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In vitro screening of different essential oils against *Alternaria solani* causing early blight of potato

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ABSTRACT

Potato (*Solanum tuberosum* L.) is a significant cash and staple crop in Meghalaya, contributing to both local food security and agricultural economies. However, its cultivation faces persistent challenges from fungal diseases, among which early blight, caused primarily by *Alternaria solani* and occasionally by *Alternaria alternata*, stands out as a major constraint to production. This disease manifests as necrotic lesions with concentric rings on leaves, stems, and tubers, leading to premature defoliation, reduced photosynthetic capacity, and substantial yield losses. In the present study, an early blight pathogen, *Alternaria solani*, was isolated from infected potato plants collected from fields in the vicinity of the College of Post Graduate Studies in Agricultural Sciences (CPGS-AS), Umiam, Meghalaya. The isolation and morphological identification of this native putative pathogen were followed by *in vitro* screening against a range of essential oils to evaluate their antifungal efficacy. Among various essential oils evaluated *via* the poisoned food technique, clove oil demonstrated complete (100%) inhibition of the pathogen at all concentrations (500, 1000, and 1500 ppm), followed by ginger oil at higher concentrations. This effort aims to identify effective, eco-friendly alternatives to synthetic fungicides for the sustainable management of early blight, tailored to the specific pathogen prevalent in the local agroecological conditions of the region.

1. Introduction

The common potato (*Solanum tuberosum* L.) is a nutritionally complex staple food, offering far more than just carbohydrates. It is a significant source of essential micronutrients and high-quality protein, defying its common reputation as merely an energy-dense comfort food. For instance, a single medium-sized potato

provides a substantial amount of vitamin C (approximately 27 mg of ascorbic acid), meeting nearly half of the recommended daily intake, and stands as a leading dietary source of potassium, which is vital for maintaining cardiovascular health [2]. Additionally, the potato's skin contributes valuable dietary fiber, and the tuber itself is notable for its high-quality protein; its protein-to-calorie ratio is superior to that of maize and soybeans, featuring a well-balanced and complete amino acid profile [1].

Cultivated across diverse global agro-climatic zones, from temperate plains to tropical highlands at elevations up to 4,000 meters, the potato demonstrates remarkable adaptability. Its global cultivation covers approximately 17.3 million hectares, yielding an annual production that exceeds 374 million tonnes. The People's Republic of China ranks as the world's largest producer, with an output of over 94 million tonnes per year, followed by India, which produces approximately 54 million tonnes annually [5].

1.1 Importance of Early blight of potato

Early blight, caused by the fungal pathogen *Alternaria solani*, is a devastating foliar disease of potato (*Solanum tuberosum*) and poses a significant and recurring threat to potato cultivation in the state of Meghalaya. The disease is characterized by the appearance of small, dark, target-board-like concentric rings with a yellow halo on the leaves, which can rapidly lead to extensive defoliation. This loss of photosynthetic area directly impacts tuber bulking, resulting in substantial yield losses ranging from 30% to 50% annually, and can reach as high as 70-80% in severe, unmanaged epidemic conditions [3]. The importance of this disease is magnified by the socio-economic role of potato in Meghalaya. Potato is a major cash crop for the small and marginal farmers of the state, cultivated extensively in the East and West Khasi Hills, Ri-Bhoi, and East Jaintia Hills districts. It is a crucial component of local food security and a primary source of income, making any threat to its production a direct threat to rural livelihoods.

The agro-climatic conditions of Meghalaya, while ideal for high-quality potato production, are paradoxically also highly conducive for the proliferation of early blight. The disease becomes a major constraint primarily due to a combination of climatic and operational factors. The state's high annual rainfall, often exceeding 2500 mm, coupled with persistent mist, fog, and high relative humidity (frequently above 80%), creates a prolonged leaf wetness period that is perfect for spore germination and infection. Furthermore, the moderate temperatures prevalent in the Meghalayan plateau (15°C to 25°C) fall squarely within the optimal range for the growth and sporulation of *Alternaria solani*.

2. MATERIALS AND METHODS

2.1 Collection of disease sample

The isolation of *Alternaria solani* has been done with the collection of locally infected potato leaves exhibiting characteristic symptoms of early blight, specifically those with dark brown, concentric ring lesions near to College of Post Graduate Studies in Agricultural Sciences (CPGS-AS), Umiam, Meghalaya campus. These symptomatic leaf samples are first surface sterilized to eliminate epiphytic microorganisms and saprophytic fungi.

2.2 Isolation

A standard protocol involves rinsing the tissue in 70% ethanol for 30 seconds, followed by immersion in a 1-2% sodium hypochlorite solution for 1-2 minutes, and a final rinse in three changes of sterile distilled water to remove any residual sterilant [4]. The sterilized leaf pieces, typically cut from the margin between diseased and healthy tissue, are then aseptically placed onto Petri plates containing potato dextrose agar (PDA) medium, amended with antibiotics like streptomycin sulphate (50 mg/L) to suppress bacterial growth.

2.3 Purification

The plates are incubated at room temperature (approximately 25±2°C) under a 12-hour photoperiod using light to stimulate sporulation. Fast-growing greyish to black colonies with woolly aerial mycelium typically emerge within 3-5 days. However, these initial isolates was contaminated with other fungi or bacteria, necessitating a purification step. Purification is achieved by sub-culturing a single hyphal tip or a single conidium onto fresh PDA plates. The hyphal tip method involves transferring a tiny fragment of the growing edge of the colony under a stereomicroscope to a new plate [6].

2.4 Identification

The identification of the purified isolate has been done based on a combination of macroscopic cultural characteristics and meticulous microscopic morphology. Macroscopically, *Alternaria solani* colonies on PDA are typically greyish-olive to black with a suede-like to cottony texture and often produce a dark brown pigment that diffuses into the medium. The definitive identification, however, relies on microscopic examination. Slide cultures are prepared to observe the conidiophores and conidia without disturbing their natural arrangement. The conidia are large, obclavate to muriform (beak-like), with a long tapering beak that is often as long as or longer than the body of the conidium. The conidial body is dark brown, divided by both transverse and longitudinal septa, giving it a brick-walled appearance. The size, shape, and septation pattern, particularly the elongated filiform beak, was observed in the pathogen [6].

2.5 Screening of essential oils against the pathogen

In vitro efficacy of essential oils against *Alternaria solani* was performed by poisoned food technique [8]. The efficacy of the commercially available essential oils *viz.,* Tea tree, Garlic, Clove, Lavender, Eucalyptus, Cinnamon, Ginger Peppermint and Mogra (Table.1 and Fig.1) were tested at three different concentrations (500 ppm, 1000 ppm and 1500 ppm) against the pathogens under laboratory conditions. Specific initial concentrations were prepared by adding appropriate quantity of essential oils containing 0.05 % to cooled molten PDA media followed by manual rotation in a sterile conical flask to disperse the oil in the medium which was carried out in laminar air flow chamber. The medium was allowed to solidify at room temperature. All the treatments and control plates were aseptically inoculated by placing a 7 mm mycelial disc in the centre obtained from seven days old culture. All these inoculated plates were incubated in BOD incubator at 26 ± 1 °C till the untreated control plates got full coverage with mycelial growth of the test fungal isolates.

Observation on colony diameter was recorded when the mycelial growth of *Alternaria solani* attained full growth in the control plates. Per cent inhibition of *Alternaria solani* were calculated by the formula-

$$I = (C-T/C) X 100$$

Where, I = Per cent inhibition of mycelial growth,
C = Growth diameter in control plate (mm) and
T = Growth diameter in treated plate (mm)

Table. 1 List of essential oils evaluated against *Alternaria solani* of potato

Sl. No.	Treatments		Concentration (ppm)		
1.	T_1	Tea tree oil	500	1000	1500
2.	T_2	Garlic oil	500	1000	1500
3.	T_3	Clove oil	500	1000	1500
4.	T_4	Lavender oil	500	1000	1500
5.	T_5	Eucalyptus oil	500	1000	1500
6.	T_6	Cinnamon oil	500	1000	1500
7.	T_7	Ginger oil	500	1000	1500
8.	T_8	Peppermint oil	500	1000	1500

9.	T_9	Mogra oil	500	1000	1500
10.	T_{10}	Control			

3. RESULTS AND DISCUSSION

An *in vitro* investigation was conducted to evaluate the antifungal efficacy of nine distinct essential oils against *Alternaria solani*, the causal agent of potato early blight. The oils were tested at three concentrations: 500, 1000, and 1500 parts per million (ppm). The results, measured as the percentage inhibition of fungal mycelial growth, demonstrated a significant variation in efficacy among the different oils and across the tested concentrations. Clove oil (T_3) exhibited the most potent and consistent antifungal activity, achieving complete (100%) inhibition of *Alternaria solani* at all three concentrations (500, 1000, and 1500 ppm). This superior performance indicates its potential as a highly effective botanical fungicide. Garlic oil (T_2) also showed strong efficacy, achieving 64.44% inhibition at 500 ppm and complete (100%) inhibition at the two higher concentrations (1000 and 1500 ppm). Ginger oil (T_7) demonstrated a dose-dependent response, with its inhibitory effect increasing from 50.00% at 500 ppm to 57.58% at 1000 ppm, culminating in complete (100%) inhibition at the highest dose of 1500 ppm. The remaining essential oils displayed varying levels of effectiveness as Peppermint oil (T_8) provided good inhibition that increased with concentration (67.77%, 70.00%, and 85.55%), Tea tree oil (T_1) showed moderate to good activity (47.77%, 68.89%, and 73.33%), Cinnamon oil (T_6) displayed a strong dose-response, with efficacy rising sharply from a weak 8.88% at 500 ppm to 74.44% at 1500 ppm, Eucalyptus oil (T_5) followed a similar pattern, with inhibition increasing from 13.33% to 67.77% across the concentration range, Mogra oil (T_9) exhibited relatively weak activity at all doses (21.11%, 25.56%, and 43.33%), Lavender oil (T_4) was found to be the least effective treatment, showing negligible to very low inhibition (2.22%, 21.11%, and 20.00%), suggesting it has minimal utility for controlling this pathogen as shown Table. 2 and Fig.1.

In vitro studies have established the potent efficacy of clove oil against *Alternaria solani*, the fungal pathogen responsible for potato early blight. Research demonstrates that clove oil application on Potato Dextrose Agar (PDA) medium results in a significant dose-dependent suppression of mycelial growth. For instance, [7] reported inhibition rates exceeding 60% at higher concentrations, with complete (100%) inhibition frequently observed at concentrations of 500 ppm and above.

This pronounced antifungal property is primarily attributed to eugenol, the major bioactive constituent of clove oil. Eugenol compromises the pathogen's viability by disrupting the integrity of the cell membrane and effectively inhibiting the process of spore germination. Due to its consistent and high efficacy in controlled environments, clove oil is recognized as a highly promising botanical fungicide and a viable alternative to synthetic chemicals for the management of early blight disease.

4. CONCLUSION

In conclusion, this *in vitro* investigation successfully identified clove oil as the most effective treatment, demonstrating complete inhibition of *Alternaria solani* at all tested concentrations. Garlic and ginger oils also exhibited strong, dose-dependent antifungal activity, achieving full inhibition at higher doses. While oils like peppermint, tea tree, cinnamon, and eucalyptus showed moderate to good efficacy that increased with concentration, mogra and lavender oils proved to be largely ineffective. These findings strongly advocate for the potential of clove oil as a potent botanical fungicide and highlight the need for further research to develop it into a sustainable alternative for managing potato early blight of potato and help the farming communities of Meghalaya.

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Table 2. *In vitro* efficacy of essential oils against the pathogen

	500 ppm		1000 ppm		1500 ppm	
Essential oil	**Radial growth**	**% inhibition**	**Radial growth**	**% inhibition**	**Radial growth**	**% inhibition**
Teatree oil	4.7±0.14^{e} (0.67)	47.77±1.52^{f} (43.72)	2.8±0.1^{b} (0.45)	68.89±1.12^{g} (56.09)	2.4±0.01^{d} (0.38)	73.33±0.07^{e} (58.90)
Garlic oil	3.2±0.06^{c} (0.51)	64.44±0.67^{h} (53.39)	0±0^{a} (-)	100±0.00^{h} (90.00)	0±0^{a} (-)	100±0.00^{g} (90.00)
Clove oil	0±0^{a} (-)	100±0.00^{j} (90.00)	0±0^{a} (-)	100±0.00^{h} (90.00)	0±0^{a} (-)	100±0.00^{g} (90.00)
Lavender oil	8.8±0.12^{i} (0.94)	2.22±1.32^{b} (8.57)	7.1±0.26^{g} (0.85)	21.11±2.92^{b} (27.35)	7.2±0.05^{g} (0.86)	20.00±0.50^{b} (26.56)
Eucalyptus oil	7.8±0.14^{g} (0.89)	13.33±1.56^{d} (21.41)	4.5±0.01^{d} (0.65)	50±0.13^{e} (45.00)	2.9±0.07^{e} (0.46)	67.77±0.72^{d} (55.41)
Cinnamon oil	8.2±0.07^{h} (0.91)	8.88±0.82^{c} (17.34)	5.1±0.1^{e} (0.71)	43.33±1.12^{d} (41.16)	2.3±0.07^{c} (0.36)	74.44±0.76^{f} (59.63)
Ginger oil	4.5±0.09^{d} (0.65)	50.00±0.99^{g} (45)	3.8±0.13^{c} (0.58)	57.78±1.40^{f} (49.47)	0±0^{a} (-)	100±0.00^{g} (90.00)
Peppermint oil	2.9±0.12^{b} (0.46)	67.77±1.36^{i} (55.41)	2.7±0.03^{b} (0.43)	70.00±0.35^{g} (56.78)	1.3±0.02^{b} (0.11)	85.55±0.16^{g} (67.66)
Mogra oil	7.1±0.15^{f} (0.85)	21.11±1.63^{e} (27.35)	6.7±0.28^{f} (0.83)	25.56±3.15^{c} (30.36)	5.1±0.01^{f} (0.71)	43.33±0.10^{c} (41.16)
Control	9±0^{j} (0.95)	0.00±0.00^{a} (0.00)	9±0^{h} (0.95)	0.00±0.00^{a} (0.00)	9±0^{h} (0.95)	0.00±0.00^{a} (0.00)
Sem	0.06	0.66	0.08	0.88	0.02	0.21
CD (*p=0.05*)	0.17	1.96	0.23	2.61	0.06	0.64

Note: Values are mean of three replications; Sem: Standard error mean; CD.: Critical Difference; Values in parentheses are transformed values.

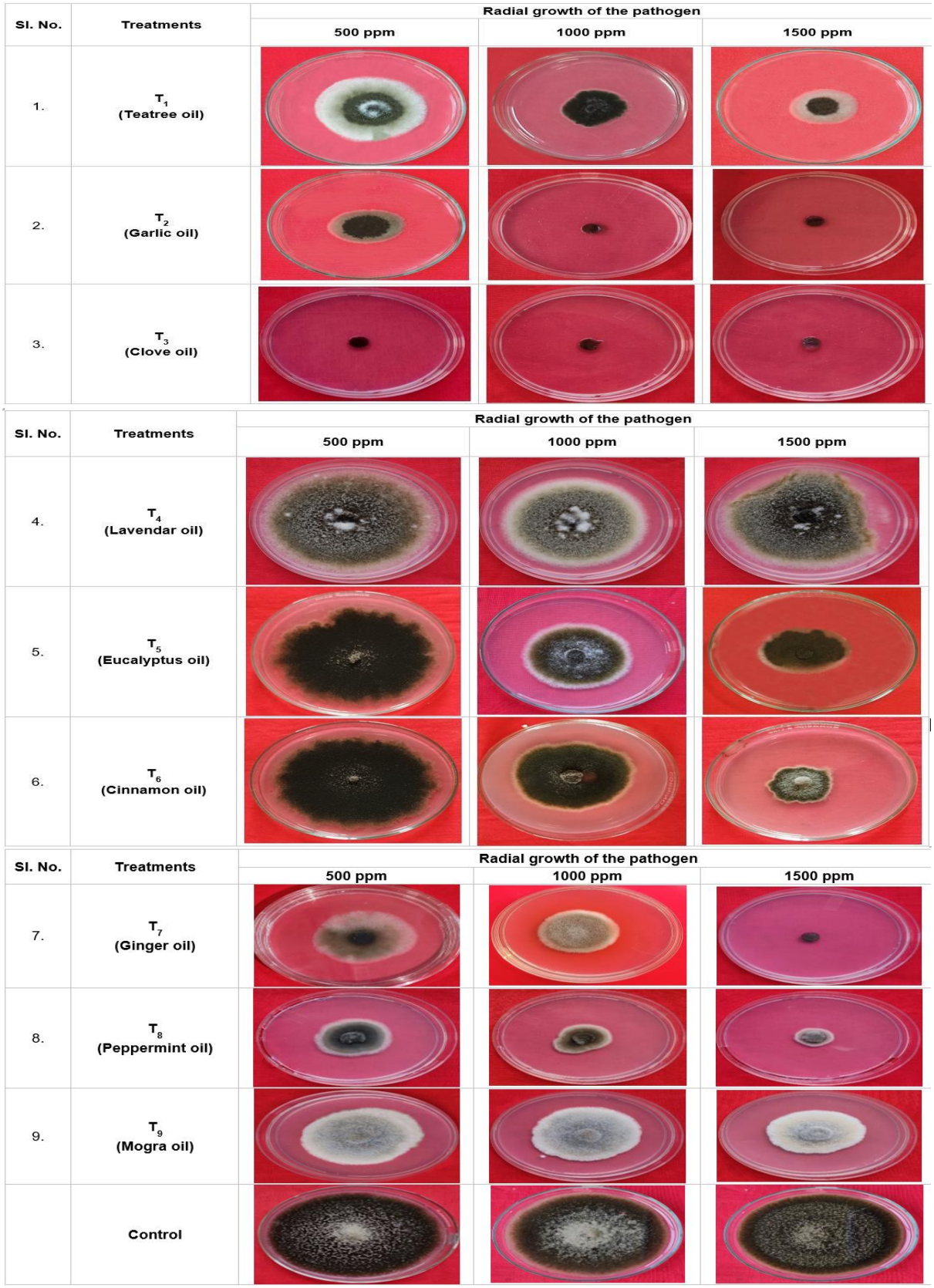

Fig.:1 *In vitro* efficacy of essential oils against the pathogen

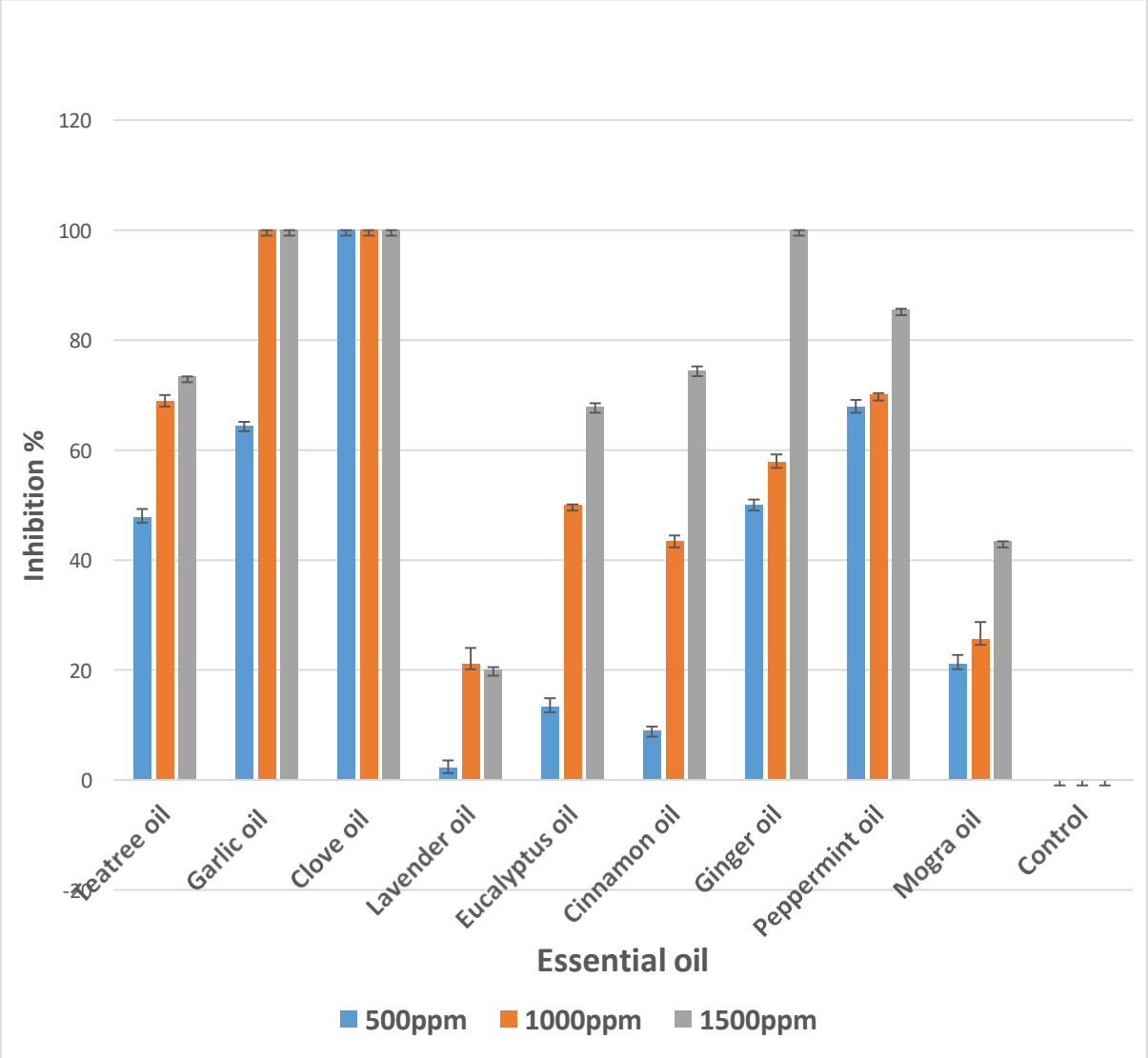

Fig.: 2 *In vitro* efficacy of essential oils against pathogen

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