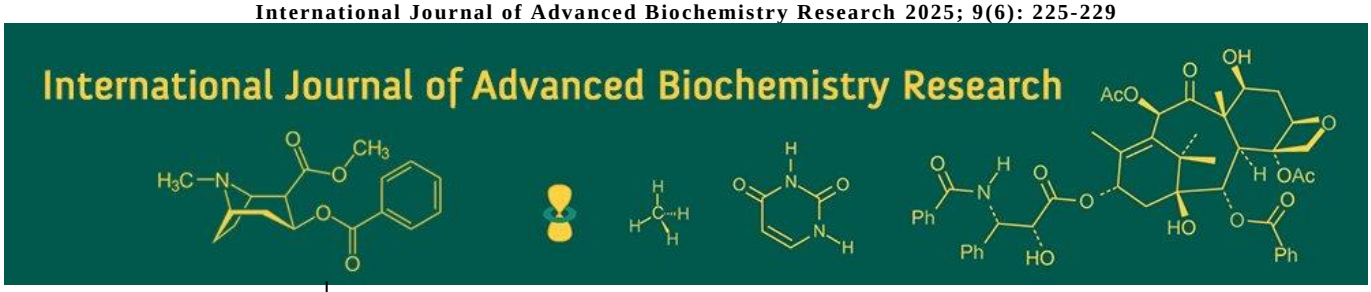

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Evaluation of antifungal efficacy of selected essential oils against *Botrytis cinerea* causing grey mould in chickpea

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Abstract
The current study investigates the antifungal properties of several plant-derived essential oils against *Botrytis cinerea*, the causative agent of grey mould in chickpea. Using a paper disc agar diffusion method, the effectiveness of various oils—including peppermint, citronella, palmarosa, geranium, menthol, neem, and jatropha—was evaluated *in vitro*. Among these, peppermint oil exhibited the strongest inhibitory effect, producing a 17 mm inhibition zone at an 8 µl concentration and 15 mm at 6 µl. In contrast, jatropha and neem oils demonstrated minimal activity. Field trials, involving three foliar sprays applied at 10-day intervals, confirmed the laboratory results, with peppermint and citronella oils showing significant disease suppression and yield enhancement. Specifically, peppermint oil reduced disease severity by 56.16% and increased grain yield by 50.14%. These findings highlight the potential of certain essential oils, particularly peppermint, as sustainable and eco-friendly alternatives to conventional chemical fungicides for managing grey mould in chickpea cultivation.

Keywords: Essential oils, *Botrytis cinerea*, peppermint, antifungal, chickpea, grey mould

Introduction
Botrytis cinerea, first identified by Micheli in 1729 (Hennebert G L., 1973) [12], is now widely recognized as a destructive fungal pathogen responsible for significant crop losses. Within this genus, *B. cinerea*—commonly referred to as Botrytis grey mould (BGM)—is notably problematic in chickpea (*Cicer arietinum* L.), particularly in regions where cool, humid, and cloudy conditions prevail during the growing season. The pathogen's broad host range and its capacity to survive on plant debris and infected seeds complicate effective disease management.

Chemical fungicides have long been the cornerstone of grey mould control; however, issues such as pathogen resistance and environmental concerns have reduced their reliability. Over the past decade, there has been growing interest in identifying sustainable alternatives or supplements to traditional chemical control methods. Natural plant extracts and essential oils have emerged as promising options due to their antifungal properties and biodegradability.

For instance, compounds derived from *Allium*, *Capsicum*, and various aromatic plants have been used to suppress *B. cinerea*-induced diseases in multiple crops (Wilson *et al.*, 1997; Sesan, 2003) [29, 25]. However, managing this pathogen remains a challenge due to its genetic variability and high adaptability. The emergence of fungicide-resistant strains has limited the effectiveness of commonly used chemical groups, including benzimidazoles and dicarboximides (Brent & Hollomon, 1998) [6].

Essential oils, known for their diverse bioactive constituents, offer an eco-friendly alternative. These oils can disrupt fungal growth and toxin production, and their vapor-phase activity further enhances their appeal for use in both pre-and post-harvest disease management. Given the increasing resistance of *B. cinerea* to synthetic fungicides, evaluating essential oils as natural antifungal agents is both timely and necessary.

2.1 Selection of Test Oils
Seven oils—six essential oils and one non-essential oil—were assessed for antifungal activity. These included:

- Palmarosa (*Cymbopogon martini*)

- Geranium (*Pelargonium graveolens*)
- Menthol (*Mentha arvensis*)
- Citronella (*Cinnamomum aromaticum*)
- Neem (*Azadirachta indica*)
- Peppermint (*Mentha piperita*)
- Jatropha (*Jatropha podagrica*)

Each oil was selected based on its known phytochemical profile and reported antimicrobial potential.

2.2 Media Preparation
A double-strength Potato Dextrose Agar (PDA) medium was prepared using:
- 400 g peeled, sliced potato
- 40 g dextrose
- 40 g agar-agar
- 1 L distilled water

The mixture was autoclaved at 121.6 °C for 20 minutes under 15 psi pressure to ensure sterility.

2.3 *In vitro* Antifungal Assay
The antifungal effect of each oil was evaluated using a paper disc agar diffusion method:
- Sterile 5 mm filter paper discs were placed in the center of PDA plates.
- Each disc was loaded with 4 µl, 6 µl, or 8 µl of the respective oil.
- Control discs received sterile distilled water.
- After a 30-minute absorption period, the plates were overlaid with a 10 ml fungal spore suspension.
- Plates were sealed with parafilm and incubated.
- Growth inhibition was measured as the diameter (mm) of the clear zone around each disc.
- Percent inhibition was calculated using McKinney's formula:

Inhibition (I) = (C−T C)×100

Where:
- C = colony diameter in control
- T = colony diameter in treatment

2.4 Field Trials
Field experiments were conducted at the Crop Research Centre, G.B. Pant University of Agriculture and Technology, Pantnagar, India, during the 2007-08 and 2008-09 crop seasons. Trials were arranged in a Randomized Block Design (RBD) with three replications, using the susceptible chickpea variety H-208.
Each essential oil was used both as:
- **Seed treatment:** 50 ml/kg of seed
- **Foliar spray:** 2 ml/L, applied thrice at 10-day intervals

2.5 Plot Preparation and Crop Management
- Soil type: Silty clay loam
- Field preparation: One ploughing, followed by 3-4 harrowings and leveling
- Fertilizer application (broadcast before sowing):
  - Nitrogen: 20 kg/ha
  - Phosphorus: 60 kg/ha
  - Potash: 50 kg/ha
- Sowing dates: 19 November (2007) and 21 November (2008)
- Plot size: 3.0 × 1.5 m
- Sowing depth: 3-4 cm
- Weeding: Three manual weedings
- Irrigation: One light irrigation as needed

2.6 Disease and Yield Observations
- **Thousand grain weight:** Seeds from each plot were counted and weighed (1000 seeds).
- **Grain yield:** Post-harvest, the yield per plot was measured, air-dried, and extrapolated to kg/ha.
- **Disease severity:** Assessed using a 1-9 scale (Singh *et al.*, 1991), 10 days post-inoculation:

Score	Description
1	No symptoms
3	Minor lesions on <5% tender leaves
5	Soft rot on 11-25% upper leaves
7	Extensive fungal growth on 41-55% foliage
9	Severe infection on >70% plant area

Statistical analysis was performed using Completely Randomized Design (CRD) and RBD models. Differences among treatments were tested for significance at p = 0.05 using Critical Difference (CD) values.

3.1 *In vitro* Efficacy of Essential Oils on *Botrytis cinerea*
The antifungal potential of six essential oils and one non-essential oil was assessed using the paper disc diffusion method. The data showed that peppermint oil exhibited the strongest inhibitory effect on *B. cinerea* growth. At 8 µl, peppermint produced a 17 mm inhibition zone, followed by citronella (14 mm)**,** palmarosa (12 mm)**,** and geranium (11 mm). Lower levels of inhibition were recorded for menthol (9 mm), while neem and jatropha oils showed minimal inhibition, with only 6 mm and 0 mm zones, respectively, at 8 µl concentration.
The inhibitory effect was dose-dependent, with larger inhibition zones consistently observed at higher concentrations (8 µl vs. 6 µl). The control treatment (distilled water) showed no inhibition, confirming the activity was oil-specific.

3.2 Field Performance of Essential Oils: Disease Control and Yield Parameters
Field trials conducted over two consecutive seasons demonstrated a consistent pattern of disease suppression and yield enhancement, particularly with peppermint and citronella oils.

2007-08 Season
- **Disease severity** was lowest in peppermint-treated plots (score: 3.8), followed by citronella (4.1), palmarosa (4.1), and geranium (4.3).
- **Grain yield** peaked in peppermint plots (2068 kg/ha), with citronella (2038 kg/ha) and palmarosa (1968 kg/ha) also performing well.
- **Thousand grain weight** was highest for peppermint and citronella (115 g), while jatropha-treated plots showed the lowest (100 g).

2008-09 Season

- Peppermint again provided the most effective disease control (score: 3.2), followed by citronella (4.0), palmarosa (4.2), and geranium (4.2).
- The highest yield (2099 kg/ha) and grain weight (110 g) were recorded in peppermint-treated plots.
- Compared to the untreated control, peppermint oil resulted in a 56.16% reduction in disease severity and a 50.14% increase in yield**.**

Across both seasons, peppermint consistently outperformed other treatments in all measured parameters, confirming its superior antifungal effectiveness and potential agronomic benefits.

Table 1: Effect of Essential oils on Disease Severity, grain yield and 1000-grain weight during 2007-08 and 2008-09 crop seasons

Treatment	Dose (ml/kg seed)	Disease Severity (1-9 scale)		Disease decline (%)		Grain yield (kg/ha)		% increase over check		1000-grain weight (g)	
		2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09	2007-08	2008-09
Palmarosa	50	4.1	4.2	43.84	42.46	1968	1926	40.17	37.76	105	107
Geranium	50	4.3	4.2	42.46	42.46	1900	1858	35.33	32.90	105	103
Menthol	50	5.0	4.5	31.50	38.36	1761	1677	25.43	19.96	102	105
Citronella	50	4.1	4.0	43.84	45.20	2038	2049	45.16	46.56	115	106
Neem	50	4.9	6.2	32.88	15.06	1570	1483	11.82	6.08	102	103
Peppermint	50	3.8	3.2	47.94	56.16	2068	2099	47.29	50.14	115	110
Jatropha	50	5.0	4.4	31.51	39.73	1702	1717	21.22	22.81	100	98
Check	-	7.2	7.3	-	-	1404	1398	-	-	100	90
Sem (±)	-	0.125	0.0547	-	-	11.68	15.09	-	-	2.394	1.806
CD (P=0.05)	-	0.379	0.166	-	-	35.44	45.77	-	-	7.261	5.477

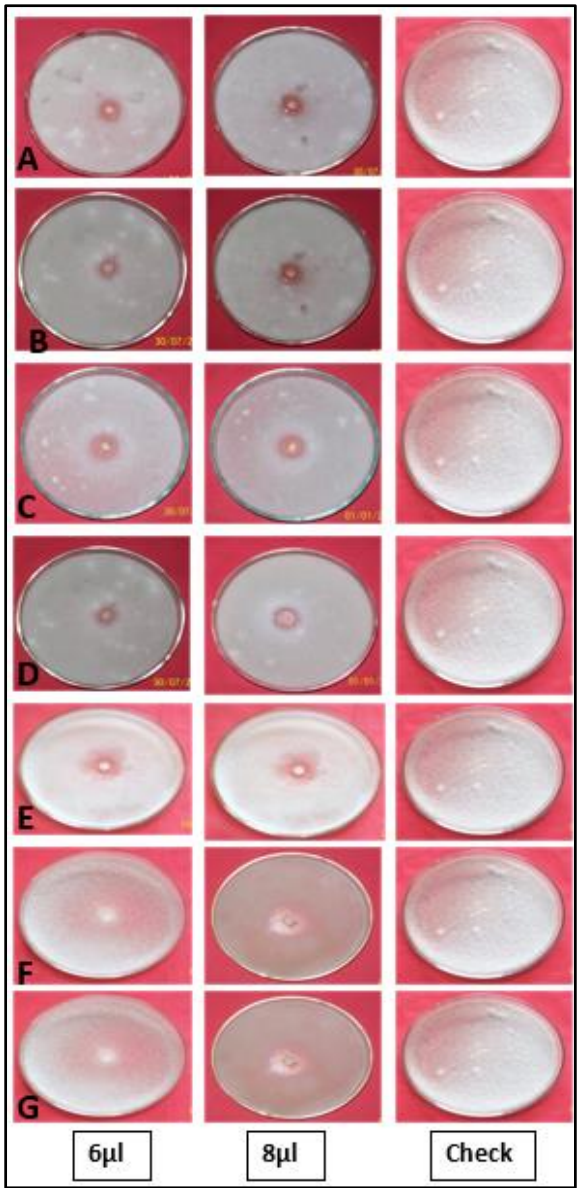

Fig 1: Antifungal activity of oils against *B. cinerea*
A. Citronella B. Geranium C. Peppermint D. Palmarosa E. Menthol F. Jatropha G. Neem oil

4. Discussion

The outcomes of both laboratory and field experiments strongly support the use of essential oils—particularly peppermint—as viable and environmentally friendly alternatives to chemical fungicides for managing grey mould in chickpea. The observed antifungal activity across multiple test oils confirms earlier findings that many essential oils possess broad-spectrum bioactivity against phytopathogens (Pavela & Benelli, 2016; Bajpai *et al.*, 2012) [18, 2].

Variability in efficacy among the oils may be attributed to differences in their chemical composition, which is influenced by factors such as plant species, geographical origin, seasonal variation, and extraction methods (Panizzi *et al.*, 1993) [19]. For example, peppermint oil consistently produced the largest inhibition zones and the most substantial disease control in the field, possibly due to higher concentrations of monoterpenes and phenolic compounds with known antifungal properties.

The field trials further reinforce these observations by showing statistically significant improvements in disease suppression, grain yield, and seed weight when essential oils were applied as seed treatments and foliar sprays. In particular, peppermint oil achieved over 50% yield increase compared to untreated controls, validating its agronomic potential.

These findings are aligned with earlier research suggesting that essential oils may trigger plant defense responses or act directly on pathogens, disrupting membrane integrity or interfering with fungal metabolism (Mihaliak *et al.*, 1991; Sharma *et al.*, 2020) [17, 26]. However, the exact mode of action remains insufficiently understood and warrants further investigation.

Despite the positive results, it is important to recognize that not all plant oils were equally effective. Oils like neem and jatropha demonstrated limited antifungal activity under the same conditions, suggesting that not all botanicals are suitable as fungicide alternatives for *B. cinerea*.

In conclusion, essential oils—especially peppermint—offer a sustainable, residue-free approach for the integrated management of grey mould. Further research is needed to

understand the molecular basis of their antifungal action and to standardize formulations for practical agricultural use.

5. Conclusion

This study clearly demonstrates the potential of essential oils, particularly peppermint, as effective antifungal agents against *Botrytis cinerea*, the causal organism of grey mould in chickpea. Both *in vitro* and field evaluations confirm that peppermint oil significantly inhibits fungal growth and improves crop performance, resulting in reduced disease severity and enhanced yield.

The findings suggest that essential oils can serve as promising alternatives to conventional chemical fungicides, offering an eco-friendly, sustainable, and residue-free strategy for managing plant diseases. Their integration into crop protection programs could reduce dependency on synthetic chemicals, minimize environmental impact, and support organic or low-input agricultural systems.

Future research should focus on optimizing application methods, understanding the biochemical pathways involved in antifungal action, and evaluating the long-term effects of essential oil treatments on crop health and soil ecology.

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