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Antifungal potential of allelopathic plant extracts of *Ageratum conyzoides* L. and *Parthenium hysterophorus* L. on Phytophthora blight of *Capsicum annuum* L.

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ABSTRACT

We studied the allelopathic effects of aqueous extracts of two weed species *viz*., *Ageratum conyzoides* L. and *Parthenium hysterophorus* L. on seed germination and early seedling growth of bell pepper plants infected by *Phytophthora capsici.* The parameters studied were: Percent disease index (PDI), seed germination, seedling fresh weight and seedling height of bell pepper. The inhibition (%) of different aqueous extracts of both donor weed plants ranged from 74.82 % to 91.16 %. Of all 4-aqueous extracts, ethanolic extract of leaves showed the best antifungal effects. The GC-MS analysis revealed that these extracts had anti-fungal properties due to the presence of distinct groups of active compounds, suggesting their applied role in disease management. These allelochemicals can be potential tool to manage important plant diseases and replace synthetic fungicides for crop disease management.

Key words: *Ageratum conyzoides*, Allelochemicals, Allelopathy, Antifungal, Bell pepper, Blight management, *Capsicum annuum, Parthenium hysterophorus, Phytophthora capsici.* weed plants

INTRODUCTION

Bell pepper (*Capsicum annum* L. Solanaceae family) is annual vegetable crop (51). It is known as "Shimla Mirch" in North India and has red or yellow colour. Bell peppers are and excellent source of vitamins A, B6, C, calcium and folic acid (28).

The reason for low production of bell pepper is becoming infected with bacteria, fungi, viruses, and mycoplasmas (20,52). Bell pepper is susceptible to several fungal diseases (fruit rot, stem blight, leaf spot, and root rot caused by *Phytophthora capsici* these kill seedlings (45). *P. capsici* can induce different types of symptoms during various phenological stages of the plant (50), and these symptoms can change based on the host's resistance, the infected tissue and the environmental factors (46). Root is first affected tissue showing brownish rot (44). Leaf blight appeared as small, water-soaked blackish lesions with a light brown center and dark margins before turning necrotic (37,50). This oomycete pathogen additionally impacts fruits; first with little lesions these quickly progress until the infected part rots (40,44).

Allelopathy refers to the direct, indirect, stimulatory, or inhibitory impacts that plants have on one another by chemical release into the surrounding environment (9). Allelopathy may exert both positive and negative effects depending upon the concentration of allelochemicals and organisms involved (54). Allelochemicals which are typically

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secondary metabolites are released by plants (48). Numerous allelochemicals found in leaf extract of *Ageratum conyzoides* and *Parthenium hysterophorus* are involved in plant defence system (4,12,32).

Figure 1. A. *Ageratum conyzoides* L. B. *Parthenium hysterophorus* L. plants

Billy goat weed (*Ageratum conyzoides* L. Asteraceae family) (Fig.1A) can be prevalent in tropical and semitropical locations globally, notably India (25). The genus *Ageratum* has 30 species, however, only few have been studied phytochemically (7). Its leaf extract and essential oil have allelopathic effects on cultivated crops (13,22). It secretes phenolic chemicals gallic acid, coumaric acid, protocatechuic acid, p-coumaric acid, sinapic acid (21,31,49., Asteraceae family have allelopathic effects on crops (23).

Parthenium (*Parthenium hysterophorus* L. family Asteraceae) is an invasive herbaceous weed (Fig. 1B) found in 88 nations. It has major negative effects on human health and crop productivity (47) and grazing livestock. Beside roots exudates, *Parthenium* plants release allelochemicals from their leaves, stems, and flowers (49). *Parthenium* weed extracts effects have been studied on seed germination, seedling growth, shoot and root growth, plant biomass generation, chlorophyll content and nitrogen levels in the leaves of recipient plant (5). This investigation aimed to assess the effects of different ratios of *A. conyzoides* and *P. hysterophorus* leaf extract on the seeds germination, fresh weight and seedlings height of bell pepper along with their antifungal effects of against *Phytophthora capsici* causing blight of *Capsicum annuum*.

MATERIALS AND METHODS

All laboratory experiments (*in- vitro*) were done during March 2022-23 in our Central Instrumentation Laboratory. *Ageratum* and *Parthenium* leaves used in the study were collected at the seedling stage from the nearby area of University Campus.

Extracts Preparation

Certified and disease-free seeds of bell pepper were sterilized with sodium hypochloride. The seeds were treated with extracts of *A. conyzoides* and *P. hysterophrous* in various solvents *viz*., ethanol, methanol, acetone, and chloroform and at different concentrations. As a new fungicide against *P. capsici*, ethanolic and acetonic extracts of *A. conyzoides* and *P. hysterophrous* leaf powder were used. To formulate biofungicides on large scale and for its commercial manufacture, it is necessary to identify the active components of these extracts (11,14).

Bioassay

Different concentrations of leaf leachates of *A. conyzoides* and *P. hysterophrous* for each solvent were prepared. The seeds were treated with different concentration of leaf leachates using poisoned food technique (18). Two layers of filter papers were placed in Petri-dishes containing 10-seeds of bell pepper spaced equally apart. Fifteen ml leachates of *A. conyzoides* and *P. hysterophrous* were applied at 2, 4, 6, 8, and 10% to 90 mm Petri plates. In control, sterilized water was used. Treatments were replicated thrice in complete randomised design for five days, the Petri plates were kept at laboratory temperature. When the blotting paper dries distilled water was added.

Antifungal activity of extracts

The antifungal activity of *Ageratum* and *Parthenium* plant extracts were tested using the modified poison food technique. 800 µl of sabouraud broth in the 2 ml (MCT) micro centrifuge tube, then 100 µl of each solvent extract was taken by micropipette. Separately, mixed the plant extracts and Sabouraud broth thoroughly. 100 µl of test fungal microorganism inoculums (McFarland standard) were added in the Sabouraud broth. The test micro centrifuge tubes were incubated at 28 ±2 °C for 24-48 h. Sterile disc of 0.5 mm diameter was dipped in the test suspension of 800 µl SD broth + 100 µl plant extract +100 µl fungal suspension culture in micro centrifuge tube. The treated discs were placed on Sabouraud dextrose agar medium on Petri plate. All Petri plates were incubated at 28 ±2 °C. The Sabouraud broth without any aqueous extract of test plants served as control. After 48 h, mycelial growth of the test fungus was quantified and compared with the control. Vincent's formula was used to determine of mycelial growth inhibition (53).

$$\text{Growth Inhibition } \% = \frac{C - T}{C} X\ 100$$

Where, C : colony diameter in control, T : colony diameter in treatment.

Germination tests

Viable bell pepper seeds were surface sterilized with 0.1% $HgCl_2$ solution for 3 min and transferred to 1 % silver nitrate solution to remove the $HgCl_2$ and thoroughly washed in sterile distilled water. Ten healthy seeds were placed in a sterile Petri plates lined with a layer of absorbent cotton moistened with 25 ml test solutions, distilled water was used in control and incubated at 25 °C.

Emergence of radicle was considered as criteria for seed germination. The germinated seeds were counted daily and seedlings lengths were measured 35 days after sowing. This data was used to calculate seed germination (%), inhibition or stimulation (%)

of germination, viability and non-viability (%), seedling fresh weight and seedling height in different treatments. After the final count, germination percentage (GP) was calculated as under (3).

$$\text{Germination (\%)} = \frac{\text{Number of Total Germinated Seeds x 100}}{\text{Total number of seeds tested}}$$

GC-MS analysis

The chemical components in ethanolic extract of *A. conzoides* and *P. hysterphorus* leaf sections were identified using GC-MS analysis. GC analysis of ethanolic extract was done using a GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with a Rxi-5MS fused silica capillary column (5 % diphenyl/95 % dimethyl polysiloxane) and AOC20i+s (autosampler) of 0.25 mm diameter, 30 m length, and 0.25 µm film thickness. The sample size of 2 µl was supplied using an injector. Helium was used as the carrier gas. The MS was obtained at an ionisation energy of 70 eV. The overall flow was 16.3 ml/min, while the column flow was 1.21 mL/min. Flow control with linear velocity was 39.9 cm/s. Oven temperature initialization was 50 °C, followed by 250 °C for 5 min, 280 °C, 22-min, a 69.98 min hold, and ACQ mode. Scan range: 40 m/z to 700 m/z, 0.50 s scan period, 260° C, and 10:0 split ratio. The GC-MS took 65 min to complete its run. The relative % amount of each component was expressed as percentage of peak area (55).

Statistical analysis

All results were subjected to ANOVA using statistical packaged for social sciences (SPSS). The (DMRT) Duncan multiple range test at 5% level of probability was used to ascertain the significance between the different treatments used (30). F value to test significance of treatment difference (CD) was calculated at 5 % (8,17). The critical difference (CD) to compare the means of different entries was calculated using the following formula:

$$\text{Critical Difference (CD)} = SE \times \text{'t'}$$

Where, SE : Standard error of the difference of the treatment means was compared.

RESULTS AND DISCUSSION

(I). GROWTH INHIBITION (%) OF TEST PATHOGEN

The aqueous extracts of *Ageratum conyzoides* and *Parthenium hysterophorus* were evaluated for their anti-fungal effectiveness against *Phytophthora capsici* radial growth under *in-vitro* conditions (Table-1). There were significant ($p < 0.005$) inhibitory interactions between different extracts concentrations of *Ageratum* and *Parthenium* in inhibiting the radial growth of *P. capsici*. All aqueous extracts concentration of *Ageratum* and *Parthenium* inhibited the mycelia growth of *P. capsici* than control (Table 1) and the inhibitory effects ranged from 74.82 % to 91.16 %. According to preliminary screening, ethanolic leaf extract had the strongest antifungal activity, which is consistent with the findings of the present research (43).

Table 1. Effects of different aqueous extract of *Ageratum* and *Parthenium* on *Phytophthora capsici*

***Ageratum* Extract**	**Inhibition (%)**	***Parthenium Extract***	**Inhibition (%)**
T0	0	***T0***	0
AE	85.09	PE	87.37
AM	88.57	PM	89.16
AC	91.16	PC	74.82
AA	78.39	PA	90.01
CD at 1 %	3.1	***CD at 1 %***	***6.7***

T_0 = Control; AE = *A. conzoides* + Ethanol; AM = *A. conzoides* + Methanol; AC = *A. conzoides* + Chloroform; AA = *A. conzoides* + Acetone; PE = *P. hysterophorus* + Ethanol; PM = *P. hysterophorus* + Methanol; PC = *P. hysterophorus* + Chloroform; PA = *P. hysterophorus* + Acetone

(i). *Ageratum conyzoides* : The highest inhibition of test pathogen was recorded with *A. conyzoides* + Chloroform extract (91.16 %) followed by aqueous extract of *A. conyzoides* + Methanol extract (88.57%). Least inhibitory effect was shown by aqueous extract of *A. conyzoides* + Acetone (78.39 %). The *Ageratum conyzoides* possesses antimicrobial properties against many diseases, including those caused by *Phytophthora* genus (33). Due to abundance of bioactive chemical constituents in *A. conyzoides*, it has been used to control phytopathogenic fungi: *Aspergillus fumigatus, Phytophthora citrophthora, Pythium aphanidermadum, Fusarium solani, Phytophthora infestans, Cercospora musae* and *Cercospora capsica* (35). The leaf extract effectively controlled target fungal species. The *n*-Hexane and methanolic extracts of *A. conyzoides* showed antifungal activity against *Fusarium solani* (27) and *Macrophomina phaseolina* (4). *A. conyzoides* was most effective to reduce the growth of mycelium of *Rhizoctonia solani, Aspergillus niger* and *Pestalotiopsis theae* (25,39). High flavonoid concentration in *Ageratum* leaf extract's suppressed the plant disease (42), flavonoids at higher concentration slowed the growth of fungal mycelium (1,24).

(ii). *Parthenium hysterophorus* : The highest inhibition of test pathogen was recorded with *P. hysterophorus* + Acetone (90.01 %) followed by aqueous extract of *P. hysterophorus* + Methanol extract (89.16%). Least inhibitory effect was shown by *P. hysterophorus* + Chloroform extract (74.82 %). *Parthenium* roots, stem, leaves, inflorescence, pollen and seeds contain water-soluble phenolics and sesquiterpene lactones (29,36). Its leaf ethanolic and acetonic extracts showed strong antifungal efficacy against *Phytophthora capsici*, however methanolic extract has less antifungal activity (28). *Parthenium hysterophorus* aqueous extracts showed 100 % suppression of test pathogen (10). *P. hysterophorus* releases phytotoxic chemicals (ambrosin, coronopilin, p-hydroxybenzoic acids, coumaric and parthenin, ferulic, vanillic and caffeic acid) which has allelopathic and antifungal properties (6). *Ageratum conyzoides* and *P. hysterophorus*, have antifungal properties against *Macrophomina phaseolina*, which causes the charcoal rot disease in sunflower (38).

(II). SEED GERMINATION

The aqueous extracts of *Ageratum* and *Parthenium* species at varying concentrations significantly increased the seeds germination in infected bell pepper plants than control (Figure 2); however, their interaction was non-significant. Each of the several aqueous extracts of *Parthenium* and *Ageratum* slightly stimulated the seed germination over control. The different aqueous extracts of both donor weed plants stimulated the germination (%) of infected bell pepper plants from 69.42 % to 91.49 %. Slight decrease in bell pepper germination was recorded at 8 % and 10 % concentration of aqueous extracts from *A. conzoides* and *P. hysterophorus* might be due to allelopathic effects.

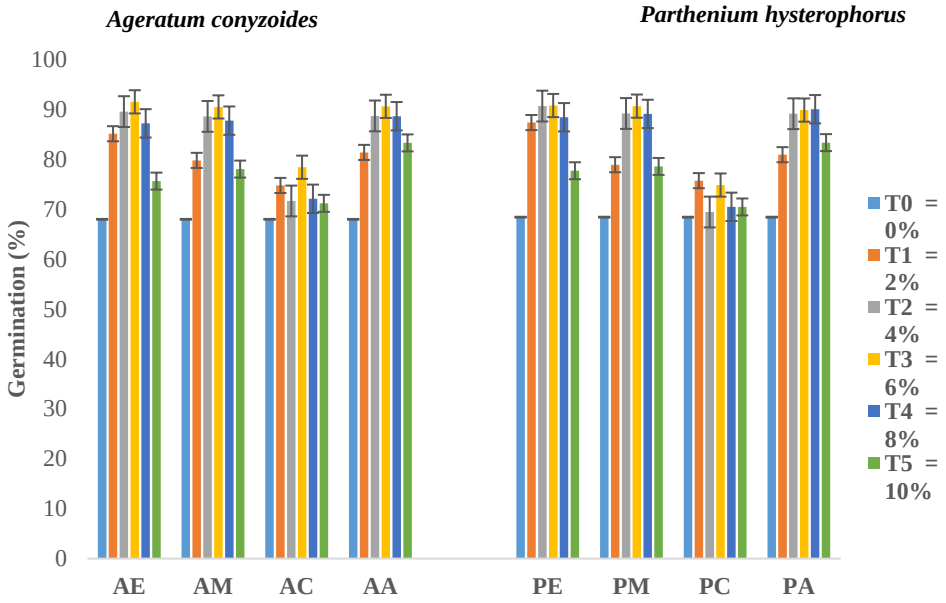

Figure 2. Stimulatory effects of *Ageratum* and *Parthenium* extract on germination (%) of infected *Capsicum annuum* at different concentrations

AE = *A. conzoides* + Ethanol; AM = *A. conzoides* + Methanol; AC = *A. conyzoides* + Chloroform; AA = *A. conzoides* + Acetone; PE = *P. hysterophorus* + Ethanol; PM = *P. hysterophorus* + Methanol

(i). *Ageratum conyzoides* : Under *in-vitro* conditions, the highest stimulatory effect was recorded in plants treated with 6 % *A. conzoides* + Ethanol extract (91.49 %) followed by 6 % aqueous extract of *A. conzoides* + Acetone (90.59%) and *A. conzoides* + Methanol (90.47 %). Least germination percent was shown by 10% aqueous extract of *A. conzoides* + Chloroform (71.16 %) over control. In rice field, applying dried *A. conyzoides* leaves boosted grain yield by 14 % (34).

(ii). *Parthenium hysterophorus*: Under *in-vitro* conditions, the highest stimulatory effect on germination percentage was recorded in plants treated with 6 % aqueous extract of *P. hysterophorus* + Ethanol (90.75 %) followed by 4 % aqueous extract of *P. hysterophorus* + Ethanol (90.64 %) and 6 % aqueous extract of *P. hysterophorus* + Ethanol (90.63 %). Least germination percent was shown by 10 % and 8 % aqueous extract of *P. hysterophorus* + Chloroform *i.e*., 70.44 % and 70.46 %, respectively. Raj and Jha (41) did not observe inhibitory effects of leaf extract of *Parthenium* on seed germination of *Phaseolus mungo*. However it is unclear, whether *Ageratum* and *Parthenium* leaf extracts inhibits bell pepper seed germination and seedling growth. *P. hysterophorus* leaves have allelopathic potential for both weeds and crops (5).

(III). SEEDLING FRESH WEIGHT

Different concentrations of extracts of *Ageratum* and *Parthenium* species stimulated the seedling fresh weight (66.33 g to 139.33 g) of infected bell pepper plants than control (Figure 3). The allelopathic effects of allelochemicals were increased by soil pH, organic matter content, nutrition, moisture content and microbes (26). There is significance of allelopathy in the relationship between weeds and crops (9).

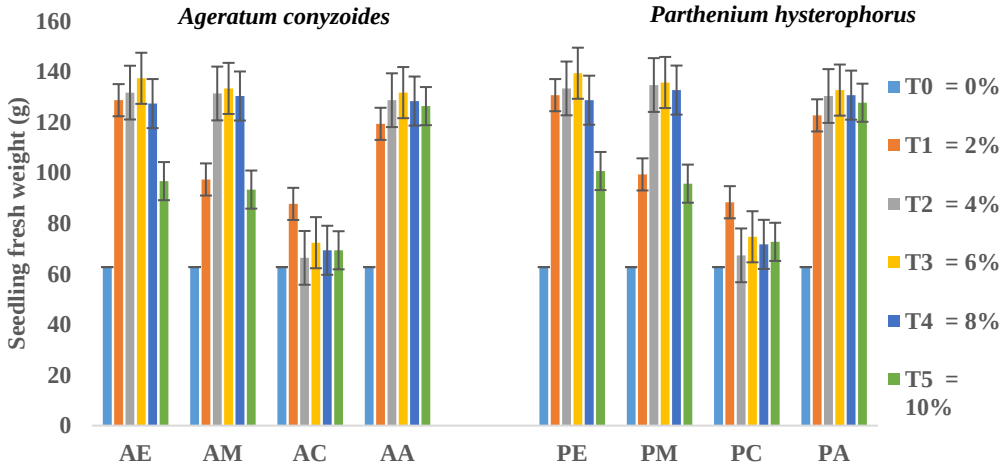

Figure 3. Stimulatory effects of *Ageratum* and *Parthenium* extract on seedling fresh weight of infected *Capsicum annuum* at different concentrations

Treatments

AE = *A. conzoides* + Ethanol; AM = *A. conzoides* + Methanol; AC = *A. conyzoides* + Chloroform; AA = *A. conzoides* + Acetone; PE = *P. hysterophorus* + Ethanol; PM = *P. hysterophorus* + Methanol

(i). *Ageratum conyzoides*: The highest fresh weight of seedling was recorded with 6 % aqueous extract of *A. conyzoides* + Ethanol (137.33 g) followed by 6 % aqueous extract of *A. conyzoides* + Methanol (133.83 g) under *in-vitro* conditions as compared to control without any aqueous extract of test weeds. The aqueous extract of *Ageratum* combined with chloroform is not effective in increasing the seedling fresh weight of bell pepper, it suggests that this combination does not promote growth or may even have neutral to slightly inhibitory effects.

(ii). *Parthenium hysterophorus*: The highest fresh weight of seedling was recorded with 6% solution of *P. hysterophorus* + Ethanol (139.86 g) followed by 6 % aqueous extract of *P. hysterophorus* + Methanol (136.39 g) under *in-vitro* conditions. Least seedling fresh weight was recorded at 8 % conc of *P. hysterophorus* + Chloroform extract (72.40 g). At higher extract concentrations of *Ageratum* and *Parthenium*, the allelopathic substances can cause direct damage to the root and shoot meristems, limiting root elongation and shoot expansion. Reduced root growth can decrease the plant's ability to absorb water and nutrients, which translates into lower seedling fresh weight (26).

(IV). SEEDLINGS HEIGHT

At 35 days after sowing, the seedling height of infected bell pepper plants was significantly affected by various concentrations of aqueous extracts from *Ageratum* and *Parthenium* species than control, but the interaction between the concentration means was non-significant (Figure 4). Different aqueous extracts of *Ageratum* and *Parthenium* slightly stimulated the seedling height at 35 DAS as compared to untreated control. At 35 DAS, the stimulatory effects of different aqueous extracts of both weed plants on seedling height of infected bell pepper plants ranged from 6.03 cm to 8.97 cm.

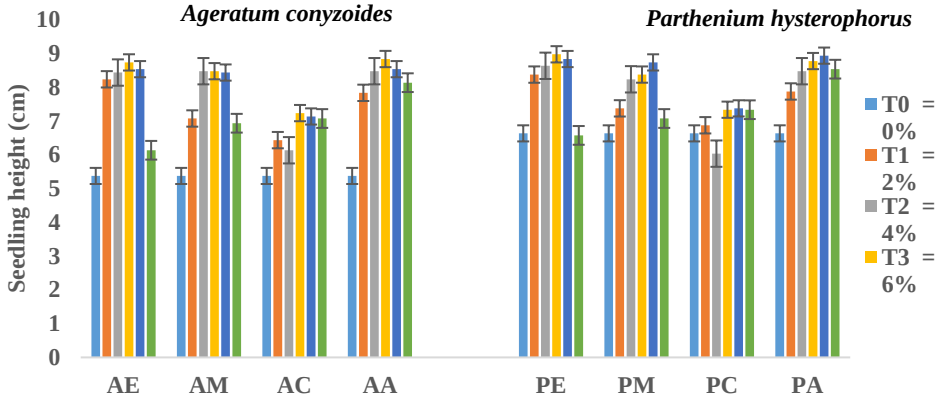

Figure 4. Stimulatory effects of *Ageratum* and *Parthenium* extract on seedling height at 35 days after sowing of infected *Capsicum annuum* at different concentrations

Treatments
AE = *A. conzoides* + Ethanol; AM = *A. conzoides* + Methanol; AC = *A. conzoides* + Chloroform; AA = *A. conzoides* + Acetone; PE = *P. hysterophorus* + Ethanol; PM = *P. hysterophorus* + Methanol; PC =*P. hysterophorus* + Chloroform

(i). *Ageratum conyzoides*: Under *in-vitro* conditions, the maximum seedling height was recorded in plants treated with 6 % *A. conzoides* + Acetone (8.83 cm) followed by 6 % *A. conzoides* + Ethanol (8.73 cm), 8 % *A. conzoides* + Ethanol (8.53 cm) and 8 % *A. conyzoides* + Acetone (8.53 cm). Least seedling height was observed at 10 % aqueous extract of *A. conzoides* + Ethanol and 4 % *A. conzoides* + Choloroform i.e., 6.13 cm. This suggests that these specific concentrations of extracts, combined with the respective solvents, have a strong inhibitory effect on seedling growth.

(ii). *Parthenium hysterophorus* : Under *in-vitro* conditions, the maximum seedling height was recorded in plants treated with 6 % *P. hysterophorus* + Ethanol extract (8.97 cm) followed by 8 % aqueous extract of *P. hysterophorus* + Acetone (8.93 cm) and 8 % *P. hysterophorus* + Ethanol (8.83 cm). The least seedling height of bell pepper was also recorded at 4 % aqueous extract of *P. hysterophorus* with chloroform. Because the diverse phytochemicals of *Ageratum* and *Parthenium* plants are valuable insecticides in agriculture and have medicinal uses in human (24). *A. conyzoides* extracts controlled weeds as well as preserved and enhanced the soil microbiota (39). *A. conyzoides* is valuable biosource to prepare formulations for industry, agriculture and medicine (25).

(V) GC-MS ANALYSIS

The comparison between the results of studies on *Ageratum conyzoides* and *Parthenium hysterophorus* extracts (Figures 5 and 6) revealed their antifungal properties against *Phytophthora capsici*. GC-MS chromatogram of leaves extract of *A. conyzoides (*Figure 2) showed the presence of several antifungal compounds. The GC-MS analysis of *Ageratum conyzoides* and *Parthenium hysterophorus* revealed the presence of 36 compounds in leaves. While both *Ageratum conyzoides* and *Parthenium hysterophorus* extracts exhibited promising antifungal properties, due to presence of active compounds, suggesting potential differences in their modes of action and plant disease management. *A. conyzoides* and *P. hysterophorus* yielded a wide range of secondary metabolites: flavonoids, alkaloids, terpenoids, coumarins and sterols (7).

(i). *Ageratum conyzoides* : *Ageratum conyzoides* antifungal activity, may be primarily attributed to major compounds : Caryophyllene, Precocene I, 1-Pentadecene, and Neophytadiene. These compounds, collectively contributed to the observed inhibitory effects against the fungal pathogen. Caryophyllene (a sesquiterpene) has antimicrobial properties, likely played a crucial role in the antifungal activity of extract. Similarly, Precocene I and the alkenes 1-Pentadecene and Neophytadiene showed substantial efficacy, suggesting a synergistic effects of multiple compounds in the extract (Figure 5).

Ageratum conyzoides L. is rich in phytoconstituents that offer numerous advantages in various contexts (27). Precocene I, Precocene II, caryophyllene, phytol, (E)-β-famesene, and 1-nonadecene are significant bioactive chemicals found in *A. conyzoides*. These compounds have antibacterial, antifungal, anticancer, anti-inflammatory, aphid-repellent, and/or antioxidant qualities (13). Five antifungal compounds from the aerial parts of *Ageratum conyzoides* L.: three polymethoxyflavones, 5′-methoxynobiletin (compound 2), nobiletin (compound 3), and 5,6,7,3′,4′,5′-hexamethoxyflavone (compound 4). In *in-vitro* studies, (compound 4) inhibited the growth of *Pyricularia oryzae* and *Rhizoctonia solani*, whereas, eupalestin (compound 5)

was only found in *P. oryzae*. Furthermore, with a ten-fold lower IC_{50}, precocene II has excellent antifungal efficacy against both fungi (35).

Table 5. Compounds in ethanolic extracts of *Ageratum conyzoides* leaves.

Peak #	*Ageratum conyzoides*	Retention Time	Area	Area (%)	Height
1	Carbonic acid, tetradecyl vinyl ester	9.202	24828	0.76	8356
2	Caryophyllene	9.851	105709	3.25	37163
3	cis-.beta.-Farnesene	10.386	42974	1.32	10884
4	Precocene I	10.691	110274	3.39	33379
5	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronapthalene	11.976	30783	0.95	5807
6	Diethyl Phthalate	13.448	61294	1.88	15367
7	1-Pentadecene	13.552	99339	3.05	33113
8	Octadecane, 1-iodo-	13.721	31037	0.95	9895
9	Cyclopropane,1-methyl-1-(1-methylethyl)-2-nonyl-	13.816	52619	1.62	6847
10	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl-	14.964	1555324	47.81	574062
11	1H-Inden-1-one,7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl-	15.423	100385	3.09	31227
12	3,5-di-tert-Butyl-4-hydroxyacetophenone	16.237	70387	2.16	28411
13	Spiro(cyclohexane-1,4'(1'H)-quinazoline)-2'(3'H)-thione, 5',6',7',8'-tetrahydro-	17.127	27519	0.85	7260
14	1-Nonadecene	17.924	62838	1.93	19915
15	Neophytadiene	18.821	47249	1.45	16338
16	Lidocaine	19.872	62319	1.92	17366
17	Butanoic acid, 2-bromo-, pentyl ester	21.828	24776	0.76	4575
18	1-Heptacosanol	21.960	38513	1.18	14473
19	Dichlone	23120	30096	0.93	3688
20	10(E), 12(Z)-Conjugated linoleic acid	23.810	25427	0.78	4553
21	5-Oxo-4-azatricyclo[4.2.1.0(3,7)]nonane-9-carboxylic acid	28.115	27340	0.84	3696
22	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate	30.843	179124	5.51	57259
23	Squalene	35.360	35438	1.09	9553
24	2,6-Dihydroxybenzoic acid, 3TMS derivative	40.779	35856	1.10	8280
25	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	41.020	28741	0.88	6848
26	1,2-Bis(trimethylsilyl)benzene	41.135	36923	1.13	4398

(ii). *Parthenium hysterophorus*

In contrast, *Parthenium hysterophorus* exhibited potent antifungal activity attributed to compounds: Neophytadiene, Caryophyllene oxide, and Squalene (Figure 6). Despite their lower abundance, these compounds were inhibitory to *Phytophthora capsici.* Additionally, lesser abundant compounds like 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-die and Phytol also showed antifungal efficacy, indicating the presence of potential novel targets for further exploration.

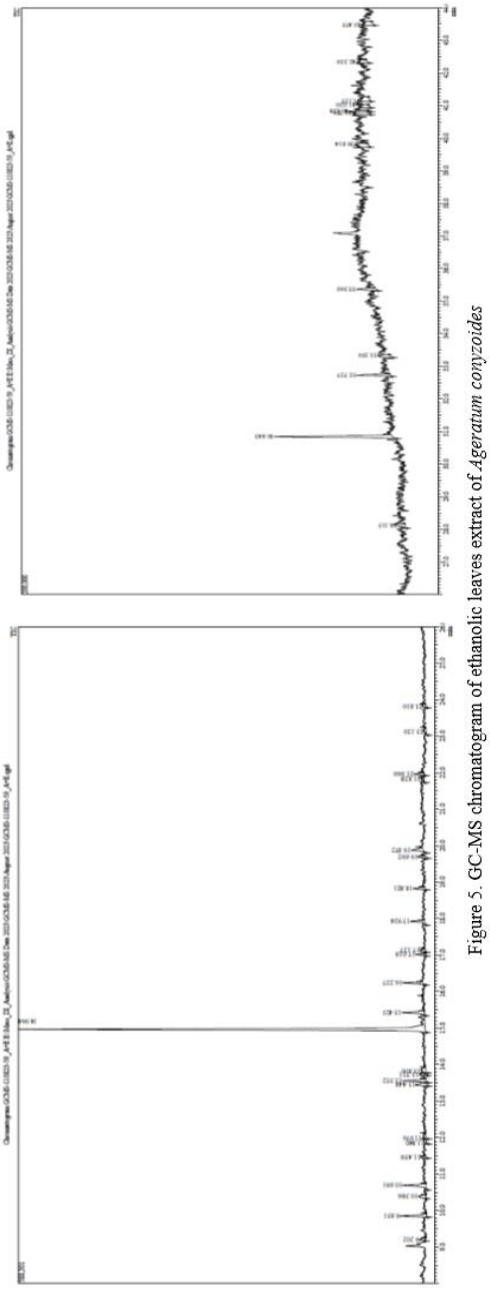

Figure 5. GC-MS chromatogram of ethanolic leaves extract of *Ageratum conyzoides*

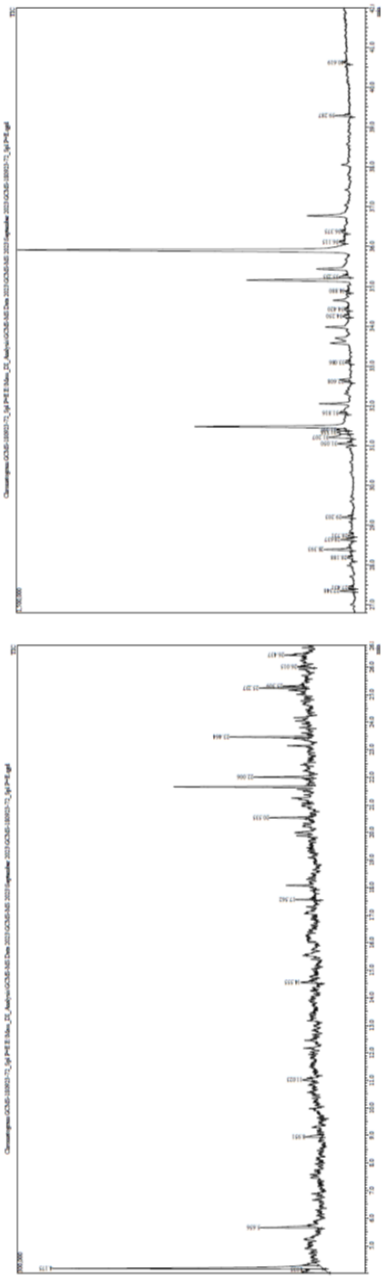

Figure 6. GC-MS chromatogram of ethanolic leaves extract of *Parthenium hysterophorus*

Table 6. Compounds on ethanolic extracts of *Parthenium hysterophorus* leaves.

Peak#	*Parthenium hysterophorus*	Retention Time	Area	Area (%)	Height
1	2-Formylhistamine	4.035	143156	3.63	20862
2	Glycerin	1.40	4.175	6.37	16.17
3	Propanoic acid, 2-oxo-, methyl ester	5.656	128567	3.26	49876
4	Butanoic acid, 4-hydroxy-	8.951	53395	1.35	13990
5	2-Piperidinecarboxylic acid	14.555	45992	1.17	13908
6	Nonane, 5-(2-methylpropyl)-	17.562	34642	0.88	17827
7	Caryophyllene	20.535	72928	1.85	34463
8	2,4-Di-tert-butylphenol	22.006	103943	2.64	50352
9	Caryophyllene oxide	23.464	171053	4.34	69846
10	Eicosane	25.237	83899	2.13	39375
11	2,6,10-Trimethyltridecane	25.309	44758	1.14	18566
12	s-Triazine, 2-amino-4-(piperidinomethyl)-4-p	26.437	46930	1.19	16989
13	Neophytadiene	27.348	114680	2.91	57734
14	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	28.393	25396	6.42	116778
15	Hexadecanoic acid, methyl ester	28.637	114159	2.90	49704
16	Benzenepropanoic acid, 3,5-bis(1,1-dimethyl)-4-hydroxy-methyl ester	28.731	68861	1.75	21042
17	16-Pregnen-3,20-dione	29.203	98906	2.51	43753
18	8,11,14-Docosatrienoic acid, methyl ester	31.050	146145	3.71	62244
19	Phytol	31.207	347138	8.80	93725
20	Decane, 1-iodo-	31.335	205600	5.21	47846
21	Methyl stearate	31.397	114534	2.90	43134
22	4-Octyloxybenzoic acid	31.816	73558	1.87	34963
23	Ambucetamide	32.608	60619	1.54	28455
24	Corymbolone	34.250	79095	2.01	28244
25	(E)-Dodec-5-en-4-olide	35.235	111647	2.83	40957
26	Benzyldiethyl-(2,6-xylylcarbamoylmethyl)-ammonium benzoate	36.115	59123	1.50	24212
27	Dotriacontane	36.375	48556	1.23	18389
28	Squalene	39.287	139471	3.54	66412
29	Acetic acid (4-chlorophenoxy)-tetradecyl ester	40.619	33513	0.85	14065

The leaf extracts from the *Parthenium hysterophorus* plant possess antibacterial and antifungal compounds against the range of tested bacterial and fungal strains (10). *P. hysterophorus* leaves are rich in phytol, may be related to the leaves' increased diterpene concentration. *P. hysterophorus* leaf proteins are superior than cereals and legumes (16). Neophytadiene is a diterpenoid molecule found in *P. hysterophorus* leaf extract, it has antimicrobial, anti-inflammatory, antibiofilm, and antioxidant activities (15).

CONCLUSIONS

The crude leaf extracts of *Ageratum conyzoides* and *Parthenium hysterophorus* had strong antifungal activities against *Phytophthora capsici* and also increased the yield of bell pepper plants. These plants contain distinct sets of active compounds, causing potential differences in their modes of action and use for plant disease control.

Standardizing extraction procedures and *in-vitro* antimicrobial activity screening would improve the evaluation of results and make the search for new biologically active plant products more methodical. Further research is warranted to elucidate the specific mechanisms underlying the antifungal activity of these compounds and optimize their efficacy for practical applications in agriculture.

CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors agree to publish it.

DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

ETHICAL STATEMENT

This is to inform you that in this study, we have not been involved in any animal and human studies.

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