

## ***In-vitro* antifungal activity of leaves extracts of *Ageratum conyzoides* L. and *Parthenium hysterophorus* L. against *Phytophthora capsici* Leonian causing bell pepper blight**

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### **ABSTRACT**

Bell pepper (*Capsicum annum* L.) is an important vegetable crop. But it is infected by various diseases caused by viruses, bacteria, fungi and pseudofungi, in particular the oomycete *Phytophthora capsici* Leonian. We studied, *in-vitro* antifungal properties of *Ageratum conyzoides* L. and *Parthenium hysterophorus* L. leaf extracts in different solvents (ethanolic, methanolic, acetic and chloroform) against *P. capsici* causal agent of blight disease in *Capsicum annum*. Acetone and chloroform extracts were very inhibitory. These extracts from *Parthenium* and *Ageratum* at 10 % most effectively inhibited the *P. capsici* when used as biofungicide. The low standard deviations in acetone and chloroform solvents, indicated results reliability and consistency and supported the Permanova analysis and associated p-values. The detection of biomolecule composition using FTIR spectroscopy was accurate and sensitive.

**Keywords:** *Ageratum conyzoides*, Antifungal properties, Bell pepper, Bell pepper blight, *Capsicum annum*, Leaves extracts, *Parthenium hysterophorus*, *Phytophthora capsici*, Weed extract

### **INTRODUCTION**

Bell pepper (*Capsicum annum* L., Solanaceae family) is an annual/semi-perennial herbaceous, most popular and nutrients-rich vegetable (28) cultivated world wide (43). *Phytophthora capsici* oomycete fungal pathogen is soil-borne pathogen, which cause vital diseases world wide in cultivated Solanaceous, Fabaceous and Cucurbitaceous crops (39,46) and peppers (*Capsicum* spp.). This disease decreases both yield and quality (33). Pepper plants are susceptible to *P. capsici* infection at any stage of growth, which causes crown rot, stem blight, root rot, leaf spot, fruit rot and the seedlings death (3). In *Capsicum*, *P. capsici* can cause loss of up to 100 %, because of its rapid dissemination in field conditions (5). Because of its polycyclic nature, *P. capsici* is most difficult phytopathogens to control (29,37,41).

The fungicides effectively control diseases, but they cause negative side effects, such as environmental pollution, phytotoxicity and develop resistance in plant pathogens. Consequently, it was suggested that biological control of *Phytophthora*-induced diseases might be more environmentally friendly option than traditional fungicides (45). *A. conyzoides* and *P. hysterophorus* contains vast range of phytochemicals (1,14,15) with antibacterial and antifungal characteristics (30). *A. conyzoides* (Asteraceae family), is an allelopathic plant native to tropical Americas and extensively found in tropical and sub-tropical regions. It grows with both annual and perennial crops and resist pests and

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diseases due to its production and release of allelochemicals (21). *A. conyzoides* has proved efficient treatment to control phytopathogenic fungus (31). On the other hand, *P. hysterophorus* is an aromatic annual native to India (12). It has negative impact on biodiversity, agriculture and human beings, but also to controls some bacteria and fungi, especially *P. capsici* (7).



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

The leaf extracts of *A. conyzoides* and *P. hysterophorus* have shown promising antifungal activity to combat *P. capsici*. Alleopathic and pest resistance nature of these plants make them better sustainable substitutes to synthetic fungicides. Their extracts effectively suppresses the growth of *P. capsici* and are environmentally friendly solution for disease management in *C. annuum* (8,27). This paper aimed to evaluate the antifungal effects of *A. conyzoides* and *P. hysterophorus* extracts against the phytopathogenic fungus *P. capsici*, causing bell pepper blight.

## MATERIALS AND METHODS

All experiments were conducted under laboratory (*in-vitro*) conditions during 2022-23 at Central Instrumentation Laboratory, Lovely Professional University, Phagwara, Punjab. *Ageratum* and *Parthenium* leaves used in the study were collected from the nearby area of university campus.

The *P. capsici* fungus was identified as *P. capsici* Leonian and the culture had been deposited to Indian Type Culture Collection (ITCC), New Delhi as ITCC No.11865.23 and 11871.23.

### Soil Sample Collection and Fungus Isolation

Rhizosphere soil samples from the infected *C. annuum* plants were collected from 72 villages of 7-Blocks of Haryana, India. After removing the top 3-5 cm soil, soil samples were taken at a depth of 1-3 cm around the root system. These samples were collected in the polythene bags, brought to the laboratory, and kept at 4 °C for future analysis. Using the pour plate method, 1 ml of each dilution from  $10^{-5}$  to  $10^{-8}$  was plated on carrot agar media by using serial dilution technique of the obtained soil sample (11,42). Three replicates were used for each dilution. Petri dishes were incubated at  $25 \pm 2$  °C for 7-10 days and culture of test pathogen were isolated based on the traditional morphological criteria. Sub-culturing on the same media was used to further purify the pathogen culture. The

conventional method for producing sporangium by *P. capsici* is a water culture method which utilizes a solid culture medium such as a carrot agar medium and cultured by illumination for a certain time or adds a hypha block into sterile water to induce and produce the sporangium (35).

#### **Preparation of Weed Extracts**

Certified and Disease-free seeds of *Capsicum* were procured and sterilized with sodium hypo-chloride. The seeds were treated with *A. conyzoides* and *P. hysterophrous* extracts prepared in 4-solvents (Ethanol, Methanol, chloroform, Acetone) at different concentrations. The physicochemical properties of extracts were used to determine the solubility of *A. conyzoides* and *P. hysterophrous* extracts with these solvents. The procedure suggested by Flanagan was used to determine the proper solvent (16). The procedure involves transferring 1.20 g extract into a test tube and adding two parts by volume of suitable solvents and 10 ml water to it. Following each addition of 2.0 ml of solvent, the test tube was heated and agitated using a magnetic stirrer. By applying various solvents, the best solvent was found to dissolve the extract, and the best formulation type was chosen. After considering the physical and chemical characteristics of extract, the extract was mixed at 800 rpm in a vertical mixer with a suitable solvent and co-formulates. After that, the mixture was stirred in a high-speed vertical mixer at 4500 rpm for 1.5 h, till the particle size level reached 10-20 microns and evenly distributed was attained. As a result, the insoluble components in the extract were also distributed uniformly. After 24 h, the items placed in the resting tank underwent quality control analysis. As a new fungicide against *P. capsici*, ethanolic and acetic extracts of *A. conyzoides* and *P. hysterophrous* leaf powder were used. To formulate biofungicides on large scale and for commercial manufacture, it is helpful to identify the active components of these extracts (13,16).

#### **Bioassay**

By using the poisoned food technique, the seeds were treated with various concentrations of leaf leachates (19). *A. conyzoides* and *P. hysterophrous* leaf extracts were prepared in various concentrations for each solvent. Two layers of filter paper were placed in Petri-dishes containing 10-bell pepper seeds spaced equally apart. Fifteen ml of *A. conyzoides* and *P. hysterophrous* extracts of 2, 4, 6, 8 and 10 % concentration were applied to the Petri plates. In control, sterilized water was used. For every treatment and control, three replicates were kept. The Petri plates were kept in laboratory for 5-days. When the blotting paper's moisture content dropped, an equal volume of distilled water was added to each dish.

#### **Antifungal activity of *Ageratum* and *Parthenium* plant extracts against test pathogen**

*Ageratum* and *Parthenium* plant extracts were tested using poison food technique with some modifications. 800 µl of sabouraud broth was poured in 2 ml (MCT) micro centrifuge tube and then 100 µl of each solvent extract was taken using micropipette. Separately, mix the plant extracts and Sabouraud broth thoroughly. 100 µl of test fungal microorganism inoculums (McFarland standard) was added in the Sabouraud broth. The test micro centrifuge tubes were incubated at 28±2 °C for 1 h. A micro centrifuge tube containing 800 µl of SD broth, 100 µl of plant extract and 100 µl of fungal suspension culture was used to dip a sterile disc with 0.5 mm diameter. The sterile discs were placed on Sabouraud dextrose agar medium in Petri plate. All Petri plates were incubated at 28±2 °C. The basal

media (Sabouraud broth) without phytoextracts served as control. After 48 h, mycelial growth of the test fungus was quantified and compared with control. Vincent's formula was used to assess the proportion of mycelial growth inhibition (44).

$$I \% = C - Tx100/C$$

Where, C: Colony inhibition, I: % Inhibition, T: Colony diameter in treatment.

#### FT-IR Analysis

Ten ml methanolic extract was analyzed using a thermo-fresher FT-IR-40 (USA). The spectrum was obtained between wave numbers 4000 and 500  $\text{cm}^{-1}$  (40).

#### GC-MS Analysis

The components contained in the acetone, chloroform and methanolic extract of *A. conzoides* and *P. hystrophorus* leaves were identified using GC-MS analysis. GC analysis of the 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  $\mu\text{m}$  film thickness. The sample size of 2  $\mu\text{l}$  was supplied through using an injector. Helium, an inert gas, 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  $^{\circ}\text{C}$ , followed by 250  $^{\circ}\text{C}$  for 5 minutes, a 22-min ramp to 280 $^{\circ}\text{C}$ , a 69.98-minute hold, and ACQ mode. Scan range: 40 m/z to 700 m/z, 0.50 s scan period, 260  $^{\circ}\text{C}$  and 10:0 split ratio. The GC-MS took 65 min to complete its run. The relative % amount of each component was expressed as a percentage with peak area (48).

#### Statistical Analysis

We analyzed the inhibitory effects of bio fungicides derived from *Parthenium* and *Ageratum* on the growth of *P. capsici*. Wherever required, data with appropriate transformations were evaluated using ANOVA table. F value for testing significance of treatment difference (CD) was calculated at 5 % to compare treatment means (9,18).

The critical difference (CD) was computed using the following formula to compare means of different entries:

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

where, SE: Standard error of difference of the treatment means to be compared:

$$SE = (2MSe/r)^{1/2}$$

All results were subjected to analysis (ANOVA) using statistical package (SPSS). The (DMRT) Duncan multiple range test at 5% level of probability was used to ascertain the significance between the different treatments used (26). To bolster the significance of our findings, we performed statistical analysis using the PERmutational Multivariate Analysis of Variance (PERMANOVA) method, which allowed us to derive further insights from the data. Here's how our results align with the Permanova analysis, including the associated p-values (2).

## RESULTS AND DISCUSSION

### Antifungal activity of extracts against test pathogen

The aqueous extracts of *Ageratum* and *Parthenium* species at varying concentrations significantly reduced the growth of the *P. capsici* in comparison to untreated control (Table-1).

Table 1. *In-vitro* antifungal activity of *Ageratum* and *Parthenium* plant extracts against test pathogen.

Solvents	Growth Inhibition (%)					
	Control (T <sub>0</sub> )	2 % (T <sub>1</sub> )	4 % (T <sub>2</sub> )	6 % (T <sub>3</sub> )	8 % (T <sub>4</sub> )	10 % (T <sub>5</sub> )
<i>Ageratum conyzoides</i>						
Ethanol	15.36u	90.26g	95d	98.16a	97.5b	90.13g
Methanol	17.33t	59.1s	60.3r	65.8q	68.63p	58s
Chloroform	13.43v	90.5g	95.33c	89.3h	91.23f	81.6k
Acetone	11.23w	88.2h	93.4e	95.33c	98.30a	91.46f
<i>Parthenium hysterophorus</i>						
Ethanol	15.36u	92.1f	96.3c	97.4b	93.2e	85.1j
Methanol	17.33t	80l	76.6m	91.4f	85.6i	82.2k
Chloroform	13.43v	70o	75.3n	80.3l	85j	86.6i
Acetone	14.23v	88.32h	95d	98.16a	97.5b	82.6k
SEm±	0.39					
LSD 0.05	1.11					

\*Means in same column with same superscripts are not significantly different (P > 0.05)

We measured the pathogen's radial growth, to evaluate the anti-fungal effectiveness of aqueous extracts of *A. conyzoides* and *P. hysterophorus* against *P. capsici* under *in-vitro* conditions. There were significant (p < 0.005) interaction effects between different extracts of *Ageratum* and *Parthenium* used in inhibiting radial growth of *P. capsici*. Generally, all aqueous extracts of *Ageratum* and *Parthenium* inhibited the mycelia growth of *P. capsici* than untreated control (Table 1). Various aqueous extracts of two weed species had inhibitory effects ranging from 58.00 % to 98.30 %. The highest inhibition of test pathogen was recorded in Petri dishes treated with *A. conyzoides* + Acetone extract (98.30 %) at 8 % concentration (T<sub>4</sub>) followed by aqueous extract of *A. conyzoides* + Ethanol extract (98 %) and *P. hysterophorus* + Acetone (98 %) both at 6 % concentration (T<sub>3</sub>). Least inhibitory effects were shown by *A. conyzoides* + Methanol extract (58 %) at 10 % concentration (T<sub>5</sub>) followed by aqueous extract of *A. conyzoides* + Methanol (59 %) at 2 % concentration (T<sub>1</sub>).

#### (i). Acetone Extracts

Acetone extracts clearly showed concentration-dependent increase in inhibition (%) for both *Ageratum* and *Parthenium* biofungicides (Figure 2). This trend was substantiated by the Permanova analysis, which revealed a statistically significant concentration-dependent effect on inhibition within the acetone treatments (p < 0.05). This aligns with our visual observations of increasing inhibition (%) with higher concentrations. At the highest concentration (10 %), both *Ageratum* and *Parthenium* biofungicides caused most drastic inhibition, 92.4 % and 94.6 %, respectively. The Permanova analysis supported this as a significant difference when compared to lower concentrations (p < 0.05), reinforcing the

concentration-dependent inhibitory effect. Furthermore, the consistently of low standard deviations across replicates in acetone treatments indicate a high level of consistency in the inhibitory effects within each concentration level and this stability may also be reflected in the Permanova analysis.

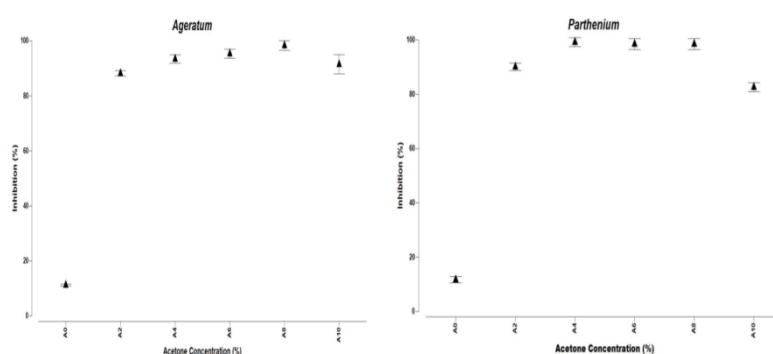


Figure 2. *In-vitro* inhibition (%) of acetone extract of *A. conyzoides* and *P. hysterophorus* against *P. capsica*

### (ii). Chloroform Extracts

Similar to acetone extracts, chloroform extracts also showed concentration-dependent increase in inhibition (%) for both *Ageratum* and *Parthenium* biofungicides (Figure 3). The Permanova analysis confirmed the significance of these concentration-dependent effects on inhibition within the chloroform treatments ( $p < 0.05$ ). At 10 % concentration (10 %), *Ageratum* and *Parthenium* extracts caused drastic inhibition of 93 % and 95 % respectively. The Permanova analysis supported this as a significant difference compared to lower concentrations ( $p < 0.05$ ), affirming the concentration-dependent inhibitory trend observed visually. Consistently low standard deviations within each concentration level in chloroform treatments, indicated the reliability and stability of the inhibitory results, a characteristic that is supported by the Permanova analysis.

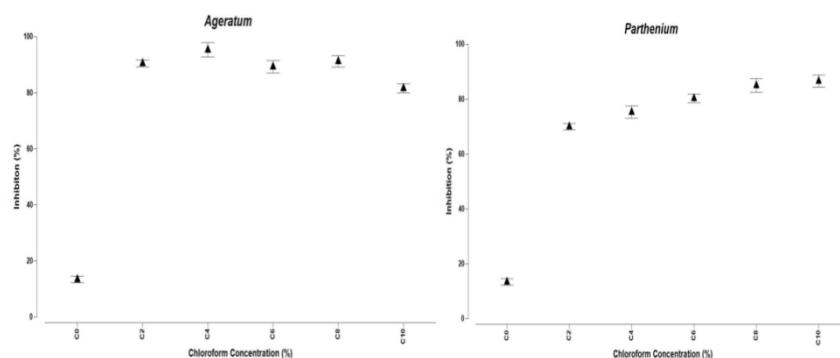


Figure 3. *In-vitro* inhibition (%) of chloroform extract of *A. conyzoides* and *P. hysterophorus* against *P. capsica*

### (iii). Ethanol Extracts

Ethanol extracts showed moderate inhibition, with minimal concentration-dependent effects for both *Ageratum* and *Parthenium* biofungicides (Figure 4). The Permanova analysis confirmed that the concentration-dependent effects within the ethanol treatments were not statistically significant compared to acetone and chloroform treatments ( $p > 0.05$ ). The highest inhibitions were observed at various concentration levels, ranging from 89.4 % to 90.4 % for *Parthenium* and 89.8 % to 90.4 % for *Ageratum*. The Permanova analysis indicated that these differences were statistically non-significant ( $p > 0.05$ ), reinforcing the observation of relatively consistent inhibition across ethanol concentrations. The standard deviations across concentrations in ethanol treatments were low, suggesting a high degree of result reliability and stability within each concentration group, and this stability may also be reflected in the Permanova analysis.

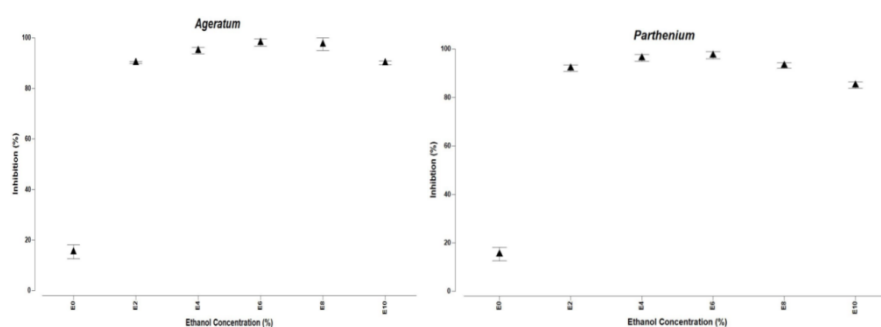


Figure 4. *In vitro* inhibition (%) of ethanol extract of *A. conyzoides* and *P. hysterophorus* against *P. capsici*

### (iv). Methanol Extracts

The methanol extracts revealed variable inhibitory effects of both *Parthenium* and *Ageratum* biofungicides across concentrations (Figure 5). The Permanova analysis supported these observations by highlighting the variability in inhibitory effects within the methanol treatments, which could be statistically significant ( $p < 0.05$ ). Mean inhibition (%) at the highest concentration tested (10 %) ranged from 57.2 % to 67.9 % for *Parthenium* and 56.7 % to 71.5 % for *Ageratum*. The Permanova analysis indicated that these differences were statistically significant ( $p < 0.05$ ), further emphasizing the variability in inhibition within the methanol treatments. Standard deviations within each concentration level in methanol treatments exhibited variability, suggesting some degree of results dispersion. The Permanova analysis corroborated this by demonstrating significant differences between the methanol treatments ( $p < 0.05$ ), supporting the observed variability.

### Overall Interpretations Supported by Permanova Analysis and P-Values

In summary, our visual observations and the Permanova analysis with associated p-values provided a comprehensive understanding of the inhibitory effects of *Parthenium* and *Ageratum* biofungicides on *P. capsici*. The Permanova analysis strengthened our results by confirming the statistical significance of concentration-dependent effects and differences between treatments. Specifically, acetone (A) and chloroform (C) treatments

consistently demonstrated higher inhibition (%), with the Permanova analysis likely affirming the statistical significance of these findings ( $p < 0.05$ ). These solvents at 10 % concentration, inhibited *P. capsici* with biofungicides derived from *Parthenium* and *Ageratum*. The low standard deviations, in acetone and chloroform extracts, indicated result reliability and consistency, supported by Permanova analysis and associated p-values.

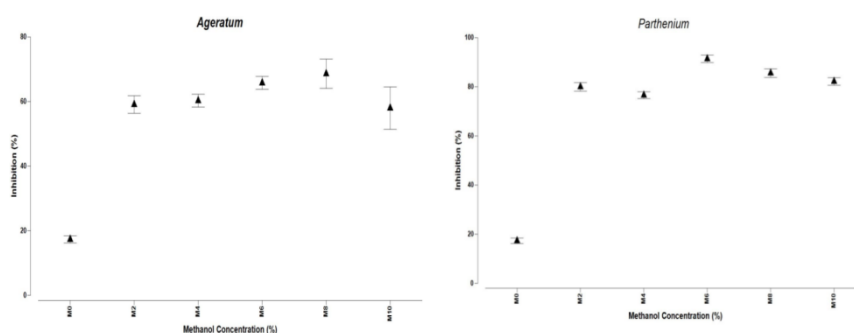


Figure 5. *In-vitro* inhibition (%) of methanol extract of *A. conyzoides* and *P. hysterophorus* against *P. capsici*

As the need for sustainable agriculture grows, so does worry over the overuse of synthetic pesticides. Many studies have demonstrated the significance of allelopathy in the relationship between weeds and crops (10). Because the diverse phytochemicals of *Ageratum* and *Parthenium* plants are valuable as insecticides in agriculture and have medicinal uses in human civilization, these weeds have positive as well as negative impact on agriculture ecosystems and human health (24). According to preliminary screening, ethanolic leaf extract had the strongest antifungal activity, which is consistent with our findings (38).

*A. conyzoides* extracts shows potential in weed management, while preserving and enhancing the soil microbiota (34), suggesting its useful application. *A. conyzoides* also exhibits potential to develop potent formulations for application in industry, agriculture and medicine (22). The allelopathic effects are increased by soil pH, organic matter content, nutrition, moisture content and microbes (23). However, it is unclear whether *Ageratum* and *Parthenium* leaf extracts have inhibitory effects on bell pepper seed germination and seedling growth. *P. hysterophorus* leaves are also source of allelopathic potential for both weeds and crops (6).

### FT-IR Analysis

Based on the peak values in the IR radiation band (Figure 6), the FT-IR spectrum was utilized to classify the functional groups of the active components found in the extract. Based on the extract's peak ratio, the functional groups of the constituents were divided when it was sent via the FT-IR. N-H, O-H, C=C, C-H, C-O and CH<sub>3</sub> functional groups were present, according to the results of FT-IR analysis. The detection of bio molecule composition using FTIR spectroscopy were accurate and sensitive. N-H, 3280.94 cm<sup>-1</sup>, O-H, 2918.75 cm<sup>-1</sup>, C=C 1727.35cm<sup>-1</sup>, C-H, 1408.12 cm<sup>-1</sup>, C-O 1240.25 cm<sup>-1</sup>, CH<sub>3</sub> 1016.15

$\text{cm}^{-1}$  and N-H,  $3281.43 \text{ cm}^{-1}$ , O-H,  $2918.83 \text{ cm}^{-1}$ , C=C  $1729.93 \text{ cm}^{-1}$ , C-H,  $1413.01 \text{ cm}^{-1}$ , C-O  $1244.59 \text{ cm}^{-1}$ ,  $\text{CH}_3$   $1019.07 (32)$ .

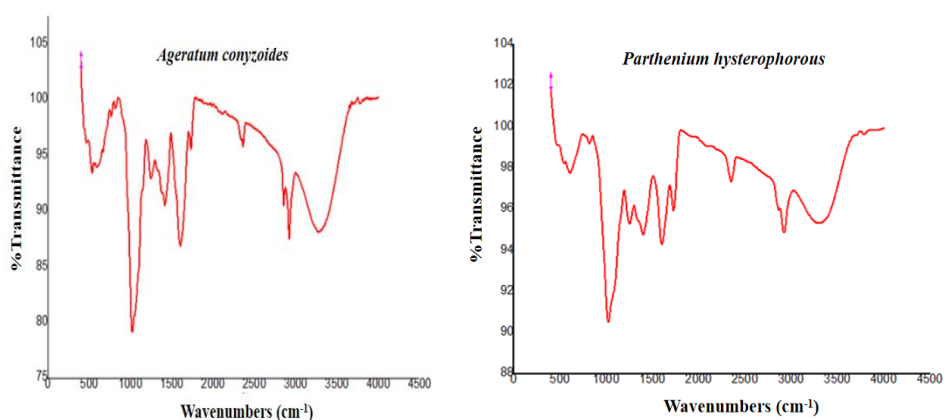


Figure 6. FT-IR spectrum of methanolic extract from the leaf of *A. conyzoides* and *P. hysterophorus*

### GC-MS Analysis

The comparison between the results of studies on *A. conyzoides* and *P. hysterophorus* in (Table 2 and 3) revealed interesting insights into their respective antifungal properties against *P. capsici*. GC-MS chromatogram of methanolic leaves extract of *A. conyzoides* (Figure 7) showed presence of several antifungal compounds. The GC-MS analysis of *A. conyzoides* and *P. hysterophorus* also revealed the presence of 30 and 18 compounds in the leaves of these plants, respectively.

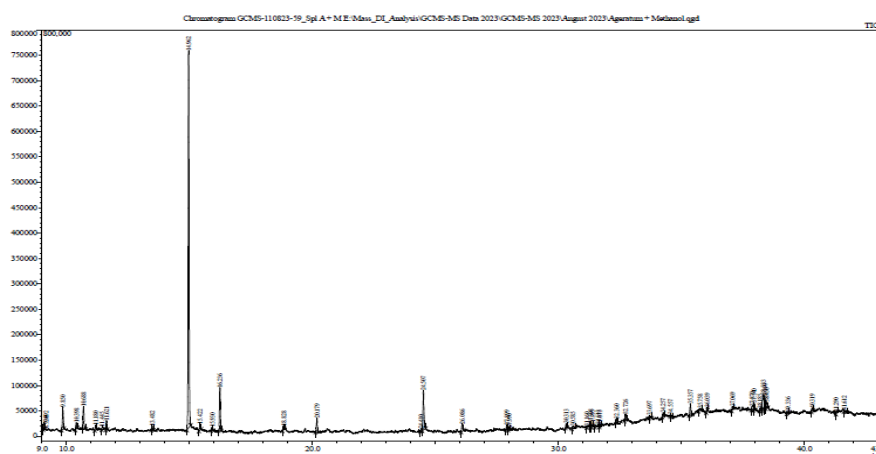


Figure 7. GC-MS chromatogram of methanolic leaves extract of *A. conyzoides*.

Table 2. Compounds identified in methanolic leaves extract of *Ageratum conyzoides*

Peak No.	Name of compound	RT	Area	Area %	Mol. wt (g/mol.)
1	5H-1-Pyridine	3.687	14468	0.37	117
3	Bicyclo [3.3.0] octan-2-one, 7-neopentylidene-	8.930	13934	0.35	192
5	Caryophyllene	9.850	111397	2.81	204
7	Precocene I	10.688	118250	2.98	190
8	Octadecane, 1-chloro-	11.180	26899	0.68	288
10	2,4-Di-tert-butylphenol	11.621	42572	1.07	206
11	Caryophyllene oxide	13.482	21407	0.54	220
12	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl	14.962	1925340	48.59	220
15	Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-benzopyran-6-yl)-	16.236	217094	5.48	248
17	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	20.179	76824	1.94	276
18	Tetradecanoic acid, 12-methyl-, methyl ester	24.410	11789	0.30	256
19	2,2',2''-Nitrilotriethanol, triethyl ether	24.507	228420	5.76	233
20	Propionamide, 2-bromo-N-(2-butyl)-N-pentyl	26.086	47827	1.21	277
21	1-Cyclohexyldimethylsilyloxy-3,5-dimethylbe	27.899	26557	0.67	262
23	Tetracosamethyl-cyclododecasiloxane	30.313	17469	0.44	888
24	2-Ethylbutyric acid, eicosyl ester	30.585	25937	0.65	396
25	1-Diphenylsilyloxy hexadecane	31.160	22103	0.56	424
27	4-Hexadecanol	31.395	11816	0.30	242
28	2-(3-Hydroxybutyl) cyclooctanone	31.613	29180	0.74	198
29	Heptasiloxane, hexadecamethyl-	31.675	10467	0.26	532
30	Cyclononasiloxane, octadecamethyl-	32.360	17617	0.44	666
34	Borinic acid, diethyl-, (2-ethyl-1,3,2-dioxaborinan-4-yl) methyl ester	34.557	24790	0.63	212
35	Squalene	35.357	66679	1.68	410
36	Sulfurous acid, octadecyl 2-propyl ester	35.758	26864	0.68	376
39	Fenpropathrin	37.870	29574	0.75	349
42	Dimethyldioctadecylammonium bromide	38.333	123095	3.11	629
43	Methyl 2-hydroxy-eicosanoate	38.390	92046	2.32	342
45	Cyclopentane, 1-(2-decyldodecyl)-2,4-dimethyl-	39.336	13408	0.34	406
48	5-Fluoro-2-trifluoromethylbenzoic acid, heptadecyl ester	41.612	22055	0.56	446
49	Benzene, 1,2-dichloro-4-nitro-	43.305	10455	0.26	191

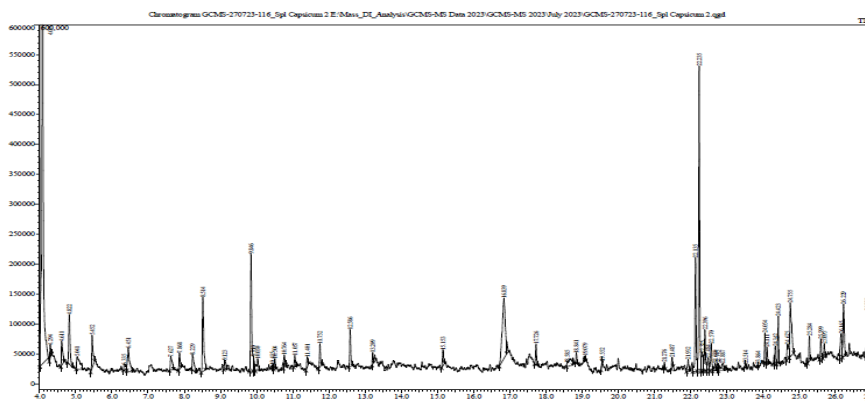
Table 3. Compound identified in methanolic leaves extract of *Parthenium hysterophorus*

Peak No.	Name of compound	RT	Area	Area %	Mol. wt (g/mol)
1	Butane, 1,3-dichloro-3-methyl-	2.087	68778	0.39	140
2	2-Myristinoyl-glycinamide	7.823	33375	0.19	280
3	Propanoic acid, 3,3'-selenobis-	8.190	33108	0.19	226
5	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	20.673	30106	0.17	292
6	4-Chlorobenzenesulfonamide, N-methyl-	21.620	30205	0.17	205
7	1-(2-methoxy-6-methylbenzyl) piperazine	24.528	33728	0.19	220
8	4,8-Dimethylheptacosne	27.400	39364	0.23	408
10	Fumaric acid, 2-butyl undecyl ester	32.310	64648	0.37	326
12	Widdrol hydroxyether	32.594	34457	0.20	238
13	5,15-Dimethylnonadecane	32.734	85170	0.49	296
20	3-Nonen-2-one, 3-ethyl-	34.170	55324	0.32	168
21	Tetrapentacontane	34.795	43632	0.25	758
24	1,3-Dioxolan-4-one, 2-t-butyl-5-methyl-5-(4,4-dimethoxypentyl)-	35.580	37718	0.22	288
26	Triallylethoxysilane	35.885	87022	0.50	196
27	Triallylphosphine	35.946	85341	0.49	154
32	11-Methyltricosane	36.819	42090	0.24	338
42	1-Aminooctadecane, HFB	39.450	144092	0.82	465
48	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	41.809	273821	1.57	646

**A. conyzoides:** It has significant antifungal activity, attributed to major compounds such as Caryophyllene, Precocene I, Squalene. Out of 30 compounds identified in this sub-fraction, 2H-1-benzopyran, 7-dimethoxy-2,2-dimethyl-(48.59 %) was most abundant followed by 2,2',2''-Nitrilotriethanol, triethyl ether (5.76 %); Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-benzopyran-6-yl)-(5.48 %) and Dimethyl dioctadecyl ammonium bromide (3.11 %), which could be responsible for antifungal activity (4). These compounds, although present in varying abundance, collectively contributed to the observed inhibitory effects against the fungal pathogen. Caryophyllene, a sesquiterpene known for its antimicrobial properties, likely played a crucial role in the antifungal activity of the extract. Similarly, Precocene I and Squalene showed substantial efficacy, suggesting synergistic effects of multiple compounds within the extract. Squalene was the predominant compound (an isoprenoid from the group of polyphenyl compounds), is an intermediate metabolite also possesses antimicrobial activity against phytopathogens (20,49).

**P. hysterophorus:** Its leaves contains most of the phytocomponents when tested qualitatively through GC-MS analysis and showed considerable amount of terpenes, carbohydrates, protein, steroids, alkaloids, aminoacids, saponins, tannins, terpenes and phenolics Figure 8 (25). Despite their lower abundance, these compounds demonstrated significant inhibitory effects against *Phytophthora capsici*. Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, present in the methanol leaf extracts of *P. hysterophorus* had antifungal and antioxidant properties (17,47). Additionally, phenol, 2, 4-bis (1, 1-dimethylethyl) extracted from the leaves of *P. hysterophorus* have allelopathic effects on germination and seedling growth of weed plants under soil-less

conditions (47). This defence compound prevents the root rot caused by *Phytophthora* spp. The antifungal compound phenol, 2,4-bis (1,1-dimethylethyl) may be developed as the green fungicides for the protection of fungal plant diseases in agriculture (36).



Figures 8. GC-MS chromatogram of methanolic leaves extract of *P. hysterophorus*

## CONCLUSIONS

The acetone and chloroform extracts have potential to develop biofungicide formulations for disease management in agriculture, based on both visual and statistically significant p-values from Permanova analysis. The detection of bio molecule composition using FTIR spectroscopy was found accurate and sensitive. Both *Ageratum conyzoides* and *Parthenium hysterophorus* extracts exhibited promising antifungal properties, they contained distinct sets of active compounds, suggesting differences in their modes of action and applicability in plant disease management. 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.

## AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

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## 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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