AATCC Review

27 January 2024: Received
10 February 2024: Revised
22 February 2024: Accepted
09 March 2024: Available Online

www.aatcc.peerjournals.net

Research Article Open Access

Preparation of ready-to-use formulations from clove oil and their antifungal activity against *Colletotrichum gloeosporioides,* the cause of post-harvest anthracnose disease in mango

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ABSTRACT

Clove oil has demonstrated insecticidal, antimicrobial, antifungal, and nematicidal activity, but its use is limited due to its insolubility in water, high volatility, rapid oxidation, and degradation upon exposure to air. To overcome these constraints, an experiment was conducted to prepare water-soluble formulations from clove oil and evaluate their antifungal activity against Colletotrichum gloeosporioides, the causative agent of post-harvest anthracnose disease in mango. The main challage during the study was to prepare water souble and ready to use formulation from clove oil. In vitro testing showed that all concentrations of clove oil tested resulted in 100% inhibition of C. gloeosporioides. Ready-to-use formulations were prepared by mixing clove oil with various adjuvants, including Tween 20, Tween 80, silicon antifoaming agent, castor oil, xanthan gum, and gum arabic. Of the formulations prepared, two were found to be water-soluble: CT-20 (20% clove oil + 70% Tween 80 + 10% water) and CT-10 (10% clove oil + 80% Tween 80 + 10% water). The physical parameters of both CT-20 and CT-10 met the criteria for EC formulations according to World Health Organization (WHO) specifications (2016). Of the two water-soluble formulations, CT-20 at 0.5% and higher concentrations resulted in 100% inhibition of C. gloeosporioides. The study highlights the potential of clove oil as a natural alternative to chemical pesticides for controlling post-harvest anthracnose disease in mango caused by C. gloeosporioides. The preparation of water-soluble formulations from clove oil will also help to overcome the limitations of clove oil's water insolubility, high volatility, rapid oxidation, and degradation on exposure to air.

Keywords: *Clove oil, Colletotrichum gloeosporioides, Anthracnose, Ready to use formulation, Water soluble*

INTRODUCTION

Clove bud (*Syzygium aromaticum* L.) essential oil is recognized for its insecticidal, antimicrobial, and nematicidal activity, primarily due to its main active component, eugenol [3,5, 11,13,10]. Despite the demonstrated antimicrobial and insecticidal properties of essential oils, their use is limited by their water insolubility, high volatility, rapid oxidation, and degradation upon exposure to air [4,16]. To overcome these limitations, methods that can enhance the physical stability, protect against evaporation, and increase water solubility of essential oils are necessary.

Anthracnose, caused by *C. gloeosporioides*, is a major fruit disease that affects both pre-harvest and post-harvest quality [20]. Severe infections can lead to the destruction of entire inflorescences, resulting in no fruit setting. Young fruits infected with the disease develop black spots, shrivel, and drop off, while mature fruits infected with the disease can cause considerable loss during storage, transit, and marketing [1]. This disease is a major limitation to mango production in countries where mangoes are grown, particularly in areas with high humidity during the growing season. Post-harvest is the most damaging and economically significant phase of this disease worldwide, as it directly affects the fruits, rendering them unmarketable and reducing the income of mango growers [2].

Chemical pesticides are widely used for controlling anthracnose disease, due to their effectiveness and ease of availability. However, the overuse of these synthetic pesticides can have harmful effects on living organisms and the environment. The post-harvest use of chemicals as fungicides is also restricted in many countries, as consumers demand agricultural products without pesticide residues. Pesticides can also kill beneficial organisms and their toxic forms can persist in soil, leading to increased resistance among pathogens towards synthetic chemicals [7]. To address these issues, scientists are turning to natural plant products, such as essential oils, as alternative solutions. These products are biodegradable and eco-friendly, making them ideal candidates for use as agrochemicals. They are bio-efficacious, economical, and environmentally safe, and can be a promising solution to this problem [15].

The present study aimed to prepare biopesticide from the clove oil using different adjuvants that will improve water solubility and ensure the prolonged efficacy of the oil, and check their antifungal activity against *Colletotrichum gloeosporioides* causes anthracnose disease in mango.

MATERIALS AND METHODS

Location

The investigation was carried out during 2021–22 at Department of Plant Pathology, N. M. College of Agriculture,

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DOI: https://doi.org/10.21276/AATCCReview.2024.12.01.264
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Navsari Agricultural University, Navsari, Gujarat, India.

Material used

The clove oil used in present study was purchased from the Qualigens firm, Surat, Gujarat (India).

Isolation of *Colletotrichum gloeosporioides*

The infected fruits showing typical symptoms like dark brown, sunken spot on the fruit were collected from college farm of NAU, Navsari and pathogen was isolated by following standard isolation procedure.

In vitro* evaluation of clove oil against *C. gloeosporioides

Three concentrations (0.50, 1.0 and 1.5 per cent) of clove oil was evaluated under *in vitro* condition against *C. gloeosporioides* by Poisoned Food Technique (PFT). The diameter of the mycelial growth (mm) of pathogen was measured after 7 days of incubation. Antifungal activity were recorded in terms of inhibition of mycelial growth (%)calculated using following formula.

$$\textbf{Inhibition of mycelial growth } (\%) = \frac{\mathbf{C - T}}{\mathbf{C}} \times \mathbf{100}$$

Where,

C = Average diameter of fungal colony in control

T = Average diameter of fungal colony in poisoned plates

Preparation of ready-to-use formulation from clove oil

Ready to use formulations were prepared by mixing clove oil and other adjuvants (Tween 20, Tween 80, Xanthan gum, silicon etc.) at room temperature in a certain weight ratio. Intensive stirring was applied after the addition of each component. The solubility test of the prepared products were carried out, only water soluble formulations were selected for further studies.

Physical parameters of prepared water-soluble formulation

The physical properties like colour, pH and persistent foam etc. of prepared water soluble formulations were determined according to the method of FAO/WHO specification, 2016.

Antifungal activities prepared water-soluble formulations against *C. gloeosporioides*

Two concentrations (0.50 and 1.0 per cent) of water soluble prepared formulation of clove oil were evaluated under laboratory condition against*C. gloeosporioides* by poisoned food technique as mentioned above.

RESULTS AND DISCUSSION

In vitro* evaluation of clove oil against *C. gloeosporioides

Three concentrations *viz.,* 0.50, 1.0 and 1.5 per cent of clove oil was evaluated under *in vitro* condition against *C. gloeosporioides* and depicted in Plate 1. All the tested concentrations of clove oil exhibited cent per cent inhibition of *C. gloeosporioides* compared to the control. Similar results were reported by earlier scientist [9]. They reported that, clove essential oil significantly inhibited the mycelial growth of *C. gloeosporioides*. The inhibition increased along with the increased concentration of oil. It was also in agreement with findings of earlier worker [23]. They also reported antifungal properties of clove oil against *C. gloeosporioides* and clove oil was found to be the most promising inhibitor of mycelium growth of *C. gloeosporioides*. Antifungal activities of the clove oil has also been reported against *Fusarium* sp.[14].

Preparation of ready-to-use formulation from clove oil

The ready to use formulation were prepared by combining clove oil (CO) with different adjuvants such as Tween 20, Tween 80, silicon, xanthan gum, and water. Formulations CT-20 and CT-10 showed solubility in water, while the other formulations (CTSX-60 to CTSX-10 and CT-60 to CT-30) were not soluble in water(Table 1 &Plate 2). The water-soluble formulations were used for further studies. The present findings were following the earlier studies [8]. They prepared water-soluble neem oil nanoemulsion formulations by using neem oil, Tween 80, Agnique® MBL 510H, and deionized water. Three clove essential oil (CEO) based emulsifiable concentrate (EC) formulations by using clove essential oil as an active ingredient, synthetic zeolite, natural zeolite, and bovine gelatin as a carrier material, rapeseed oil as a solvent and Tween 20 as an emulsifier[17].

Physical Parameters of water-soluble prepared formulations of clove oil

The physical parameters like colour, pH and persistent foam of water soluble prepared formulations of clove oil are presented in Table 2.The obtained results fulfill the criteria for EC formulation given by FAO/WHO, 2016.

The present findings were in accordance with the earlier studies [17,12]. They studied the different physical parameters such as color, pH, density, persistent foam, stability, surface tension and viscosity of their own synthetized formulations prepared from the different essential oils.

Antifungal activities prepared water-soluble formulations against *C. gloeosporioides*

In this study, the antifungal activities of two water-soluble clove oil formulations (CT-20 and CT-10) and their concentrations (0.5 and 1.0%) were evaluated against *Colletotrichum gloeosporioides* (Table 3 & plate 3). The results showed that CT-20 at both concentrations showed complete inhibition of the fungus, while CT-10 at 1.0 percent concentration showed 6.00 mm mycelial growth and at 0.5% concentration showed 22.00 mm mycelial growth with 93.33% and 75.55% inhibition of the fungus, respectively.

The efficacy of clove oil as a good source of antifungal components has been previously reported [21,19,25]. Clove oil completely inhibited the mycelial growth of *Rhizopus stolonifer* and *Fusarium solani* [22] and clove oil had significant antimicrobial activities against mycelia growth and spore germination of *Phytophthora megakarya* [18]. The mycelial growth and the conidial germination of *C. gloeosporoides* and *Fusarium sp.* inhibited at 50 µg/ml, by EO of *S. aromaticum* [6].

CONCLUSIONS

In conclusion, the results of the present study suggest that clove oil can be an effective antifungal agent against *C. gloeosporioides*, the cause of anthracnose disease in mango. The combination of clove oil, Tween 80, and water in a formulation (CT-20) showed strong antifungal activity and was found to be water-soluble, making it a promising alternative to synthetic fungicides for the management of the disease.

FUTURE SCOPE OF THE STUDY

This study will helpful for preparation of water soluble formulation from clove oil and their application in field condition.

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CONFLICT OF INTEREST

All authors declare that they have no conlicts of intrest.

ACKNOWLEDGMENT

Authors are thankful to Director and Dean, Navsari Agricultural University, Navsari (Gujarat) for providing the facilities to conduct the research.

Table 1: Different adjuvants combinations used for preparation of ready to use formulations

Sr. No.	Formulation code	Adjuvant combinations	Solubility in water
1	CTSX-60	CO (60%) + Tween 20 (5%) + silicon (5%) + Xanthan gum (5%) + water (25%)	Not soluble
2	CTSX-50	CO (50%) + Tween 20 (5%) + silicon (5%) + Xanthan gum (5%) + water (35%)	Not soluble
3	CTSX-40	CO (40%) + Tween 20 (5%) + silicon (5%) + Xanthan gum (5%) + water (45 %)	Not soluble
4	CTSX-30	CO (30%) + Tween 20 (5%) + silicon (5%) + Xanthan gum (5%) + water (55 %)	Not soluble
5	CTSX-20	CO (20%) + Tween 20 (5%) + silicon (5%) + Xanthan gum (5%) +water (65%)	Not soluble
6	CTSX-10	CO (10%) + Tween 20 (5%) + silicon (5%) + Xanthan gum (5%) + water (75%)	Not soluble
7	CT-60	CO (60%) + Tween 80 (30%) + water (10%)	Not soluble
8	CT-50	CO (50%) + Tween 80 (40%) + water (10%)	Not soluble
9	CT-40	CO (40%) + Tween 80 (50%) + water (10%)	Not soluble
10	CT-30	CO (30%) + Tween 80 (60%) + water (10%)	Not soluble
11	CT-20	CO (20%) + Tween 80 (70%) + water (10%)	Soluble
12	CT-10	CO (10%) + Tween 80 (80%) + water (10%)	Soluble

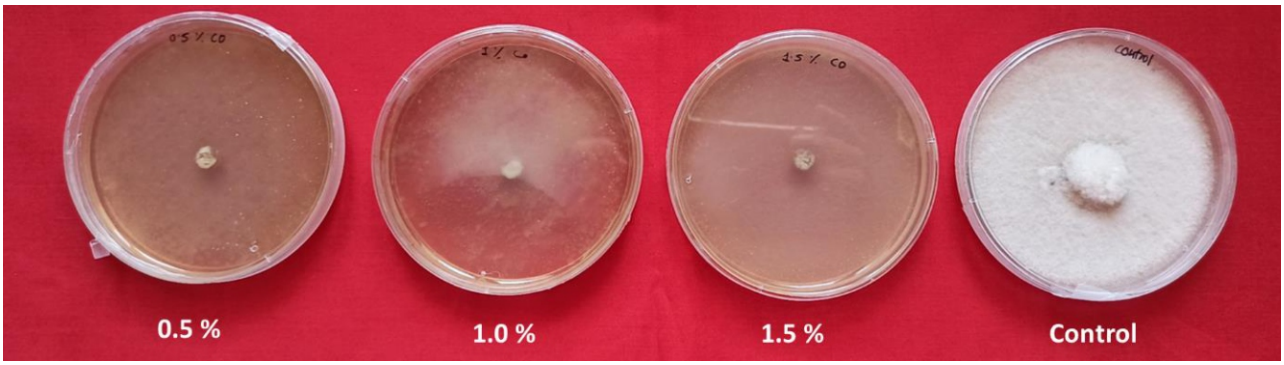
CO- clove oil

Table 2: Physical parameters of water soluble prepared formulations from clove oil

Sr. No.	Parameters	Formulations	
		CT-20	CT-10
1.	Colour	Light Yellow	Light Yellow
2.	pH	6.4	6.8
3.	Persistent foam		
a)	10s	0.1 ml	0.1 ml
b)	60s	0.0 ml	0.1 ml
c)	120s	0.0 ml	0.0 ml
d)	180s	0.0 ml	0.0 ml
e)	720s	0.0 ml	0.0 ml

Table 3: Antifungal activities of water soluble formulations of clove oil against Colletotrichum gloeosporioides

Tr.No.	Treatment Details	Conc. (%)	Mycelial growth (mm)	Per cent growth inhibition
T_1	CT-10	0.5	22.00 (4.73)	75.55
T_2	CT-10	1.0	6.00 (2.546)	93.33
T_3	CT-20	0.5	0.00 (0.70)	100
T_4	CT-20	1.0	0.00 (0.70)	100
T_5	Control	-	90.00 (9.51)	-
SEm±			0.07	
CD at 5%			0.23	
CV			4.30	



Figures in parentheses are √x +0.5 transformation values

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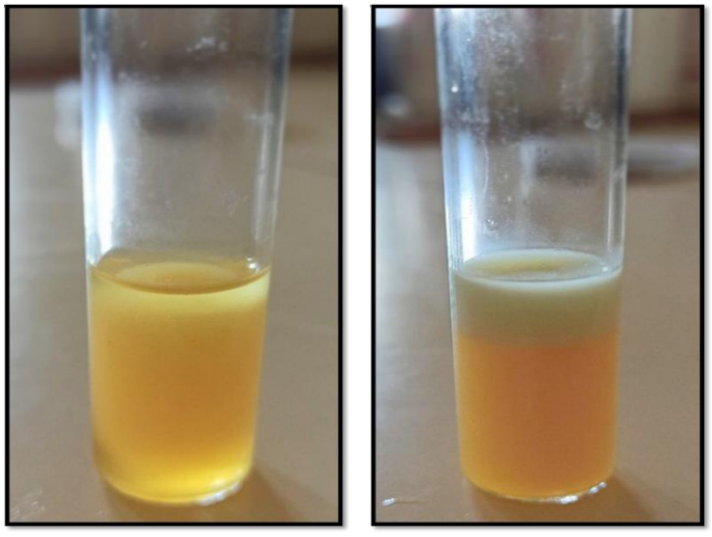

Water soluble formulation Water insoluble formulation
Plate 2:Water soluble and water insoluble prepared formulations from clove oil

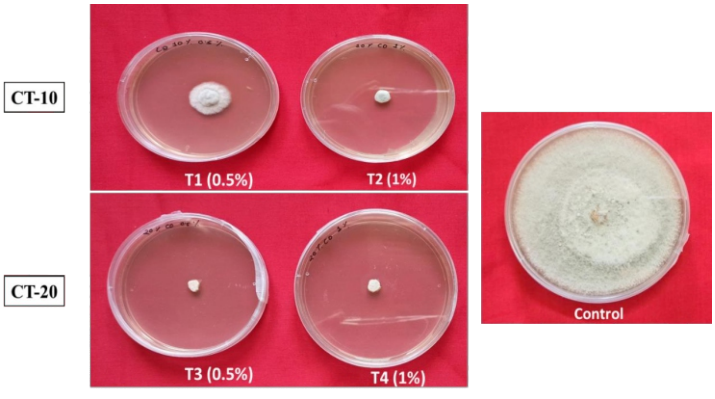

Plate 3: In vitro evaluation of water soluble prepared formulations of clove oil against C. gloeosporioides

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