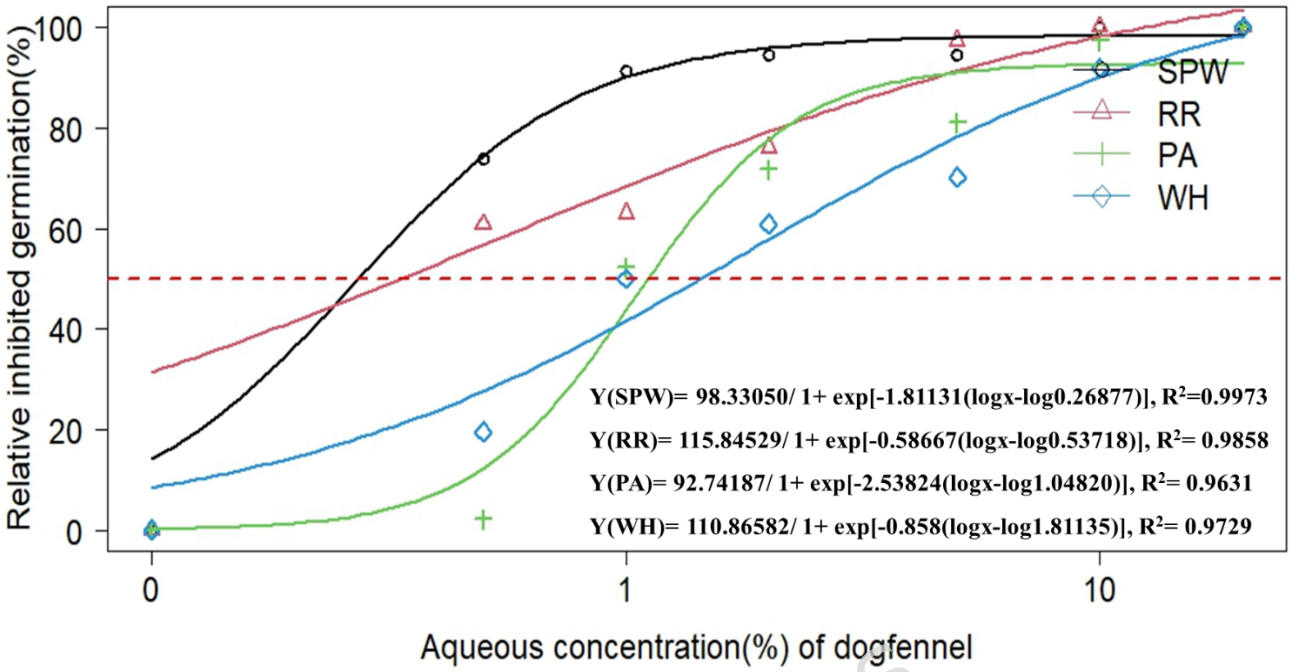


Fig. 5. Dose-response analysis of various pigweeds with different concentrations of *Eupatorium capillifolium* aqueous extracts. *Dogfennel (Eupatorium capillifolium), Amaranthus palmeri* (PA), *Amaranthus tuberculatus* (WH), *Amaranthus retroflexus* (RR), and *Amaranthus hybridus* (SPW)

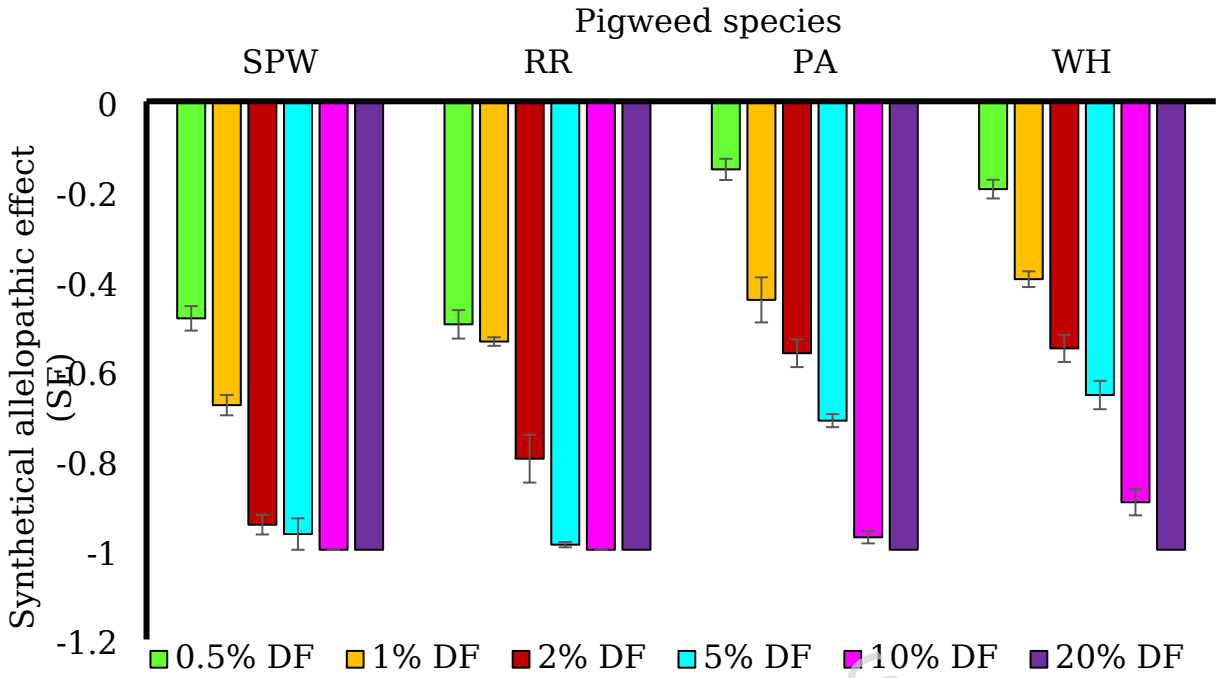


Fig. 6. The synthetical allelopathic effects (SE) of various concentrations of *Eupatorium capillifolium* aqueous extracts on different pigweed species. *Eupatorium capillifolium (DF), Amaranthus palmeri* (PA), *Amaranthus tuberculatus* (WH), *Amaranthus retroflexus* (RR), and *Amaranthus hybridus* (SPW)

Table 1. Effect of *Eupatorium capillifolium* aqueous extracts (5 and 10%) on relative inhibited germination (RIG%) of selected weed species and crops*

Species	Aqueous extract (%)		Trend equation	R^2
	5	10		
	RIG_5 (%)	RIG_{10} (%)		
Broadleaf weeds				
Amaranthus retroflexus	97.22	100.00	y= -7.666x + 68.89	0.846
Amaranthus hybridus	94.57	100.00	y= -6.133x + 52.22	0.791
Amaranthus palmeri	85.10	97.87	y= -6x + 50.557	0.771
Amaranthus tuberculatus	73.17	92.68	y= -6.33x + 62.22	0.899
Erigeron canadensis	26.91	60.87	y= -4.22x +70.143	0.996
Sida spinosa	60.09	52.96	y= -2.26x +37.89	0.651
Rumex crispus	15.38	11.54	y= -0.8x + 67.111	0.519
Ipomoea lacunose	28.13	40.63	y= -3.467x + 85.41	0.953
Chenopodium album	62.96	88.52	y= -3.187x + 33.75	0.944
Grass weeds				
Lolium multiflorum	7.40	14.87	y= -1.31x +88.11	1.000
Dactyloctenium aegyptium	32.31	75.64	y= -6.556x +88.26	0.993
Digitaria sanguinalis	14.29	28.57	y= -0.267x +9.333	1.000
Echinochloa crus-galli	25.00	25.00	y= -0.133x + 5.111	0.750
Crops				
Arachis hypogaea	0.00	6.25	y= -0.5x +80.83	0.750
Zea mays	0.00	6.12	y= -0.5x +82.5	0.750
Gossypium hirsutum	16.66	25.00	y= -2x +78.89	0.964
Glycine max	21.43	28.57	y= -2x +68.33	0.923

*At the end of a 21-day germination test. Different superscript letters on mean values in a specific row indicate significant differences in seed germination among the doses within a species at $P < 0.05$. x represents concentration of *Eupatorium capillifolium* in the regression equation.

Table 2. Effect of *Eupatorium capillifolium* (DF) aqueous extracts (5 and 10%) on seed germination and seedling growth parameters* of various weeds

Treatments	Seed germination parameters						Seedling growth parameters			
	G(%)	RI(G)	SG	RI(SG)	MGT	RI(MGT)	R (mm)	RI(R)	S (mm)	RI(S)
Amaranthus hybridus										
Control (0.0%)	61.33[a] (±0.267)	-	11.25[a] (±0.301)	-	45.21[a] (±0.993)	-	31.33[a] (±1.526)	-	16.09 (±0.423)	-
5% DF	2.67[b] (±0.600)	-0.948 (±0.52)	0.424[b] (±0.424)	-0.963 (±0.037)	1.94[b] (±1.937)	-0.959 (±0.041)	1.69[b] (±1.693)	-0.833 (±0.096)	0.000	-1.00
10% DF	0.000	-1.000	0.000	-1.000	0.000	-1.000	0.000	-1.000	0.000	-1.00
Amaranthus retroflexus										
Control (0.0%)	60.00[a] (±1.29)	-	8.21[a] (±0.470)	-	34.95[a] (±3.257)	-	19.89 (±1.120)	-	11.43 (±0.73)	-
5% DF	1.67[b] (±0.373)	-0.976 (±0.024)	0.115[b] (±0.115)	-0.987 (±0.013)	0.841[b] (±0.841)	-0.979 (±0.021)	0.00	-0.951 (±0.049)	0.00	-1.00
10% DF	0.000	-1.000	0.000	-1.000	0.000	-1.000	0.000	-1.000	0.00	-1.00
Amaranthus palmeri										
Control (0.0%)	78.33[a] (±0.373)	-	15.59[a] (±0.654)		47.70[a] (±1.065)	-	36.41[a] (±4.420)	-	16.09[a] (±0.423)	-
5% DF	11.67[b] (±0.373)	-0.851 (±0.020)	1.80[b] (±0.148)	-0.885 (±0.005)	6.87[b] (±0.826)	-0.856 (±0.016)	13.55[b] (±0.423)	-0.614 (±0.056)	10.37[b] (±0.763)	-0.354 (±0.054)
10% DF	1.68[c] (±0.33)	-0.979 (±0.021)	0.420[c] (±0.420)	-0.975 (±0.025)	1.03[c] (±1.032)	-0.979 (±0.021)	2.54c	-0.928 (±0.009)	0.000	-1.00
Amaranthus tuberculatus										

Control (0.0%)	68.33[a] (±0.373)	-	11.84[a] (±0.366)	-	41.44[a] (±0.962)	-	24.13[a] (±0.733)	-	15.66[a] (±0.423)	-
5% DF	18.33[b] (±0.745)	-0.852 (±0.074)	1.30[b] (±0.651)	-0.892 (±0.054)	10.65[b] (±0.117)	-0.744 (±0.043)	11.43[b] (±0.733)	-0.524 (±0.045)	7.83[b] (±1.120)	-0.500 (±0.072)
10% DF	5.00[c] (±0.65)	-0.923 (±0.038)	0.594[c] (±0.306)	-0.951 (±0.025)	2.87[c] (±1.623)	-0.931 (±0.038)	2.12c (±1.12)	-0.915 (±0.044)	3.39[c] (±0.423)	-0.784 (±0.025)
Erigeron canadensis										
Control (0.0%)	69.33[a] (±0.267)		9.81[a] (±0.391)		47.81[a] (±0.886)		19.05[a] (±1.404)		12.56[a] (±0.580)	
5% DF	50.67[b] (±0.533)	-0.268 (±0.047)	5.84[b] (±0.287)	-0.401 (±0.044)	32.64[b] (±1.541)	-0.316 (±0.046)	11.96[a][b] (±0.643)	-0.228 (±0.046)	9.24[ab] (±0.632)	-0.261 (±0.057)
10% DF	27.13[c] (±0.422)	-0.480 (±0.035)	4.54[c] (±0.320)	-0.533 (±0.051)	24.33[c] (±1.60)	-0.490 (±0.042)	8.18[b] (±0.662)	-0.468 (0.064)	6.90[b] (±1.04)	-0.452 (±0.075)
Sida spinosa										
Control (0.0%)	42.67[a] (±0.533)		10.98[a] (±0.359)		31.62[a] (±1.67)		47.41[a] (±1.85)		22.44[a] (±1.85)	
5% DF	17.03[b] (±0.27)	-0.367 (±0.067)	5.32[b] (±0.385)	-0.513 (±0.045)	20.00[b] (±1.00)	-0.361 (±0.061)	49.53[a] (±2.64)	0.040 (±0.052)	19.89[a] (1.53)	-0.107 (±0.064)
10% DF	20.07[b] (±0.961)	-0.367 (±0.133)	4.60[b] (±0.985)	-0.584 (±0.082)	19.59[b] (±3.60)	-0.376 (±0.126)	44.45[a] (±3.67)	-0.063 (±0.063)	17.78[a] (±1.27)	-0.206 (±0.024)
Rumex crispus										
Control (0.0%)	69.33[a] (±0.267)		11.94[a] (±0.147)		51.05[a] (±0.906)		34.29[a] (±1.27)		12.28[a] (±0.423)	
5% DF	58.67[b]	-0.154	8.57[b]	-0.282	41.71[b]	-0.183	35.14[a]	0.022	11.85[a]	-0.037

	(±0.267)	(±0.018)	(±0.303)	(±0.025)	(±1.090)	(±0.022)	(±1.845)	(±0.022)	(±0.847)	(±0.037)
10% DF	61.33[b] (±0.462)	-0.115 (±0.002)	8.55[b] (±0.118)	-0.283 (±0.017)	43.21[b] (±0.546)	-0.153 (±0.004)	25.40[b] (±1.47)	-0.260 (±0.023)	11.85[a] (±0.847)	-0.037 (±0.037)
Ipomoea lacunose										
Control (0.0%)	85.33[a] (±0.705)		26.40[a] (±1.11)		65.94[a] (±2.73)		95.25[a] (±3.67)		51.65[a] (±2.96)	
5% DF	61.33[b] (±0.267)	-0.279 (±0.035)	18.66[b] (±0.767)	-0.290 (±0.047)	47.24[b] (±1.14)	-0.281 (±0.036)	61.38[b] (±5.60)	-0.358 (±0.036)	44.45[b] (±3.65)	-0.142 (±0.024)
10% DF	50.67[b] (±0.961)	-0.408 (±0.037)	14.67[b] (±1.27)	-0.447 (±0.028)	38.76[c] (±3.69)	-0.414 (±0.037)	47.41[c] (±1.84)	-0.502 (±0.018)	29.63[c] (±7.63)	-0.439 (±0.112)
Chenopodium album										
Control (0.0%)	36.00[a] (±0.800)		4.51[a] (±0.594)		24.59[a] (±2.87)		38.95[a] (±2.96)		19.35[a] (±1.94)	
5% DF	13.33[b] (±0.533)	-0.629 (±0.072)	1.15[b] (±0.229)	-0.744 (±0.051)	8.41[b] (±1.68)	-0.657 (±0.067)	30.16[b] (±1.20)	-0.215 (±0.072)	16.44[b] (±0.484)	-0.129 (±0.104)
10% DF	4.13[c] (±0.533)	-0.856 (±0.075)	0.459[b] (±0.229)	-0.901 (±0.052)	3.36[c] (±1.68)	-0.867 (±0.069)	23.71[b] (±0.847)	-0.384 (±0.051)	15.66[b] (±1.53)	-0.190 (±0.028)
Dactyloctenium aegyptium										
Control (0.0%)	86.67[a] (±0.267)	-	17.34[a] (±0.526)	-	62.65[a] (±1.11)	-	19.05[a] (±2.78)	-	16.09[a] (±1.53)	-
5% DF	58.67[b] (±0.267)	-0.323 (±0.005)	9.99[b] (±0.303)	-0.423 (±0.024)	42.60[b] (±1.02)	-0.320 (±0.005)	13.97[a][b] (±0.733)	-0.248 (±0.126)	12.28[a][b] (±1.12)	-0.222 (±0.111)
10% DF	21.11[c]	-0.756	2.99[c]	-0.827	16.37[c]	-0.738	9.59[b]	-0.491	8.75[b]	-0.448

	(±0.444)	(±0.028)	(±0.284)	(±0.021)	(±1.64)	(±0.029)	(±0.43)	(±0.060)	(±0.267)	(±0.044)
Lolium multiflorum										
Control (0.0%)	88.09[a] (±2.08)		10.02[a] (±0.245)		42.29[a] (±2.18)		79.59[a] (±1.84)		74.93[a] (±2.64)	
5% DF	81.59[a] (1.38)	-0.059 (±0.138)	9.03[a] (±0.080)	-0.097 (±0.029)	38.24[a] (±1.46)	-0.090 (±0.063)	76.43[a] (±2.58)	-0.038 (±0.042)	68.93[a] (±1.24)	-0.077 (±0.044)
10% DF	75.00[a] (±0.645)	-0.137 (±0.059)	8.97[a] (±0.201)	-0.105 (±0.011)	42.71[a] (±1.32)	0.015 (±0.054)	47.84[b] (±1.85)	-0.372 (±0.041)	46.57[b] (±5.60)	-0.372 (±0.099)
Digitaria sanguinalis										
Control (0.0%)	9.33[a] (±0.267)		1.09[a] (±0.073)		6.33[a] (±0.714)		43.60[a] (±2.96)		24.98[a] (±1.53)	
5% DF	8.00[a] (±0.800)	-0.250 (±0.250)	0.966[a] (±0.455)	-0.220 (±0.280)	5.52[a] (±2.714)	-0.236 (±0.264)	25.35[b] (±3.15)	-0.423 (±0.041)	17.78[b] (±0.733)	-0.280 (±0.070)
10% DF	6.67[a] (±0.267)	-0.278 (±0.147)	0.573[a] (±0.115)	-0.478 (±0.099)	4.21[a] (±0.841)	-0.334 (±0.130)	13.12[c] (±0.423)	-0.696 (±0.021)	15.24[b] (±1.27)	-0.387 (±0.058)
Echinochloa crus-galli										
Control (0.0%)	5.33[a] (±0.267)		1.18[a] (±0.080)		3.94[a] (±0.841)		35.69[a] (±1.58)		23.63[a] (±0.851)	
5% DF	4.00[a] (±0.462)	-0.333 (±0.441)	0.455[b] (±0.248)	-0.607 (±0.203)	2.71[a] (±1.54)	-0.027 (±0.266)	34.53[a] (±1.79)	-0.032 (±0.027)	23.36[a] (±1.07)	-0.011 (±0.028)
10% DF	4.00[a] (±0.462)	0.000 (±0.577)	0.455[b] (±0.248)	-0.639 (±0.197)	2.71[a] (±1.54)	-0.224 (±0.415)	35.60[a] (±1.07)	-0.002 (±0.037)	22.30[a] (±1.27)	-0.055 (±0.058)

[*]At the end of a 21-day germination test. Different superscript letters on mean values for each weed species in a specific column indicate significant differences in seed germination among the doses within a species at $P < 0.05$.

G(%)= % germination, RI(G%)= Response index for G(%), SG = Speed of germination, RI(SG) = Response index for SG, MGT = Mean germination time, RI(MGT) = Response index for MG, R = Root length (mm), RI(R) = Response index for R, S = Shoot length (mm), RI(S) = Response index for S.

Table 3. Effect of *Eupatorium capillifolium* (DF) aqueous extract on seed germination and seedling growth parameters* of various pigweeds

Treatments	Seed germination parameters						Seedling growth parameters			
	G(%)	RI(G)	SG	RI(SG)	MGT	RI(MGT)	R (mm)	RI(R)	S (mm)	RI(S)
Amaranthus hybridus										
Control	61.33[a] (±0.267)	-	11.25[a] (±0.301)	-	45.21[a] (±0.993)	-	31.33[a] (±1.53)	-	16.09[a] (±0.423)	-
0.5% DF	16.00[b] (±0.462)	-0.740 (±0.033)	3.13[b] (±0.188)	-0.707 (±0.18)	11.33[b] (±1.327)	-0.750 (±0.025)	28.36[b] (±0.423)	-0.09	14.05[a] (±1.86)	-0.131 (±0.095)
1% DF	5.33[c] (±0.267)	-0.914 (±0.019)	0.625[c] (±0.115)	-0.941 (±0.011)	3.65[c] (±0.841)	-0.920 (±0.017)	25.82[b] (±1.12)	-0.18	8.89[b] (±1.94)	-0.440 (±0.139)
2% DF	2.67[c] (±0.533)	-0.956 (±0.044)	0.424[c] (±0.424)	-0.960 (±0.040)	1.94[c] (±1.94)	-0.956 (±0.044)	4.66[c] (±0.423)	-0.85	0.00	-1.00
5% DF	2.667[c] (±0.600)	-0.948 (±0.52)	0.424[c] (±0.424)	-0.963 (±0.037)	1.94[c] (±1.937)	-0.959 (±0.041)	1.69[d] (±1.693)	-0.833 (±0.096)	0.00	-1.00
10% DF	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00
20% DF	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00
Amaranthus retroflexus										
Control	60.00[a] (±1.29)	-	8.21[a] (±0.470)	-	34.95[a] (±3.257)	-	19.89[a] (±1.120)	-	11.43[a] (±0.73)	-
0.5% DF	24.00[b] (±0.65)	-0.607 (±0.085)	3.74[b] (±0.590)	-0.649 (±0.055)	16.27[b] (±1.993)	-0.579 (±0.084)	13.12[b] (±1.12)	-0.335 (±0.068)	11.01[a] (±0.423)	-0.316 (±0.009)
1% DF	22.67[b] (±0.27)	-0.619 (±0.019)	3.42[b] (±0.079)	-0.679 (±0.007)	15.29[b] (±0.522)	-0.661 (±0.017)	12.28[b] (±0.423)	-0.377 (±0.053)	10.58[a] (±0.423)	-0.342 (±0.023)
2% DF	14.67[c]	-0.764	1.48[c]	-0.861	9.41[c]	-0.789	8.47[c]	-0.571	0.00	-1.00

	(±1.16)	(±0.092)	(±0.670)	(±0.063)	(±3.88)	(±0.088)	(±0.423)	(±0.037)		
5% DF	1.67[d] (±0.373)	-0.976 (±0.024)	0.115[d] (±0.115)	-0.987 (±0.013)	0.841[d] (±0.841)	-0.979 (±0.021)	0.00	-1.00	0.00	-1.00
10% DF	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00
20% DF	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00
Amaranthus palmeri										
Control	78.33[a] (±0.373)	-	15.59[a] (±0.654)		47.70[a] (±1.065)	-	36.41[a] (±4.420)	-	16.09[a] (±0.423)	-
0.5% DF	60.00[b] (±0.462)	-0.234 (±0.017)	13.65[b] (±0.678)	-0.125 (±0.011)	45.03[a] (±1.95)	-0.057 (±0.024)	28.36[b] (±0.423)	-0.198 (±0.95)	13.76[b] (±0.560)	-0.145 (±0.015)
1% DF	29.33[c] (±0.961)	-0.623 (±0.067)	6.96[c] (±0.870)	-0.547 (±0.074)	22.06[b] (±03.48)	-0.535 (±0.081)	25.40[bc] (±1.47)	-0.287 (±0.071)	12.49[bc] (±0.212)	-0.222 (±0.030)
2% DF	17.33[d] (±0.267)	-0.778 (±0.022)	4.05[d] (±0.385)	-0.737 (±0.036)	12.90[c] (±1.01)	-0.728 (±0.028)	24.77[c] (±1.32)	-0.302 (±0.078)	11.85[c] (±0.423)	-0.263 (±0.023)
5% DF	11.67[e] (±0.373)	-0.851 (±0.020)	1.80[e] (±0.148)	-0.885 (±0.005)	6.87[d] (±0.826)	-0.856 (±0.016)	13.55[d] (±0.423)	-0.614 (±0.056)	10.37[d] (±0.763)	-0.354 (±0.054)
10% DF	1.68[f] (±0.33)	-0.979 (±0.021)	0.420[f] (±0.420)	-0.975 (±0.025)	1.03[e] (±1.032)	-0.979 (±0.021)	2.54[e]	-0.928 (±0.009)	0.000	-1.00
20% DF	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00
Amaranthus tuberculatus										
Control	68.33[a] (±0.373)	-	11.84[a] (±0.366)	-	41.44[a] (±0.962)	-	24.13[a] (±0.733)	-	15.66[a] (±0.423)	-
0.5% DF	49.33[b] (±0.267)	-0.276 (±0.038)	10.22[a] (±0.340)	-0.135 (±0.047)	36.33[b] (±1.32)	-0.121 (±0.053)	20.53[b] (±0.923)	-0.119 (±0.026)	10.50[b] (±0.339)	-0.328 (±0.034)

1% DF	30.67^{c} (±0.706)	-0.553 (±0.042)	5.92^{b} (±0.660)	-0.502 (±0.043)	22.68^{c} (±2.55)	-0.455 (±0.050)	20.11^{b} (±1.18)	-0.134 (±0.113)	10.37^{b} (±0.56)	-0.339 (±0.020)
2% DF	24.00^{d} (±0.800)	-0.650 (±0.054)	3.54^{c} (±0.735)	-0.704 (±0.052)	16.86^{d} (±2.58)	-0.595 (±0.062)	13.34^{c} (±0.367)	-0.427 (±0.009)	9.74^{b} (±0.423)	-0.378 (±0.024)
5% DF	18.33^{e} (±0.745)	-0.852 (±0.074)	1.30^{d} (±0.651)	-0.892 (±0.054)	10.65^{e} (±0.117)	-0.744 (±0.043)	11.43^{d} (±0.733)	-0.524 (±0.045)	7.83^{b} (±1.120)	-0.500 (±0.072)
10% DF	5.00^{f} (±0.65)	-0.923 (±0.038)	0.594^{d} (±0.306)	-0.951 (±0.025)	2.87^{f} (±1.623)	-0.931 (±0.038)	2.12^{e} (±1.12)	-0.915 (±0.044)	3.39^{c} (±0.423)	-0.784 (±0.025)
20% DF	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00	0.00	-1.00

*At the end of a 21-day germination test. Different superscript letters on mean values for each weed species in a specific column indicate significant differences in seed germination among the doses within a species at $P < 0.05$. *G(%)= % germination, RI(G%)= Response index for G(%), SG = Speed of germination, RI(SG) = Response index for SG, MGT = Mean germination time, RI(MGT) = Response index for MG, R = Root length (mm), RI(R) = Response index for R, S = Shoot length (mm), RI(S) = Response index for S.*

Table 4. LC-MS analysis of *Eupatorium capillifolium* aqeous extract

Sl. No.	Name of the compound	Molecular formula	Molecular weight	RT (min)	% area
1	Gallic acid	$C_7H_6O_5$	170.0215	1.954	4.50
2	Hydroxy-1,4-benzoquinone	$C_6H_4O_3$	124.0161	1.961	4.18
3	7-(2-hydroxypropan-2-yl)-1,4a-dimethyl-decahydronaphthalen-1-ol	$C_{15}H_{28}O_2$	222.1981	13.049	3.25
4	(-)-alpha-Cedrene	$C_{15}H_{24}$	204.1875	13.055	3.22
5	Acetophenone	C_8H_8O	120.0574	1.814	2.99
6	Cyclononyne	C_9H_{14}	122.1095	13.05	1.76
7	Gentisic acid	$C_7H_6O_4$	154.0268	3.795	1.59
8	(2E)-4-Hydroxy-3,7-dimethyl-2,6-octadien-1-yl beta-D-glucopyranoside	$C_{16}H_{28}O_7$	378.1882	8.964	1.28
9	(-)-Caryophyllene oxide	$C_{15}H_{24}O$	220.1825	11.346	1.24
10	(1S,4R,6S)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]oct-6-yl hexopyranoside	$C_{16}H_{28}O_7$	332.1828	8.957	1.03
11	1-phenylpropane-1,2-dione	$C_9H_8O_2$	148.0523	3.127	1.01
12	L-Phenylalanine	$C_9H_{11}NO_2$	165.0788	3.1	1.01
13	(5-methyl-3-isoxazolyl)[4-(5-propyl-2-pyrimidinyl)piperazino]methanone	$C_{16}H_{21}N_5O_2$	315.1675	8.207	0.94
14	Phenylacetylene	C_8H_6	102.0468	3.11	0.73
15	Capsidiol	$C_{15}H_{24}O_2$	236.1775	11.131	0.62
16	1,4-dihydroxy-1,4-dimethyl-7-(propan-2-ylidene)-decahydroazulen-6-one / Zedoarondiol	$C_{15}H_{24}O_3$	252.1722	10.433	0.62
17	Caffeic acid	$C_9H_8O_4$	180.0423	8.404	0.57
18	2-Acetamidophenol	$C_8H_9NO_2$	151.0632	10.487	0.52
19	4-Hydroxyindole	C_8H_7NO	133.0526	10.489	0.51
20	2,4,6-Trihydroxy-2-(4-hydroxybenzyl)-1-benzofuran-3(2H)-one	$C_{15}H_{12}O_6$	288.0632	10.238	0.50
21	(1S,4R,6S)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]oct-6-yl hexopyranoside	$C_{16}H_{28}O_7$	332.1829	10.522	0.48
22	Acrylic acid	$C_3H_4O_2$	72.02133	1.737	0.46
23	DL-Erythrono-1_4-lactone;Erythrono-1_4-lactone	$C_4H_6O_4$	118.0268	1.827	0.45
24	(2R,3R)-3,5-dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-3,4-dihydro-2H-1-benzopyran-4-one	$C_{16}H_{14}O_6$	302.0786	11.568	0.44
25	DL-Erythrono-1,4-lactone; Erythrono-1,4-lactone	$C_4H_6O_4$	118.027	1.726	0.42

26	1-(1-Isobutyl-4-piperidinyl)-3-[4-methoxy-6-(trifluoromethyl)-3-pyridinyl]urea	$C_{17}H_{25}F_3N_4O_2$	374.1936	9.513	0.38
27	T-2 Triol	$C_{20}H_{30}O_7$	399.225	10.41	0.38
28	Pyrogallol	$C_6H_6O_3$	126.0319	1.961	0.34
29	p-cymene	$C_{10}H_{14}$	134.1095	13.049	0.32
31	(+)-exo-5-Hydroxycamphor	$C_{10}H_{16}O_2$	168.115	8.682	0.32
32	(-)-trans-Carveol	$C_{10}H_{16}O$	152.1201	8.967	0.32
33	2,3-Dihydro-1-benzofuran-2-carboxylic acid	$C_9H_8O_3$	164.0475	9.166	0.31
34	(1S,4R,6S)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]oct-6-yl hexopyranoside	$C_{16}H_{28}O_7$	332.1833	9.103	0.30
35	3,4-Dihydroxy-L-phenylalanine (L-DOPA)	$C_9H_{11}NO_4$	197.0688	2.541	0.29
36	Quercetin	$C_{15}H_{10}O_7$	302.0424	10.707	0.26

Sl. No. - serial number, RT (min) – retention time (in minutes) of the coumpound in the total ion chromatocgram of LC-MS analysis, % area- indicates area wise contribution of a particular compound in the TIC of LC-MS analysis.