PLANT SCIENCE TODAY
Vol 12(sp4): 1-13
https://doi.org/10.14719/pst.9535

eISSN 2348-1900

RESEARCH ARTICLE

Potential role of wheat endophytes and weed leaf extracts in the management of *Fusarium* head blight

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Received: 20 May 2025; Accepted: 30 August 2025; Available online: Version 1.0: 29 October 2025

Cite this article: Aspak, Singh JP, Khanday MA, Chandrapati A, Kumar R, Kaushik D, Nath BC, Rana M, Srivastava S. Potential role of wheat endophytes and weed leaf extracts in the management of *Fusarium* head blight. Plant Science Today. 2025; 12(sp4): 1-13. https://doi.org/10.14719/pst.9535

Abstract

Wheat (*Triticum* spp.) was known to be mostly grown cereal crops globally and consumed as staple food by 35 % population of the world. The average productivity may decrease throughout the world due to outbreak of *Fusarium graminearum* which cause *Fusarium* head blight disease in wheat crop. This research was conducted to find potential of these 11 endophytic fungus and bacteria isolated from wheat crop and 2 plant extracts which were evaluated as biological control agents and leaf leachates against *Fusarium graminaearum* as an alternative eco-friendly approach for chemical pesticides due to resistance occur in pathogens by overdose and some harmful chemical residues left by them in soil. The data revealed that maximum mycelial inhibition of test pathogen was showed by *Aspergillus niger* (93.32 %) followed by *Curvularia lunata* (90.31 %) and *Bacillus subtilis* (89.41 %). The data revealed that maximum mycelial inhibition of test pathogen was carried out by methanol + *Ageratum conyzoides* in 6 % and 4 % conc. (98.29 %) and (98.06 %) followed by ethanol + *Parthenium hysterophorus* in 6 % conc. (97.78 %).

Keywords: endophytes; *Fusarium graminearum*; *Fusarium* head blight; leaf leachates; management

Introduction

Wheat (*Triticum* sp.) is known to be mostly grown cereal crops globally and consumed as staple food by 35 % population of the world, which is used to generate energy in human and provide feed for animals. Total wheat harvested area worldwide about 219 million hectares with an annual output of 760 million tonnes on average of 10-year data (1). Total production of wheat worldwide and India is about 792.2 million tonnes and 112.9 million tonnes respectively (2). 188287.99 metric tonnes of wheat with worth of ₹470.83 crores/56.66 million USD has been exported to world market by India during 2023-24 and Uttar Pradesh was the leading state in production of wheat (3). Among the biotic stresses, pathogens (i.e., fungi, viruses and bacteria) may contribute to average global losses of 21.5 % of wheat yield (4).

Fusarium Head Blight (FHB) caused by *Fusarium graminearum* is one of the most relevant fungal diseases of wheat associated with different fungal species from the genus *Fusarium* (5). Due to increase in wheat production throughout the world, the chances of disease occurrence also increase which results in yield loss in the crop due to impact of FHB disease. According to recent studies this disease incidence has increased over the years causing major outbreaks in various countries due to mycotoxins produced by the pathogen results in major losses in wheat production throughout the world (6). The pathogen produces different mycotoxins like deoxynivalenol (DON) and Zearalenone (ZEA) were toxic to both humans and animals, respectively (7). The major fungal pathogens which cause economically important diseases results in significant losses in wheat crop such as *Blumeria graminis, Fusarium graminearum, Mycosphaerella graminicola, Fusarium oxysporum, Botrytis cinerea, Puccinia* spp., *Colletotrichum* spp., *Magnaporthe oryzae*. The common species, *Fusarium graminearum* (teleomorph: *Gibberella zeae*), is presently ranked fourth among plant fungal pathogens due to its scientific and profitable value (8). Endophytes are microorganisms such as fungi and bacteria which survive symbiotically inside intercellular and intracellular tissues of various plant parts like leaves and stem (9,10).

Fungal endophytes play major role in plant growth, development, health and survivability by establishing symbiotic association with their host plant on which they colonize and inhabit. As these endophytes help the plant in improving nutrient acquisition i.e., nitrogen, phosphorus, potassium, magnesium; and phytohormones such as auxins, cytokinins, gibberellin, photosynthetic activity, siderophore production, abiotic i.e., drought, salinity, temperature, heavy metal and biotic stress tolerance i.e., pathogens, herbivores and insect pests, produce secondary metabolites i.e., alkaloids, terpenoids, steroids help in activating defence mechanism in host plant thus making it difficult for entry of pathogen inside host plant thus increasing

survival condition which leads to generating higher yield (11,12).

Enhancing plant growth, stress tolerance, pest and disease resistance, bioremediation, enhanced soil health and increased synthesis of secondary metabolites, symbiotic partnerships and sustainable agriculture are the key benefits of endophytes. In agriculture, endophytes are essential for fostering plant health and sustainability (13). Numerous plant species have symbiotic relationship with endophytes, which leads to generation of great diversity in both host plants and endophytes therefore making development of a standard technique for isolation of endophytes challenging in recent period (14). As we have studied in recent literature that over dosage of fungicides for control of *Fusarium graminearum* results in appearance of resistant strains through from one generation to other and leave chemical residues inside soil thus causing harmful effects to crop. So, in recent period use of various endophytic bio-control agents and plant extracts as an alternative to chemical pesticides as these are eco-friendly in nature and effectively control FHB disease of wheat without causing any harmful effects to the crop or environment.

Materials and Methods

All field and laboratory experiments were done during 2023-24 in Lovely Professional University, Phagwara, Punjab, India. *Ageratum* and *Parthenium* leaves used in the study were collected at the seedling stage from the nearby area of university campus.

Isolation, purification and identification of test pathogen

The typical FHB infected samples collected from the fields of Lovely Professional University, Phagwara, Punjab were subjected to the isolation technique under aseptic conditions inside laminar air flow. The infected plant parts were cleaned using sterilized distilled water and cut into small-sized pieces with the help of a sterilized blade where the cut portion should consist of both infected and healthy area and disinfected with 1 % sodium hypochlorite for 60-90 sec followed by ethanol for 30 sec and thereafter kept in autoclaved distilled water for 3 times. Finally, the samples were inoculated in Petri plates as well as on slants consists of PDA (Potato Dextrose Agar) medium and incubated in BOD incubator for 3-4 days at 26 ± 2 (15). Further purification was done by hyphal tip method, later these were sub -cultured repeatedly three to four times to get pure culture of *Fusarium graminearum* and incubated at 26 ± 2 °C for 8-10 days. The culture was preserved on PDA slants at 4 °C in a refrigerator and stored for further study. Later the test pathogen was observed under a compound microscope and identified based on the morphological and cultural characteristics recorded in standard authentic literature as the mycelium of isolated endophytes was stained with cotton blue and observed under a compound microscope at 10X and 40X objective lenses for morphological classification of isolated fungi (16).

Biocontrol agents and plant extracts

Healthy samples of wheat plants such as root, stem, leaves and seeds were collected from the wheat fields of LPU, Punjab were brought to the laboratory and processed within 24–48 hr of collection. The samples were chopped into small pieces and rinsed below running tap water for 10-20 min, later dipped into sterilized distilled H_2O and drained. All the samples were disinfected with 1 % sodium hypochlorite for 10 sec followed by ethanol for 30 sec and thereafter wash continuously with autoclaved distilled water for 3 times and the serial dilutions of 10^{-3}, 10^{-4} and 10^{-5} was done by mixing samples with autoclaved distilled water and finally, 1 mL aliquot of each of these dilutions were placed in Petri plates poured with malt extract agar medium supplemented with a pinch of streptomycin and chloramphenicol each 100 mg/L to control growth of fungus and bacteria separately, later incubated at 28 ± 2 °C for three to days and purification was done by hyphal tip method and streaking method for fungus and bacteria with sub-culturing in new Petri plates three to four times to get pure cultures. Later these were stored in test tube slants at 4 °C in refrigerator for further study and identified based on the morphological and cultural characteristics recorded in standard authentic literature under a compound microscope as the mycelium of isolated endophytes was stained with cotton blue were observed under compound microscope at 10X, 40X and 100X objective lenses for morphological classification of isolated fungi (17).

Different concentrations of leaf leachates of *A. conyzoides* and *P. hysterophorus* were prepared with ethanol. The test pathogen was treated with different concentration of leaf leachates using poisoned food technique (18). 15 mL leaf leachates of *A. conyzoides* and *P. hysterophorus* 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 randomized design for five days; the Petri plates were kept at laboratory temperature.

Evaluation of bio-control agents and plant extracts against *Fusarium graminearum* at *in vitro* using both dual culture and poison food technique

In vitro study was conducted to check the efficacy of antagonistic effect of endophytic fungi, bacteria and plant extracts against *Fusarium graminearum* with the help of dual culture and poison food technique. A 5 mm disc of both test fungus and endophytes was placed at opposite sides of the Petri plate poured with MEA media. The plates were incubated at 26 ± 2 °C in BOD incubator. By using the poisoned food technique, the PDA medium was mixed with varying concentrations of leaf leachates i.e., 2, 4, 6, 8 and 10 % were poured into Petri plates were replicated three times and one was kept as a control with only test pathogen and distilled water and incubated for 5 days. The readings of test fungus were recorded till the control plate's mycelium were full with the test pathogen and finally the percent mycelial inhibition of the test pathogen was calculated as per the formula given by Vincent (19).

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

Where, C = colony diameter in control, T = radial growth of test pathogen in treated plate (mm)

GC-MS analysis

The chemical components in ethanolic leaf extract of *Ageratum conzoides* and *Parthenium hysterphorus* were identified by using GC-MS analysis. GC-MS (Model; QP 2010 series, Shimadzu, Tokyo, Japan) which was equipped with Rxi-5MS fused silica capillary column (5 % diphenyl/95 % dimethyl polysiloxane) and AOC20i+s (autosampler) of 0.25 mm diameter, 0.25 µm thickness and 30 m length was used for GC-analysis of ethanolic extract. In

this helium gas was used as carrier gas and a 2 µL sample was supplied using injector. The GC-MS identification of compounds were identified at an ionization energy of 70 ev, scan range of 40 m/z to 700 m/z, overall flow rate of 16.3 mL/min, column flow rate of 1.21 mL/min, linear velocity of 39.9 cm/sec, injection temperature at 260 ℃, split ratio of 10:0, initial temperature at 50℃, followed by 250℃ for 5 min, 280℃ for 22 min and final hold at 69.98 min and held at acquisition mode and its total run time is 65 min and relative percent composition of each component was shown at peak area (20).

Statistical analysis

In the present study, the information obtained from the laboratory as well as field were analysed before describing the results. All the data were analyzed with the significance of results at 5 % ($p < 0.05$) by using OPSTAT software and the appropriate data have also been indicated in each table. One-way ANOVA (analysis of variance) was used for analysis of antagonistic data. F value to test significance of treatment difference (CD) was calculated at 5 % (21,22). The Critical Difference (CD) to compare the means of different entries was calculated using the following formula:

$$\text{Critical Difference (CD)} = SE \times t$$

Where,

SE: Standard error of the difference between treatment means

Results and Discussion

Isolation, purification and identification of test pathogen i.e., *Fusarium graminearum*, biocontrol agents and plant extracts

Identification was performed using both microscopic and cultural characteristics. According to their different morphology and cultural features of mycelia growth of fungi such as colony morphology, structure and shape of conidiomata, conidiophores and conidia (size and colour), characters of conidiogenous cells and mycelium colour.

The isolation and purification of test pathogen i.e., *Fusarium graminearum* was conducted by using procedure described in a previous study (15). Cultural and morphological identification of test pathogen was conducted under a compound microscope as per the method given in another study (16). The isolation, purification and identification of various endophytes both bacteria and fungi were carried according to methodology proposed in a previous study (17) with minor changes to it as author has conducted his study on various phyllosphere fungi against *Colletotrichum capsici* and we used this method to study on various fungal and bacterial endophytes against *Fusarium graminearum*.

In-vitro evaluation of biocontrol agents against *Fusarium graminearum*

In this research potential of eleven fungal endophytes were tested against *Fusarium graminearum* to check their efficacy in reducing mycelial growth rate of test pathogen. The biocontrol agents mentioned below have significantly inhibited test fungus i.e., *Fusarium graminearum* growth over control. Maximum mycelial inhibition of test pathogen was observed with dual culture by *Aspergillus niger* (93.32 %) followed by *Curvularia lunata* (90.31 %), *Bacillus subtilis* (89.41 %) and *Aspergillus flavus* (86.41 %) as per the data presented in Table 1.

This method was carried out according to the process suggested by Vincent (19). In this research antagonistic potential shown by endophytic fungi i.e., *Aspergillus niger* (93.32 %) as compared with antagonistic yeasts (87.67 %) to inhibit mycelium growth of *Fusarium graminearum* as mentioned by author in his study (23).

Some fungal endophytes were used as biofertilizers for improving crop production (24) and biocontrol of pests was investigated recently (25). Ban on methyl bromide which was used for effective management of diseases caused by *Fusarium* spp. due to this outbreak of this disease can occur, so for supressing it many researchers started using resistant cultivars and recent studies had proven the potential of endophytic fungi in managing these diseases (26,27). Endophytic bacteria such as *Bacillus subtilis* and *Bacillus majavensis* isolated from maize shows potential in effective control of pathogens i.e., *Fusarium moniliforme* and *Fusarium graminearum* which causes disease in wheat and maize crop (28,29). An endophyte like *Aspergillus* spp. was applied at *in vivo* and observed that there was a major improvement in chlorophyll, total sugar, phenol and protein content in diseased plants (30). It was observed that there was major impact of endophytes in growth and development of plants as this produce growth stimulating, antimicrobial, secondary metabolic compounds which help in triggering defensive mechanisms inside plant against various disease-causing pathogens (31–33). *Aspergillus* spp. applied as bio-stimulant reported to improve morphological growth, enzyme activities, chlorophyll content and change in osmolytes help to

Table 1. *In vitro* evaluation of various bio-control agents against *Fusarium graminearum*

Treatment	Colony diameter (mm) of *Fusarium graminearum*	Percentage inhibition of mycelia growth*
Aspergillus niger (T1)	6.010	93.32*
Aspergillus flavus (T2)	12.230	86.41
Alternaria solani (T3)	21.933	75.63
Aspergillus fumigates (T4)	17.463	80.06
Curvularia lunata (T5)	8.713	90.31
Trichoderma harzianum (T6)	26.713	70.32
Trichoderma lixii (T7)	43.297	51.89
Pseudomonas fluorescens (T8)	42.197	53.11
Colletotrichum capsici (T9)	30.990	65.56
Trichoderma asperellum (T10)	41.833	53.52
Bacillus subtilis (T11)	9.853	89.41
Control	90.000	0
C.V	2.758	
C.D	1.376	
S.E(m)	0.466	
Significance	0.00000	

develop stress tolerance in various crops (34,35). Endophytic bacteria *i.e.*, *Pseudomonas* spp. was observed to be potential biocontrol agent as it able to adapt and survive in complex environment conditions by production of various secondary metabolites which helps in plant growth and protection against different phytopathogens (36). *Fusarium graminearum* produces toxic compounds like DON and ZEA. It was found that endophytic species of *Bacillus* can not only inhibit the pathogen growth but also inhibit the accumulation of DON (37). Endophytic *Curvularia* spp. manages the temperature stress of plants, such as watermelons, tomatoes and wheat (38,39).

Bioassay of various plant extracts against *Fusarium graminearum*

Extract of 2 weed plants i.e., *Ageratum conyzoides* and *Parthenium hysterophorus* were used as leaf leachates with various concentrations to check their efficacy to control mycelium development against *Fusarium graminearum.* All the leaf leachates showed better results in inhibiting mycelial growth of test pathogen i.e., *Fusarium graminearum* growth over control. The bioassay treatment with methanol + ageratum (98.29 %) at 6 % and (98.06 %) at 4 % concentrations showed the best result in inhibiting mycelial growth followed by ethanol + *Parthenium* (97.78) at 6 % and (97.52) at 4 % concentration as per the data provided in Table 2.

This process was carried out as per the method given by Vincent (19). According to above study the plant extract of *Ageratum conyzoides* (98.29 %) shows same antagonistic potential in mycelium growth inhibition as plant extract of Chinese gall (98-100 %) as mentioned in a previous study (40).

It was studied that the use of methanol and ethanol as solvents were known to be most effective in extracting bioactive compounds like alkaloids, phenols, flavonoids and terpenoids from *Parthenium hysterophorus* as we observed that *Fusarium oxysporum* shows high sensitivity against this leaf extract as compared to other tested pathogens (41,42). *Parthenium* leaf extract was found very promising in inhibiting the growth of *Fusarium oxysporum.*

Management of *Fusarium oxysporum* can be done under *in vivo* condition by application of *Parthenium* leaf extract due to various mechanism like production of bioactive compounds, fungal metabolism interference, induction of oxidative stress and inhibit signaling pathways (quorum sensing) in test pathogen (43). A study was conducted to check the efficacy of leaf and inflorescence methanolic extract with concentration i.e., 6 % and tested result shows leaf extract was having more mycelial growth inhibition i.e., 76 % as compared to inflorescence extract i.e., 56 % against *Fusarium* spp. (44). Sukrasno (2018) investigated that *Ageratum conyziodes* consists of coumarin which exhibits antifungal properties which helps in oxidative stress to fungal cells, disruption of ergosterol synthesis, chelation of essential minerals, thus inhibit growth and development of various pathogens like *Fusarium oxysporum*, *Botrytis cinerea*, *Colletotrichum* spp., *Aspergillus* spp. (45). Coumarin which is a secondary metabolite produced by *Ageratum conyzoides* belongs to Asteraceae family consists of antifungal, antibacterial and antioxidant properties to protect these plants against various disease causing phytopathogens (46). The secondary metabolic compound coumarin plays a major role in activating defensive mechanisms against microorganisms and herbivores in plants, microbes and aquatic organisms like sponges (47).

Coumarin shows a similar effect to colchine as it hampers microtubule formation and mitotic spindle assembly by increasing mitochondrial matrix density leads to decrease in energy production, arrest cell cycle by ceasing fungal cells at specific mitosis phases which lead to irregular structure of cells and finally cell death occurs thus inhibiting growth and development of *Fusarium oxysporum* (48). The allelochemicals present in leaves such as p-coumaric acid, benzoic acid, sinapic

Table 2. *In vitro* evaluation of weed plant extracts against *Fusarium graminearum*

Treatment	Concentration (%)	Colony diameter (mm) of *Fusarium graminearum*	Percentage inhibition of mycelia growth*
Methanol + *Parthenium*	T1 – 2 %	10.000	88.63
	T2 – 4 %	2.403	97.26
	T3 – 6 %	2.343	97.33
	T4 – 8 %	5.590	93.64
	T5 – 10 %	10.923	87.58
	Control	88.000	0
Ethanol + *Parthenium*	T1 – 2 %	11.133	87.35
	T2 – 4 %	2.180	97.52
	T3 – 6 %	1.950	97.78
	T4 – 8 %	4.917	94.41
	T5 – 10 %	12.350	85.96
	Control	88.000	0
Methanol + *Ageratum*	T1 – 2 %	10.857	87.67
	T2 – 4 %	1.707	98.06
	T3 – 6 %	1.497	98.29
	T4 – 8 %	4.757	94.65
	T5 – 10 %	9.857	88.80
	Control	88.000	0
Ethanol + *Ageratum*	T1 – 2 %	13.200	85
	T2 – 4 %	2.177	97.53
	T3 – 6 %	3.407	96.13
	T4 – 8 %	4.760	94.59
	T5 – 10 %	11.747	86.65
	Control	88.000	0
C.V		7.304	
C.D		2.269	
S.E (m)		0.794	
significance		0.00000	

acid as these help in inhibiting spore germination, enzyme activity, disrupts cellular respiration, cell membrane integrity thus obstructing metabolism and colonization of target fungal pathogen like *Fusarium oxysporum* as compared to stem and root (44). *Ageratum conyzoides* consists of various fungitoxic chemicals such as coumarins, alkaloids, phenolic compounds (p-coumaric acid, sinapic acid, benzoic acid), flavonoids in each plant part like leaves, root and stem to protect plant against *Fusarium solani* which cause wilt disease (44).

GC-MS anlaysis

The comparison between the results of studies on *Ageratum conyzoides* and *Parthenium hysterophorus* extracts (Fig. 1, 2) revealed their antifungal properties against *Fusarium graminearum*. GC-MS chromatogram of leaves extract of *A. conyzoides* (Fig. 1) 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 (49).

Ageratum conyzoides

Ageratum conyzoides antifungal activity, may be primarily attributed to major compounds: Caryophyllene, Precocene I, 1-Pentadecene and Neophytadiene (50). 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 effect of multiple compounds in the extract (Fig. 1, Table 3).

Ageratum conyzoides L. is rich in phytoconstituents that offer numerous advantages in various contexts (51). Major bioactive compounds produced by *Ageratum conyzoides* were phytol, E- β-famesene, precocene 1, caryophyllene, precocene 2 and 1-nonadecene. These compounds have antibacterial, antifungal, anticancer, anti-inflammatory, aphid-repellent and/or antioxidant qualities (52).

Ageratum conyzoides L. produces multiple antifungal compounds like flavonoids like Polymethoxyflavones, Nobiletin, 5,6,7,3',4',5'-hexametoxyflavone, 5'-methoxynobbiletin, eupalestin which inhibit various phytopathogens like *Rhizoctonia solani* and *Pyricularia oryza*. These compounds were found on the aerial parts like leaves, stem, flower and fruit of this plant. Furthermore, with a ten-fold lower IC50, precocene II has excellent antifungal efficacy against both fungi (53).

Parthenium hysterophorus

In contrast, *Parthenium hysterophorus* exhibited potent antifungal activity attributed to compounds such as Neophytadiene, Caryophyllene oxide and Squalene (Fig. 2, Table 4). Despite their lower abundance, these compounds were inhibitory to *Fusarium graminearum*. Additionally, lesser abundant compounds like phytol and 7,9-Di-tert-butyl-1-oxaspiro (4.5) deca-6,9-die (54,55) and Phytol also showed antifungal efficacy, indicating the presence of potential novel targets for further exploration (50).

The leaf extracts from the *Parthenium hysterophorus* plant possess antibacterial and antifungal compounds against the range of tested bacterial and fungal strains (41). *P. hysterophorus* leaves are rich in phytol, may be related to the leaves increased diterpene concentration. *P. hysterophorus* leaf proteins are superior to cereals and legumes (56). Neophytadiene is a diterpenoid molecule found in *P. hysterophorus* leaf extract, it has antimicrobial, anti-inflammatory, antibiofilm and antioxidant activities (57).

Since plant extracts give immediate, surface-level suppression of the pathogen and endophytes create persistent defense within the plant, integrating the two offers a synergistic, integrated strategy. In order to increase the quantity and variety of endophytes as well as their growth potential when recovered following isolation, the addition of plant extract to media was studied (58). Furthermore, their different mechanisms of action

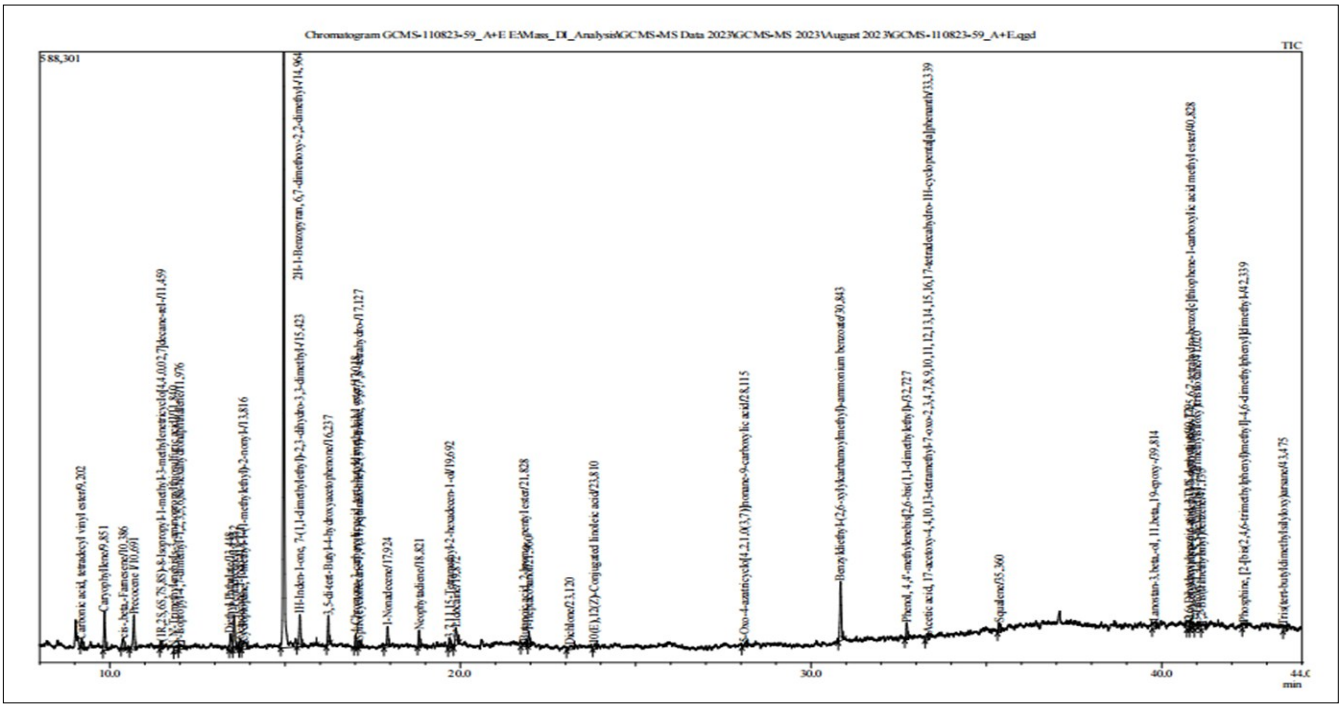
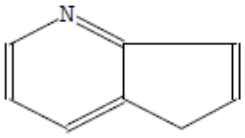
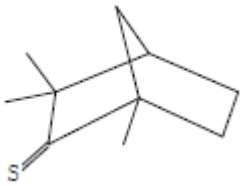
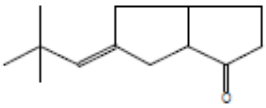
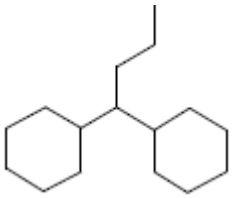
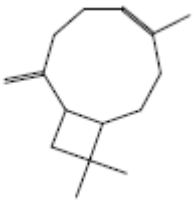
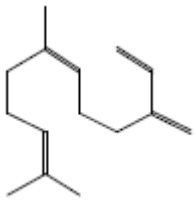
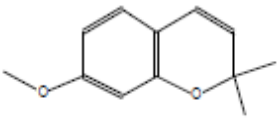
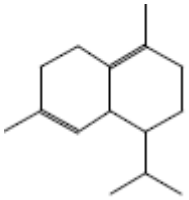
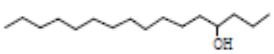
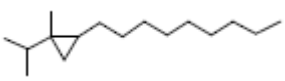


Fig. 1. GC-MS chromatogram of ethanolic leaves extract of *Ageratum conyzoides*.

Table 3. Compounds in ethanolic extracts of *Ageratum conyzoides* leaves

Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
1	5H-1-Pyrindine	3.687	14468	0.37	117	
2	2-Norbornanethione, 1,3,3 trimethyl-	3.064	62870	1.58	168	
3	Bicyclo [3.3.0] octan-2-0ne, 7-neopentylidene	8.930	13934	0.35	192	
5	1,1-Dicyclohexylbutane	5.157	31450	0.79	222	
8	1-Tridecene	9.029	30618	1.88	182	
11	Caryophyllene	9.851	105709	2.66	204	
12	cis-. beta. -Farnesene	10.386	42974	1.08	204	
13	Precocene I	10.691	110274	2.77	190	
16	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	11.976	30783	0.77	204	
17	4-Hexadecanol	31.395	11816	0.30	242	
19	Octadecane, 1-iodo-	13.721	31037	0.78	380	
20	Cyclopropane, 1-methyl-1-(1-methylethyl)-2-nonyl	13.816	52619	1.32	224	

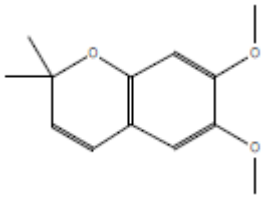
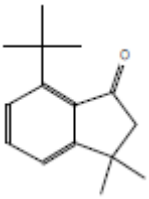
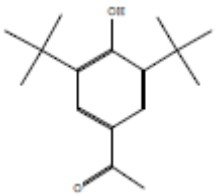
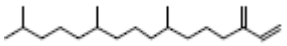
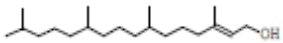
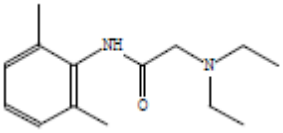
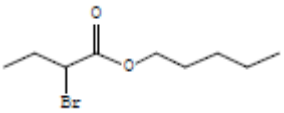
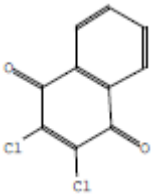
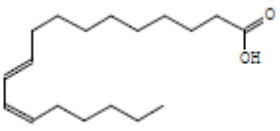
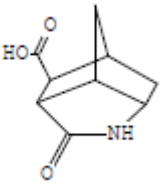
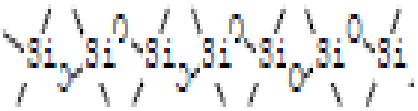
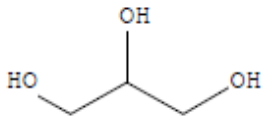
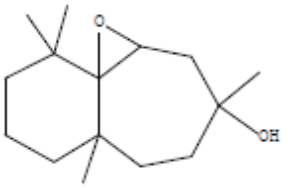
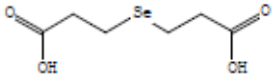
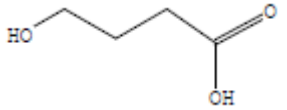
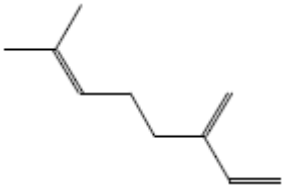
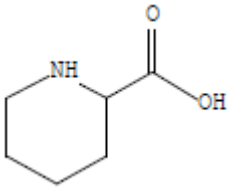
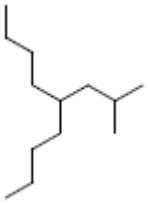
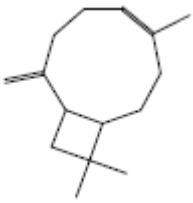
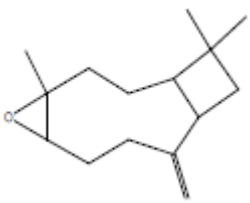
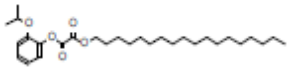
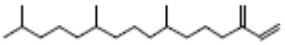
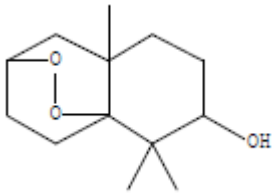
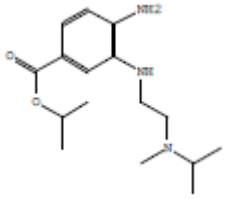
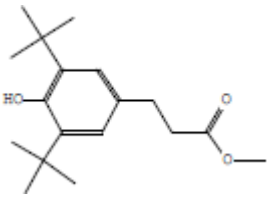
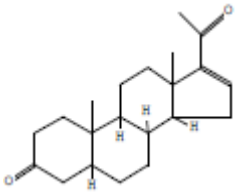
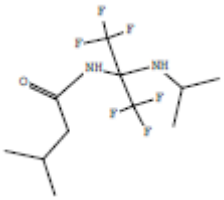
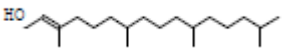
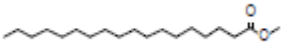
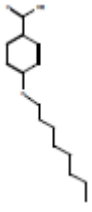
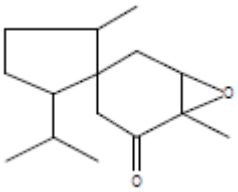
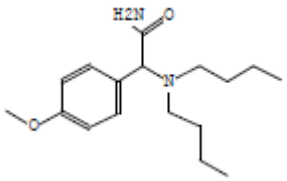
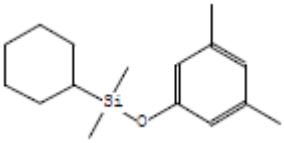
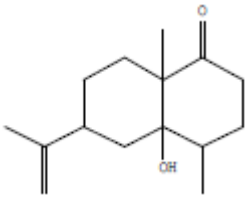
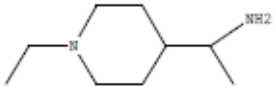
21	2H-1-Benzopyran, 6,7-dimethoxy-2,2-dimethyl	14.964	1555324	39.12	220	
22	1H-Inden-1-one, 7-(1,1-dimethylethyl)-2,3-dihydro-3,3-dimethyl	15.423	100385	2.53	216	
23	3,5-di-tert-Butyl-4-hydroxyacetophenone	16.237	70387	1.77	248	
27	Neophytadiene	18.821	47249	1.19	278	
28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.692	35642	0.90	296	
29	Lidocaine	19.872	62319	1.57	234	
30	Butanoic acid, 2-bromo-, pentyl ester	21.828	24776	0.62	236	
32	Dichlone	23.120	30096	0.76	226	
33	10(E),12(Z)-Conjugated linoleic acid	23.810	25427	0.64	280	
34	5-Oxo-4-azatricyclo [4.2.1.0(3,7)] nonane-9-carboxylic acid	28.115	27340	0.69	181	
38	Squalene	35.360	35438	0.89	410	
39	Heptasiloxane, hexadecamethyl-	38.535	32331	0.79	532	

Table 4. Compounds on ethanolic extracts of *Parthenium hysterophorus* leaves

Peak No.	Name of compound	Retention Time	Area	Area %	Mol. weight in g/mol.	Molecular Structure
1	Glycerin	4.175	637474	4.44	92	
2	Widdrol hydroxyether	32.594	34457	0.20	238	
3	Propanoic acid, 3,3'-selenobis-	8.190	33108	0.19	226	
4	Butanoic acid, 4-hydroxy-	8.951	53395	0.37	104	
5	beta.-Myrcene	11.023	27805	0.19	136	
6	2-Piperidinecarboxylic acid, (.+/-.)-	14.555	45992	0.32	129	
7	Nonane, 5-(2-methylpropyl)-	17.562	34642	0.24	184	
9	Caryophyllene	20.535	72928	0.51	204	
12	Caryophyllene oxide	23.464	171053	1.19	220	
15	Oxalic acid, 2-isopropoxyphenyl octadecyl ester	26.015	24795	0.17	476	

17	Neophytadiene	27.348	114680	0.80	278	
18	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	27.431	54559	0.38	226	
20	Isopropyl 4-amino-3-({2-[isopropyl(methyl) amino]} amino) benzoate	28.188	68349	0.48	293	
22	Hexadecanoic acid, methyl ester	28.637	114159	0.80	270	
23	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy, methyl ester	28.731	68861	0.48	292	
24	16-Pregnen-3,20-dione	29.203	98906	0.69	314	
25	N-[2,2,2-Trifluro-1-(isopropyl amino)-1-(trifluromethyl)ethyl] isovaleramide	30.805	53149	0.37	308	
27	8,11,14-Docosatrienoic acid, methyl ester	31.050	146145	1.02	348	
28	Phytol	31.207	347138	2.42	296	
29	Decane, 1-iodo-	31.335	205600	1.43	268	
30	Methyl stearate	31.397	114534	0.80	298	
32	4-Octyloxybenzoic acid	31.816	73558	0.51	250	
35	Spiro [4.5] decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl	32.055	291443	2.03	236	

36	Ambucetamide	32.608	60619	0.42	292	
38	1-Cyclohexyldimethylsilyloxy-3,5-dimethylben	33.086	41903	0.29	262	
42	Corymbolone	34.250	79095	0.55	236	
45	Piperidine N-ethyl-4-[1 -aminoethyl]-	34.880	43272	0.30	156	
47	(E)-Dodec-5-en-4-olide	35.235	111647	0.78	196	

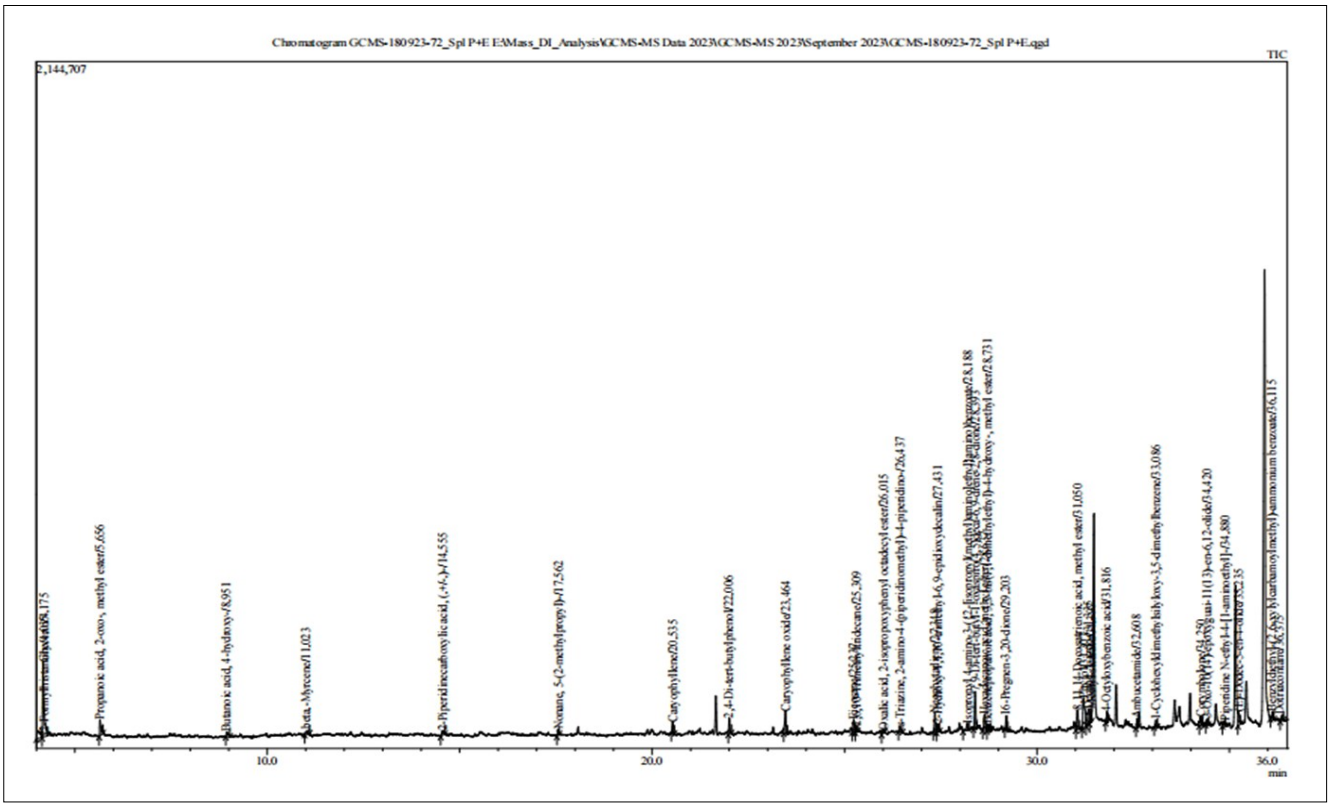


Fig. 2. GC-MS chromatogram of ethanolic leaves extract of *Parthenium hysterophorus*.

direct chemical inhibition and biological colonization can work in concert to improve suppression of DON production and lower the likelihood of resistance development. Compounds found in plant extracts and endophytic microbes can elicit responses that activate plant defense (59). The conceptual underpinnings and early data indicate that the integration of these two strategies may create a durable, multifaceted management approach, even though studies directly evaluating their effectiveness against FHB of wheat are still in their infancy. To fully grasp the potential of combining endophytes and plant-derived chemicals in FHB management, more field testing and formulation improvement are therefore necessary.

Conclusion

This research contributes valuable insights for utilization of potential endophytic bio-control agents and plant extracts in sustainable agriculture as an eco-friendly approach alternative to chemical pesticides. Endophytes play major role in enhancing plant immunity against various phytopathogens thus help host plants in inhibiting major diseases thus leading to an increase in crop production. This above study gives knowledge about potential of leaf extracts made from *Parthenium hysterophorus* and *Ageratum conyzoides* having both antifungal and antibacterial properties which helps in suppression of target pathogens. Nowadays, the use of plant-derived biocides is increasing in agriculture due to its eco-friendly nature and less harmful to both plants and environment. Identification of specific potential bioactive compound from these plants helps us to develop natural biopesticides thus decreasing usage of chemical pesticides to promote sustainable agriculture. Thus farmers can substantially benefit by using these potential endophytic bio-control agents and plant extracts to improve production of wheat and reduce losses caused by outbreak of FHB disease leads to increasing their overall profits annually.

Acknowledgements

The first author express gratitude to the Lovely Professional University for the invaluable digital assistance during the research and writing of this research paper. The library's extensive online resources, databases and support services significantly enhanced the quality and depth of the information presented in this work.

Authors' contributions

All authors made equal contributions to the conception and design, acquisition of data or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content and agreed to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflicts of interest.

Ethical issues: None

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Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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