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RESEARCH ARTICLE

Mycocidal effect of plant-based essential oil on rice brown spot / sesame leaf spot incited by *Bipolaris oryzae* (Breda de Haan) Shoemaker

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Abstract

The present research work was assumed to inspect the effect of essential oils used for biological management of sesame leaf spot / rice brown spot caused by *B. oryzae* (Breda de Haan) Shoemaker. The essential oils *viz*., cinnamon oil, clove oil, citronella oil, thyme oil and rosemary oil were examined in different concentrations against *B. oryzae* under *in vitro* conditions. The results revealed that, citronella oil was found to be more effective against brown spot pathogen and completely inhibited the mycelial growth even at 2 % concentration onwards. Whereas in field conditions, citronella oil @ 0.2 % showed minimum disease incidence which is statistically on par with the fungicide difenoconazole (0.2 %). GC - MS analysis was conducted to identify the chemical compounds present in citronella oil, revealing a total of 60 different compounds. Among them linalyl acetate, geraniol, eugenol and geranyl acetate were identified as antimicrobial secondary metabolites. FT-IR analysis exhibited a characteristic peak at 2924.40 cm^{-1}, corresponding to C-H stretching, typically observed within the 2850-2950 cm^{-1} range. This indicates the aliphatic nature of the hydrocarbons present in citronella oil. Based on the metabolomic analysis, the bioactive compounds and aliphatic hydrocarbons in citronella oil may contribute to suppressing rice brown spot or sesame leaf spot disease and enhancing biometric attributes.

Keywords: brown spot; citronella oil; essential oils; rice

Introduction

The main staple cereal food crop in Southern India is rice (*Oryza sativa* L.), belonging to the genus *Oryza* within the family Poaceae (Gramineae), with a chromosome number of 12 pairs. In the Republic of India, rice holds immense significance as a vital cereal crop, contributing substantially to national nutritional security. As one of the most important staple foods globally, rice plays a crucial role in sustaining India's food production (1). Indian farmers cultivate approximately 46 Mha of rice, with an average productivity of 4080 kg/ha and an annual production of 125 MMT. In Tamil Nadu, rice cultivation covered an area of 2.21 Mha, yielding 8.07 Mt with a productivity of 3998 kg/ha during 2022 - 2023 (2). Over the decades, paddy cultivation has evolved from traditional methods to advanced farming techniques to enhance productivity. However, despite technological advancements, the demand for rice continues to rise due to India's growing population. Alongside these changes, various challenges have emerged particularly biotic and abiotic stresses which significantly impact rice production in diverse agro-climatic conditions.

Among the major biotic stresses, plant diseases pose a significant threat, potentially reducing rice yields by 20 - 100 % depending on disease incidence and severity. Globally, rice crops are affected by approximately 70 different diseases caused by biotic, abiotic and mesobiotic agents. Among the fungal diseases in rice, the most destructive mycotic disease is brown spot or sesame leaf spot or *Helminthosporiose* or seedling blight caused by *Bipolaris oryzae*. This disease is widely distributed across rice-growing regions worldwide, leading to yield losses of up to 90 % and significantly affecting grain

quality. In Tamil Nadu, brown spot disease remains a severe threat, causing substantial quantitative and qualitative losses in rice production. The disease becomes more severe under stress conditions, leading to seed and grain discoloration, reduced germination rates, weakened seedling vigor and ultimately, yield reduction (Fig. 1, 2) (3).

Commercial agriculture mostly relies on the use of synthetic pesticides to kill or reduce the pest, diseases or the inoculum like fungal spores, bacterial colonies and cell growth to protect agricultural and horticultural crops against them. Large-scale usages of agrochemicals resulted in fungicide resistance, insecticide resistance, herbicide resistance and have negative consequences on human health, environmental sustainability, soil microbiome, phytobiomes and animal welfare. By altering physiological and metabolic processes in crops, the pesticide compounds might have a negative impact on crop growth, photosynthetic pigments and reproductive organs such as flowers, seeds, etc. (4). Recently, plant-based essential oils have been introduced to manage the fungal, bacterial plant pathogens which have no environmental toxic effects on crop health. Essential oils have gained attention as eco-friendly alternatives to agrochemicals for the management of pathogens causing fungal and bacterial diseases. Essential oils are the mixtures of natural volatile aromatic secondary metabolic constituents, characterized by a strong odour and are formed by mainly monoterpenes, sesquiterpenes (5) and their oxygenated derivatives (alcohols, aldehydes, phenols, flavonoids and tannins). The essential oils have mycotoxic properties and they are also highly safer to the eco-system than contact and systemic fungicides. From a biological perspective, the bio-active essential oils offer a wealth of substitute and potentially more sustainable environmentally manner (green pesticides) alternative of agrochemicals. There are several chances to investigate the potential benefits of natural volatiles present in plants that have mycocidal and bactericidal properties for the management of crop diseases (6, 7). Current trends in plant disease management emphasize the use of plant-based natural products, particularly essential oils, which have the potential to inhibit fungal colonization in plants while promoting growth. These essential oils are highly eco-friendly and serve as effective components in integrated plant health management strategies. They are highly non-phytotoxic in environmental sustainability as well as at the consumer level. Antimycotic constituents of essential oils are effective against fungal pathogens in plants (8 - 11). Therefore, the present scientific work is articulated with the objectives of screening of essential oils against brown spot pathogen under *in vitro* conditions and the most effective plant-based essential oil for brown spot disease management.

Materials and Methods

The present *in vitro* research works were completed in the Research Laboratory, Agricultural faculty, Annamalai University, Tamil Nadu, India (11.3921° N, 79.7147° E). The highly brown spot-susceptible variety of rice i.e. BPT 5204 was cultivated for both pot and field experiments. Paddy seeds were sourced from the Government Seed Farm, Vandrayanpattu, Chidambaram, Cuddalore District, Tamil Nadu, India (11.4070° N, 79.6912° E). Difenoconazole 25 % EC (marketed as Score by Syngenta) was applied at a 0.2 % concentration as a standard fungicide for comparison against brown spot disease under environmental field conditions. The Percent Disease Index of brown spot (PDI) was evaluated using the 0 - 9 grade scale, as described in *Phytopathometry* manual (12). Brown spot PDI was calculated based on the standard formula (13).

$$\text{Percent sesame leaf spot disease index} = \frac{\text{Sum of sesame leaf spot disease individual ratings}}{\text{Total number of rice leaves} \times \text{Maximum sesame leaf spot disease grade}} \times 100$$

Sesame leaf spot pathogenic isolate of rice, *B. oryzae* (BO_3), collected from Srimushnam village, Cuddalore District, Tamil Nadu, India (11.4017° N, 79.4061° E), was identified as the most virulent strain, recording the highest PDI in the pathogenicity test. Morphological, pathological and molecular analyses confirmed the identity of the pathogen and its partial sequence data was submitted to the NCBI database (NCBI Accession No. PQ146644). Consequently, this highly virulent isolate (BO_3) (Fig. 3) was selected for further *in vitro* and *in vivo* studies.

Fig. 1. Rice brown spot.

Fig. 2. Grain discolouration.

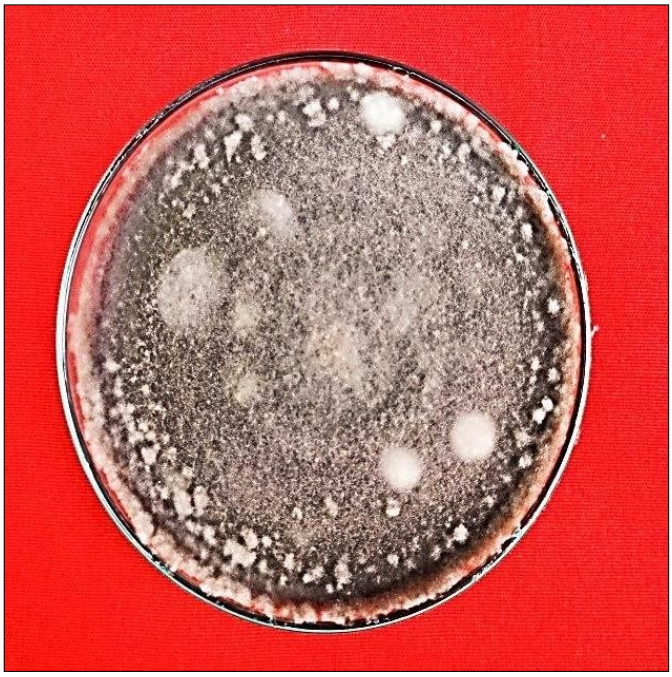

Fig. 3. Axenic culture of *B. oryzae* (Bo_3).

Collection of essential oils

The essential oils viz., cinnamon oil, clove oil, citronella oil, thyme oil and rosemary oil were purchased from the manufacturer of plant oils, Tegraj & Co., Coimbatore, Tamil Nadu and confirmed that these oils were extracted by hydrodistillation process. The efficacy of various plant oils listed in Table 1 were tested against *B. oryzae*.

Bio-efficacy of essential oils against *B. oryzae* under *in vitro* conditions

Poisoned food technique - solid bioassay

Mycocidal activities of different plant-based oils were assessed against brown spot pathogen by hyphal growth assay. The plant-based low concentration of essential oils was examined in the concentration ranges from 1 to 5 % (v/v). A 50 mL of PDA (Potato Dextrose Agar) medium with sterilized condition was taken in 100 mL conical flask. One to five percent concentration of plant-based essential oils added individually in conical flasks and the solvent was added. After mixing, the plant-based essential oils were dispensed to Petri dishes with sterile conditions. Petri dishes were then rotated gently in clockwise and anticlockwise to get the essential oil even dispersed. Petri dishes treated with 0.2 % difenoconazole were included as standard check and plates without oil served as control. A 0.8 cm hyphal disc of the brown spot pathogenic fungi was taken and placed in plant-based essential oil containing PDA medium. All the petri dishes were incubated at room temperature conditions (28 ± 2 °C) for nearly one week. Three replication treatments maintained for each treatment according to CRD (Completely Randomized Design). After seven days, the colony diameter (mm) of the test pathogen was measured and the Minimum Inhibitory Concentration (MIC) was determined. Colony diameter of the test fungus in the treatment in comparison with that of check gave growth inhibition by the following formula

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition, C = Radial growth of pathogenic fungus in check (mm), T = Radial growth in treated plates (mm).

Liquid bioassay

Essential oils, which showed effectiveness in solid assay at the lowest concentration were further tested in liquid bioassay for confirmation. The quantity of 250 mL conical flasks containing 100 mL PDB (Potato Dextrose Broth) were autoclaved separately for 1200 sec at 249.8 °F. The test oils (essential oils) at one to five per cent (v/v) were prepared and added to the conical flasks separately. 2-propanone or dimethyl ketone (acetone) at 0.01 % was used as a solvent to disperse the oil. The contents were mixed thoroughly by placing the flasks on a shaker at room temperature conditions. Pathogenic inoculum of *B. oryzae* BO_3 (0.8 cm fungal hyphal discs) was aseptically introduced into the flasks. Difenoconazole (0.2 %) was maintained as standard check/ comparison fungicide along with control plates. Three replicates of each treatment and

Table 1. Bio-efficacy of essential oils were tested against *Bipolaris oryzae*

Essential oil	Scientific name	Chemical formula	Family
Citronella oil	*Cymbopogon nardus*	$C_{10}H_{18}O$	*Graminae*
Thyme oil	*Thymus vulgaris*	$C_{10}H_{14}O$	*Lamiaceae*
Clove oil	*Syzygium aromaticum*	$C_{10}H_{12}O_2$	*Myrtaceae*
Cinnamon oil	*Cinnamon zeylanicum*	C_9H_8O	*Lauraceae*
Rosemary oil	*Rosmarinus officinalis*	Many chemical compounds including camphor, bornyl acetate, limonene and linalool	*Lamiaceae*

suitable control were arranged according to CRD. After a week, the pathogenic hyphae were collected on Whatman No. 1 filter paper with pre-weighed conditions. The mycelial mat placed in a hot air oven at 140 °F for one and a half days and weighed. The per cent mycelial inhibition was calculated using the method described by Baratta et al. (14).

$$\text{Per cent fungal inhibition (MIC)} = \frac{(C - I) - (T - I)}{(C - I)} \times 100$$

Where, T = Mean value of *B. oryzae* hyphal weight of individual treatments; C = *B. oryzae* hyphal weight in treatments without essential oils; I = Mean value of *B. oryzae* hyphal weight of initial inoculums.

Pot culture experiment

To examine the effectiveness of plant-based essential oils in reducing disease incidence in rice, pot culture experiments were conducted. The most susceptible variety of brown spot, BPT 5204 was cultivated in rectangular cement pots measuring 0.3 x 0.45 m (August - November' 2024). The rice crops were inoculated with *B. oryzae* (BO_3) by spraying conidial suspensions containing 500000 spores/ 10 mL in the evening (15). To create a suitable environment for successful colonization by *B. oryzae*, the rice plants were cultivated in a poly house and water was sprinkled regularly to maintain optimal relative humidity. The pot culture studies followed with statistically CRD with replications of thrice for all treatments, including a fungicide for comparison and a control treatment. The standard agronomical rice cultivation practices as given by the Tamil Nadu State Agricultural Department and steps to successful rice production manual were followed (16).

The most effective essential oil citronella oil (CO) spray at different concentrations @ 0.05, 0.1, 0.15 and 0.2 % individually at brown spot disease development stage and again once at fifteen days interval was given. In all the treatments, observations on the brown spot per cent disease index, grain and straw yield during the harvesting period were recorded. To determine the brown spot per cent disease index, three rice crop hills from each treatment were randomly collected and recorded the incidence. After the harvest, rice grains were winnowed and dried. The weight of the dried rice grains was expressed in g/pot. Similarly, the paddy straw was dried separately for forty-eight hr after the grains were thrashed. Once dried, the weight of the paddy straw was observed and expressed in g/pot.

$$\text{Per cent grain discolouration in rice} = \frac{\text{Number of grains discoloured / panicle}}{\text{Total number of grains / panicles}} \times 100$$

Metabolomic studies by GC-MS (Gas Chromatography-Mass Spectrometry) analysis of anti - fungal compounds present in essential oils

GC-MS qualitative analysis of secondary metabolites was performed in Shimadzu 2010 model. The instrument comprises the auto sampling unit AOC-20i. The instrument generated the GC graph and the metabolites were investigated by tagged mass spectrophotometer. GC-MS functioned with Rtx-5ms (column thickness; 0.0005 mm, column length; 300 cm and column diameter is 0.032cm,). The carrier gas, Helium (MW: 4.002 g/mol) used as a mobile phase with the injecting temperature of 482 °F and the ion-source temperature fixed at 392 °F used to facilitate ionization of the essential oil. The incubated temperature was set from 122 °F (isothermal conditions for 120 sec) to 536 °F with an increase rate of 46.4 °F/60 sec and ending with a 1200 sec isothermal at 536 °F. Spectrums were taken with scan period of 0.005 min. Total time taken for preparing the complete chromatograph was 2420 sec. Turbo Mass Ver-5.2.0 was used for conversion of analytical data to digital data. These GC-MS scientific analyses were worked out in the Biofocus Scientific Solutions Private Limited, Kumbakonam, Tamil Nadu, India.

Identification of bioactive constituents

The data collected from GC-MS secondary metabolite analysis of plant-based essential oil was interpreted with already available database of NIST (National Institute Standard and Technology). The separated secondary metabolites structure, molecular weight and chemical name were developed accordingly.

Identifying major chemical functional groups through FT-IR (Fourier Transform Infrared Spectroscopy) analysis

The essential oil citronella oil (CO) was analyzed and the chemical compounds were identified through the FT-IR analysis. Two mL of the testing sample were mixed with 200 mg Potassium bromide (FT-IR grade - KBr) and pressed into a pellet. FT-IR bonding characteristics of the thick films were analyzed by using Shimadzu IR Affinity-1 Fourier transformed infrared spectrometer (FT-IR). The FT-IR was recorded from 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1}. These FT-IR analyses were carried out at the Biofocus Scientific Solutions Private Limited, Kumbakonam, Tamil Nadu, India.

Statistical analysis

The mean values of the repeated treatment observations were used to calculate the experimental data for various growth and yield parameters. The experimental data were statistically analyzed using Indian Council of Agricultural Research – Central Coastal Agricultural Research Institute, Goa developed the Wasp version 2.0 (17). Significant levels ($P < 0.05$) and mean values were compared by DMRT (Duncan's Multiple Range Test).

Results and Discussion

Evaluation of plant-based essential oils in B. oryzae (BO_3) under *in vitro* conditions

Solid bioassay

Various plant oils tested against the pathogen *B. oryzae* (BO_3) by solid bioassay (Fig. 4) revealed that out of the five oils, citronella oil had very strong antifungal action against *B. oryzae* (BO_3) at concentrations of 2 %, 3 %, 4 % and 5 %, by totally suppressing the pathogen's growth (Fig. 5). This was followed by cinnamon and clove oils which showed maximum inhibition of hyphal growth (0 and 10 mm). However, the rosemary oil did not show any antifungal activity at all the five concentrations tested. The control plate recorded the maximum mycelial growth of 90 mm.

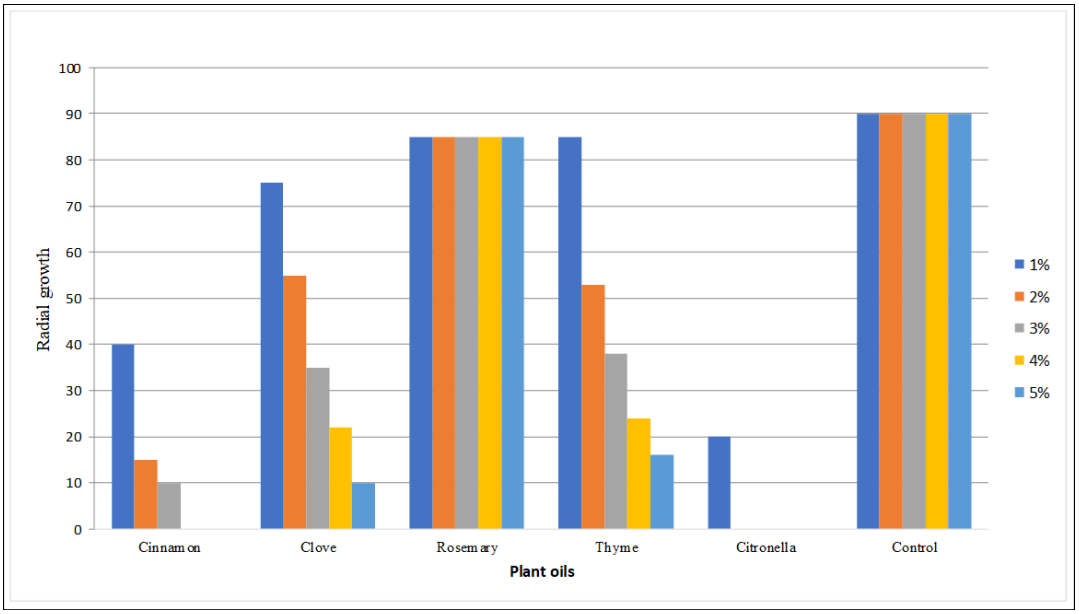

Fig. 4. Bio-efficacy of essential oils against *B.oryzae* (BO_3) (Radial growth).

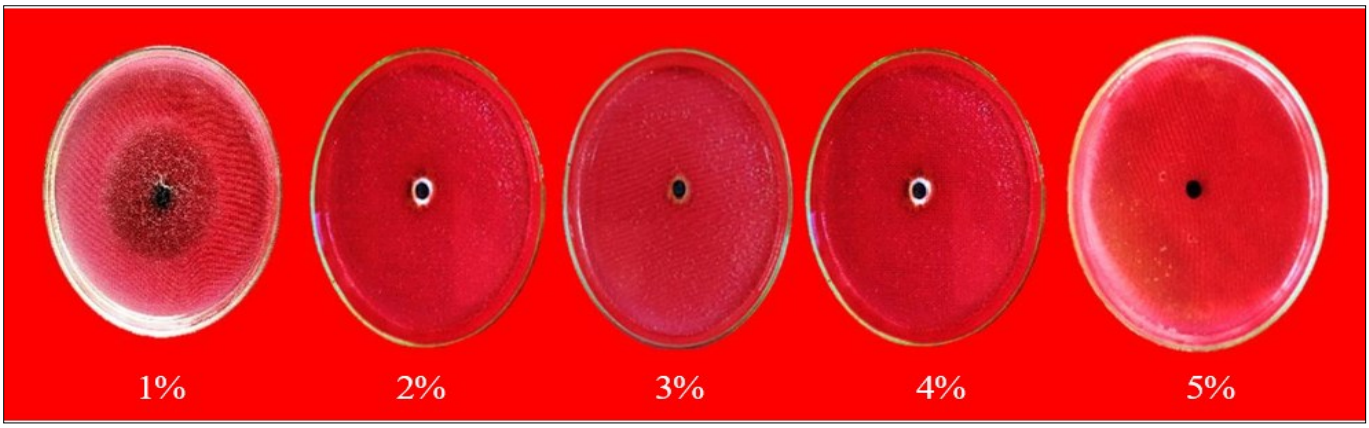

Fig. 5. Effect of citronella oil on mycelial growth of *B. oryzae* (Bo_3).

Citronella oil exhibited the maximum antimycotic effect compared to other Padang cinnamon oils, clove oils, Ceylon cinnamon oils and wild ginger oils; moreover, it was highly effective in suppressing the mycelial growth of *Sclerotium rolfsii*, *Pestalotia* sp. and *Fusarium* wilt pathogens under *in vitro* conditions (18). The presence of secondary metabolites such as eugenol and citronella which belong to the terpenoid group had proved as antimycotic compounds. The above-mentioned chemical groups arrest the fungal metabolism that leads to the disruption of mycelial growth. Plant-based cinnamon oil had the highest mycocidal activity against *Aspergillus flavus* with maximum reduction of aflatoxins B_1 and B_2 along with conidial pigmentation (19). It has also been confirmed that clove oil was highly inhibitory to *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp. and *Penicillium* sp. under *in vitro* (20).

Liquid bioassay

The results of the evaluation (Fig. 6) indicated that citronella oil completely inhibited the mycelial growth of *B. oryzae* (BO_3) at the concentration of 2 % (v/v) and the other concentrations tested recorded significant reduction in the growth of mycelium when compared to other plant oils. However, the other oils required higher concentrations even up to 5 % for recording the same effect. The rosemary oil did not show any inhibition as recorded in solid bioassay. Hence, the results indicated that citronella oil was more effective than other plant -based essential oils in liquid bioassay.

Jain and Agrawal (21) mentioned that the inhibition of fungal growth and conidial germination of plant pathogenic fungi by several essential oils. In general, the major components present in essential oils such as phenolic compound eugenol reflect their biophysical and biological characteristics. The phenolic compound denatures the fungal cell proteins and altering the fungal cell membrane leads to the fungal lysis. This brings about an alteration of the fungal growth environment, so the the fungal cell loses its metabolism (22). In addition to the above, the essential oils block the biochemical reactions and damage the pathways related to the hyphal growth as well as sexual and asexual reproduction of fungal spores (23). Many scientific reports have shown the antimycotic activity of essential oils through their inhibitory effects on mycelial dry weight of various plant pathogenic fungi (22, 24).

Efficacy of foliar spray of citronella oil for the management of rice brown spot

Among the different concentrations of citronella oil evaluated, 0.2 % concentration showed minimum disease incidence of 24.68 % which was on par with that of the test fungicide difenoconazole at 0.2 % concentration (26.80 %). The pathogen (BO_3) inoculated control exhibited the maximum disease incidence of 74.21 % (Fig. 7). Besides, the Citronella oil at 0.2 % concentration recorded a maximum of biometric attributes including panicle length (17.74), number of tillers (13.02) and productive tillers per clump (12.74) and height of the crop

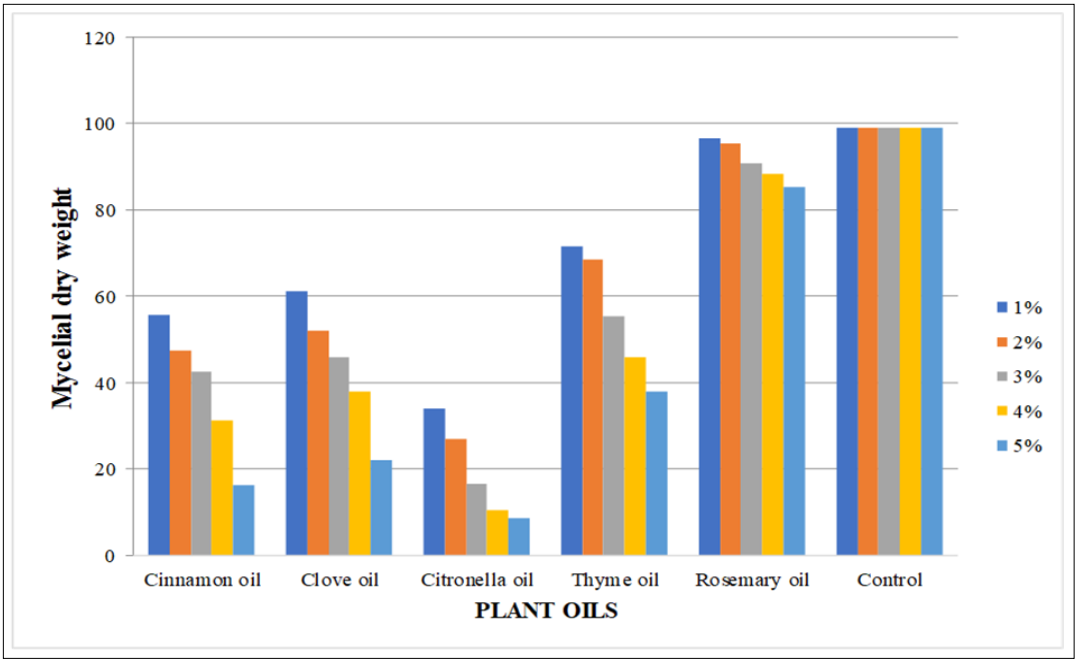

Fig. 6. Bio-efficacy of essential oils against *B. oryzae* (BO_3) (Mycelial dry weight).

(83.41 cm) (Table 2). Similarly, increase in yield attributes like percentage of filled grains (83.10 %), straw yield (83.08 g/pot), grain yield (31.89 g/pot) and lesser number of grains are discoloured (6.41 %) were also recorded at the abovesaid concentration (Table 3). This was followed by foliar application of citronella oil @ 0.15 % and 0.1 % concentration. The pathogen inoculated control treatment (BO_3) recorded the minimum yield attributes such as percentage of filled grains (59.40 %), grain yield (18.08 g/pot), straw yield (52.94 g/pot) and maximum percentage of grain discolouration (26.54 %).

Foliar application of essential oils as a bio-protectant and bio-stimulant is gaining attention due to the negative impact of agrochemicals on the ecosystem. Numerous scientific studies have demonstrated the effectiveness of essential oils in suppressing fungal diseases in crop plants while also promoting plant growth. This occurs through direct or indirect mechanisms, including the inhibition of pathogenic mycelial growth and reproduction (25). The mycocidal activity of essential oils is primarily attributed to their high eugenol concentration. These bioactive compounds penetrate fungal cell walls and membranes, disrupting mitochondrial function (26). Additionally, essential oils enhance peroxidase activity in plants, leading to the accumulation of phenolic derivatives and hydrogen peroxide, which collectively contribute to reducing fungal diseases in crops. Furthermore, foliar application of essential oils has been reported to enhance tuber yield, increase the number of main stems, promote stolon formation and improve the nutritional composition of potatoes (27).

Metabolomic studies

GC-MS analysis of citronella oil

Based on laboratory and pot culture bio-efficacy studies, citronella oil was further tested to determine the nature of volatile compounds present in it. The results revealed the presence of several compounds containing antifungal properties (Fig. 8) *viz*., 2,4-Octadiene (C_8H_{14}), linalyl acetate, geraniol, acetic acid, 2-phenylethyl ester; tetradecane, dodecane, 4, 6-dimethyl- and Citronellal. The Peak, retention time, area %, chemical name, molecular formula and activity were given in the Table 4. The identification of metabolites was confirmed through NIST Library edition, 2011.

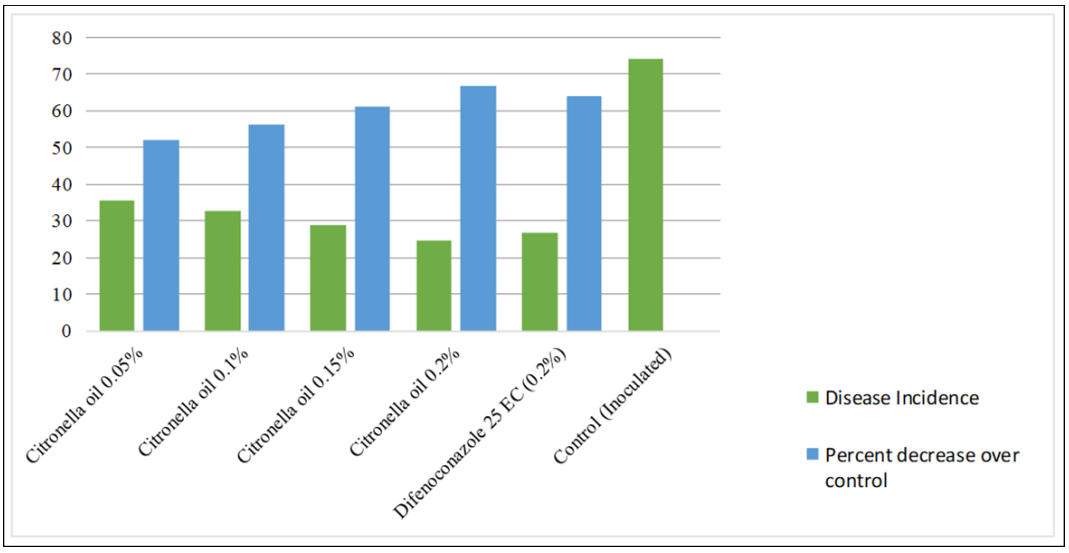

Fig. 7. Efficacy of foliar spray of citronella oil for the management of rice brown spot (Pot culture experiment).

Table 2. Efficacy of foliar application of citronella oil (CO) and *B. oryzae* (BO_3) inoculation on biometric attributes of Rice var. BPT 5204 (August - November 2024)

Treatments	Panicle length (cm) *	No. of productive tillers/ clump*	Plant height (cm) *	No. of tillers/ clump*
T_1 - Citronella oil 0.05 %	15.19^{e}	11.34^{d}	76.40^{e}	12.16^{d}
T_2 - Citronella oil 0.1 %	18.96^{c}	11.91^{c}	78.42^{d}	12.51^{c}
T_3 -Citronella oil 0.15 %	17.01^{b}	12.30^{b}	80.64^{b}	12.84^{b}
T_4 -Citronella oil 0.2 %	17.74^{a}	12.74^{a}	83.41^{a}	13.02^{a}
T_5 -Difenoconazole 25 EC (0.2 %)	15.94^{d}	10.74^{e}	79.62^{c}	11.21^{e}
T_6 -Inoculated control	12.36^{g}	8.59^{g}	68.02^{g}	9.24^{g}
T_7-Healthy control	13.84^{f}	10.19^{f}	71.78^{f}	10.36^{f}

Table 3. Efficacy of foliar application of citronella oil (CO) and *B. oryzae* (BO_3) inoculation on yield attributes of rice var. BPT 5204 (Pot culture) (August - November 2024)

Treatments	Grain yield (g/pot)*	Filled grain (%)*	Grain discolouration (%)	Straw yield (g/pot)*
T_1 - Citronella oil 0.05 %	24.81^{e}	80.32^{e}	10.29^{e}	75.16^{e}
T_2 - Citronella oil 0.1 %	27.10^{c}	81.56^{c}	9.21^{c}	78.73^{c}
T_3 -Citronella oil 0.15 %	28.35^{b}	81.94^{b}	7.60^{b}	80.64^{b}
T_4 -Citronella oil 0.2 %	31.89^{a}	83.10^{a}	6.41^{a}	83.08^{a}
T_5 -Difenoconazole 25 EC (0.2 %)	25.94^{d}	76.50^{d}	9.59^{d}	76.08^{d}
T_6 -Inoculated control	18.08^{g}	59.40^{g}	26.54^{g}	52.94^{g}
T_7-Healthy control	21.93^{f}	67.10^{f}	16.57^{f}	60.14^{f}

Secondary metabolites such as citronellal, citronella were identified through GC-MS study on citronella oil and the citronella essential oil is highly effective against fungus Fusarium under *in vitro* conditions (28). Similarly, the major constituents in citronella oil are citronellal, geraniol and eugenol which is mainly responsible for pathogenic inhibition of *F. oxysporum f. sp. Lycopersici* (29). Eugenol act as an elicitor molecule and induced H_2O_2 accumulation in crop plants. Foliar application of eugenol increases the plant immunity activities through improving the enzymes of phenylalanine ammonia lyase (PAL) and peroxidase (POD) while compared to control treatments. The foliar application of eugenol also increases the amount of salicylic acid (SA) and exhibition of Pathogenesis Related Protein (PR-1 proteins) in tomato (30).

FT-IR analysis of citronella oil

FT-IR spectroscopy was employed to identify the major chemical functional groups based on peak values and wavelengths in the infrared (IR) region. The FT-IR results, along with the corresponding wavelengths and chemical functional groups, are presented in Table 5 and Fig. 9. The analysis of citronella oil extract revealed the presence of hydrocarbons, aldehydes, alcohols and alkenes. A characteristic peak at 2924.40 cm^{-1}, corresponding to C-H stretching was observed. This peak typically appears within the 2850 - 2950 cm^{-1} range, indicating the presence of aliphatic hydrocarbons in citronella oil. For analyzing molecular variations in the major bioactive chemical groups of rice grains affected by *Ustilaginoidea virens*; a pathogen responsible for false smut or green smut disease, FT-IR spectroscopy was utilized and specific FT-IR bands corresponded to primary as well as secondary metabolites were found out. (46).

Wavelengths between 1200 and 1000 cm^{-1} highlighted diverse chemical modes, including carbohydrates and polysaccharides. In the present FT-IR analysis, the strongest absorption bands were observed between 1450 and 1000 cm^{-1}, corresponding to starch and lignin. In addition to that, the bioactive molecules of citronella oil exhibited a characteristic peak within a similar wavelength range of phenolic groups and carbohydrates (47). These major chemical groups present in citronella oil may contribute to the suppression of brown spot disease in rice.

Conclusion

In these current research evaluations, foliar application of citronella oil @ 0.2 % sprayed at brown spot disease imitation stage and repetitive another one time at flowering stage of rice crop reduces the disease incidence and increasing the plant growth promotional characters. The bio-active components in citronella oil identified through GC-MS analysis may be applied as an antimycotic action against brown spot and improving yield attributes in rice. The spectrum of FT-IR mentioned that major chemical functional groups of citronella oil are hydrocarbons, aldehyde, alcohol and alkene chemicals which might be the reduction of brown spot incidence.

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Authors' contributions

PK, VJ, ND, CK planned the work and coordinated the research activities. JS and NR contributed to the supervision of research work. The manuscript was completely revised and finalized by MT, JS and NR. The experimental data obtained under *in vitro* and *in vivo* conditions were statistically analyzed by SRRR. SS and RSR helped to shape the final research manuscript. All authors read and approved the final manuscript.

Table 4. Identification of antimicrobial components of citronella oil in GC-MS

Peak	R. Time	Area%	Chemical name	Chemical formula	Activity	Reference
1	5.163	0.05	2,4-Octadiene	C8H14	Antimicrobial	[31]
3	5.378	0.18	Linalyl acetate	C12H20O2	Antimicrobial	[32]
4	5.433	0.11	Geraniol	C10H18O	Antifungal	[33]
5	5.551	0.06	Acetic acid, 2-phenylethyl ester	C10H12O2	Antifungal	[34]
6	5.869	0.01	Tetradecane	C14H30	Antifungal	[35]
9	6.202	0.01	Dodecane, 4,6-dimethyl-	C14H30	Antimicrobial and Antioxidant	[36]
11	6.500	0.01	Sulfurous acid, dodecyl 2-ethylhexyl ester	C20H42O3S	Antifungal	[35]
16	6.890	0.09	Eugenol	C10H12O2	Antifungal and Antibacterial	[37]
17	6.983	0.03	Geranyl acetate	C12H20O2	Antifungal	[38]
21	7.402	0.00	Tetradecane, 4-methyl-	C15H32	Antimicrobial	[39]
22	7.467	0.00	Octacosane	C28H58	Antifungal	[40]
25	7.771	0.19	Trans(β)-caryophyllene	C15H24	Antifungal	[41]
47	13.883	4.47	Tetradecane (CAS) n-Tetradecane	C14H30	Antifungal	[42]
48	14.431	6.52	Hexadecane	C16H34	Antifungal	[35]
50	15.409	7.96	Iron, tricarbonyl[N-(phenyl-2 pyridyl methylene) benzenamine -N,N']-	C21H14FeN2O3	Antifungal	[43]
54	16.313	12.01	Cetane (n-Hexadecane)	$CH_3(CH_2)_{14}CH_3$	Antifungal	[44]
55	17.908	18.46	Citronellal	C10H18O	Antimicrobial	[45]

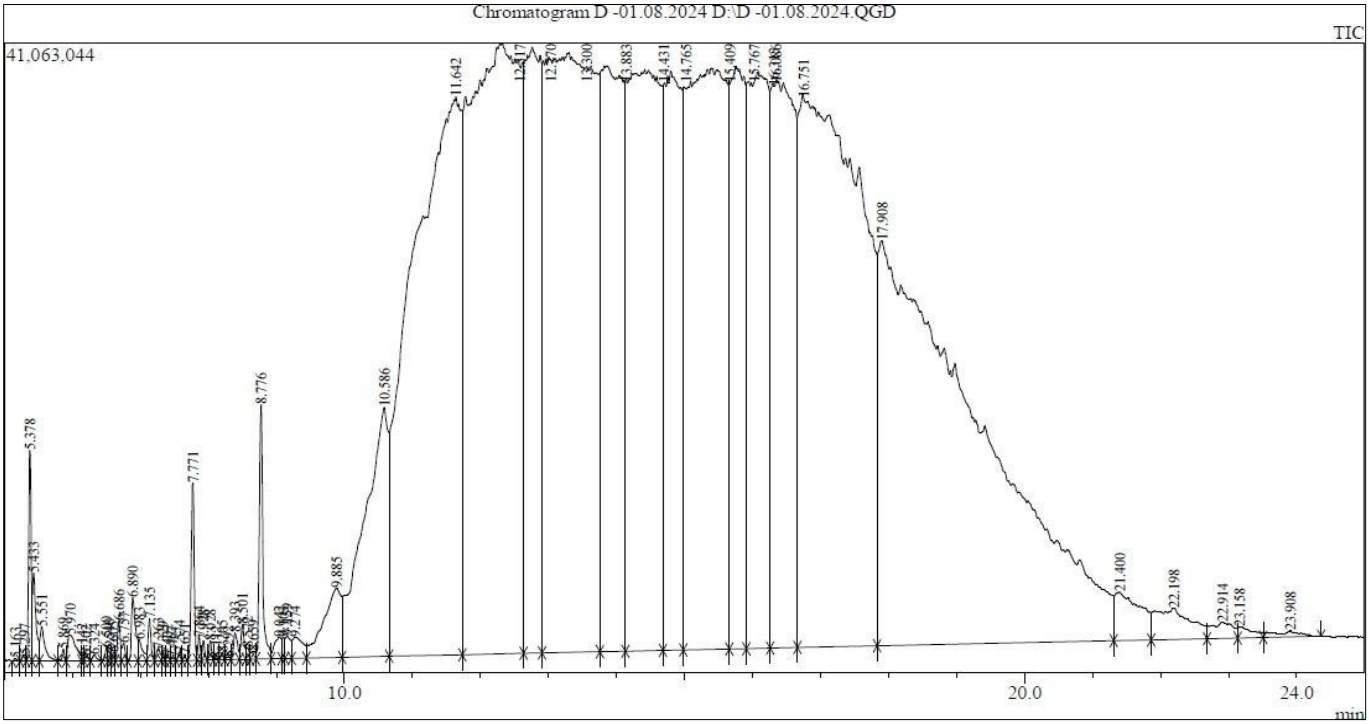

Fig. 8. Gas chromatogram of antimicrobial compounds identified from citronella oil through GC-MS analysis.

Table 5. FT-IR analysis of citronella oil

Peak number	X (cm^{-1})	Major functional groups of chemicals
1	2924.40	Stretching C–H, Aliphatics
2	2855.43	C-H stretching (Aldehydes)
3	2725.66	C-H Stretching (Aldehydes)
4	1749.08	C-O stretching
5	1460.28	C-C stretching
6	1376.71	C-O Stretching
7	1161.01	C-H bending, methyl (CH_3)
8	723.46	C-H Stretching

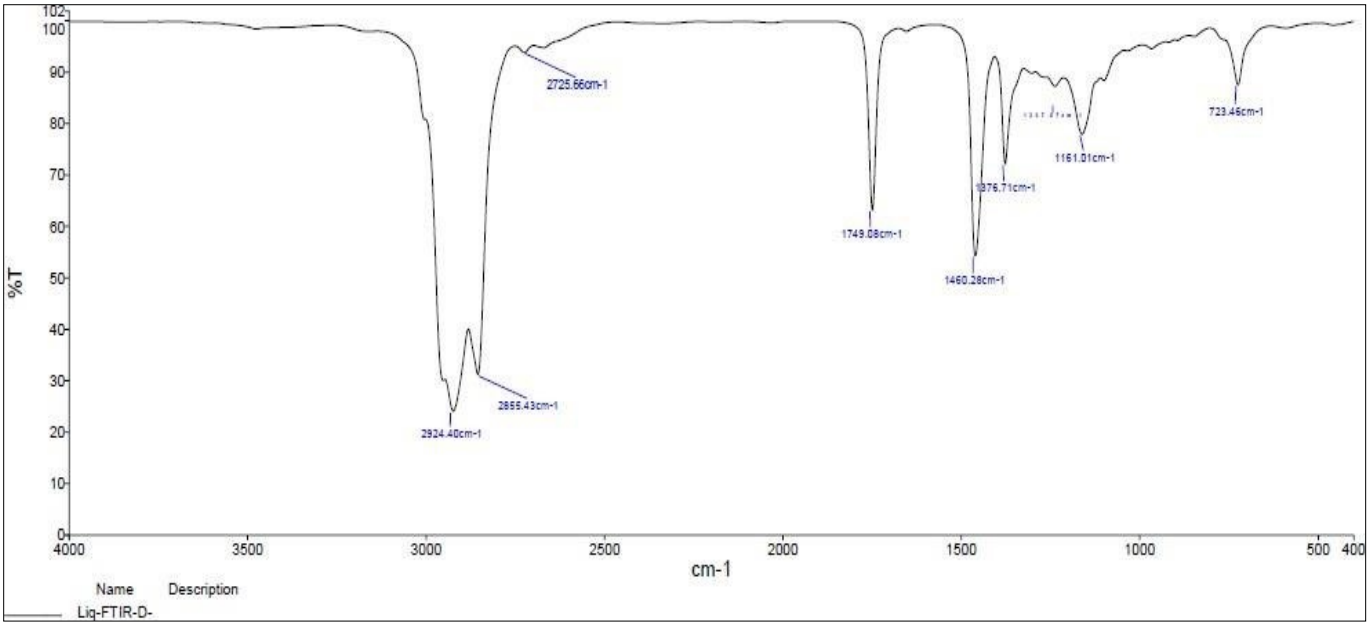

Fig. 9. FT-IR spectrum of citronella oil.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or non-financial/ personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues: None

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