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Effect Of Clove Oil and Thyme Oil on Seed Born Fungal Contaminants

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ABSTRACT:
Seed-borne fungi are the serious threats to several agricultural as well as horticultural crops. These fungi reduced the yield, quality and can cause many deformities in seeds. Over several years, much attention has been paid to environmentally-friendly solutions for plant disease management. Natural sources like essential oils could be alternative sources for biopesticides and help to control the seed-borne pathogens. This study aimed to evaluate the effect of Clove oil and Thyme oil on the growth of seed-borne fungi of economically important crops viz., Rice, Wheat and Corn. The presence of seed borne fungi was determined by blotter method and PDA method. The present study showed that essential oils specially clove oil used have a high potential to control seed borne fungal pathogens and can be incorporated in biocontrol and application in biopesticides formulations in future.

KEYWORDS: Seed borne fungi, Plant disease management, Blotter method, PDA method, Biocontrol.

INTRODUCTION:

There is an increasing demand to reduce the use of chemicals as antimicrobial agents in the field of nutrition and to combat various infections due to increasingly aggressive and increasingly endogenous microorganisms that are resistant to the use of synthetic antimicrobials. In this direction, substances derived from plants, such as hydro-alcoholic extracts or essential oils, can certainly play a fundamental role (Nazzaro *et al*., 2017).

Both humans and plants are susceptible to fungal infections by pathogenic fungi and some synthetic fungicides are known to be effective in their control. However, the use of synthetic fungicides is limited by the emergence of resistant fungus strains and some fungicides possess considerable toxicity. Moreover, there is a growing public concern over the increased health and environmental hazard associated with synthetic molecules. Recently, there has been a great interest in using essential oils as possible natural substitutes for conventional synthetic fungicides (Isman *et al*., 2020).

Rice, Wheat and Corn are the most important agricultural crops since times immemorial. (Soto-G´omez and P´erez-Rodríguez, 2022). They are considered as staple food in many parts of India. Diseases, especially caused by seed borne fungal agents, are among the main factors reducing yield and quality of these crops. Seed borne pathogens reduce viability, vigor, and germination capability of the seeds resulting in lower yield. Some of them may also cause losses with the diseases on plants growing from the infested seeds. Seed borne fungi not only decrease yield and seed quality, but they also produce mycotoxins which cause diseases on human or animals feeding with these seeds or food made with them. More than 50 fungal pathogens found to be seed-borne and it's deteriorated both quantity and quality aspects of rice (Janardhana *et al*., 1998; Kavitha *et al*., 2005).

Common seedborne fungi in corn include *Fusarium*, *Aspergillus*, and *Penicillium* species. These pathogens can cause seed rot, seedling blight, and ear mold, which not only reduce crop yields but can also produce mycotoxins harmful to humans and animals. Effective management of seed-borne fungi is crucial for ensuring healthy crops and food safety (Silva *et al*., 2019).

REVIEW OF LITERATURE:

Fungal infections are more difficult to ascertain their presence and apply the appropriate therapeutic treatment compared to bacterial infections. Chemical treatments are largely effective, but resistant strains and intrinsically resistant species can be developed (Nazzaro et al., 2017). In vitro antibacterial activity of essential oils (EOs) against several bacteria was explained by Burt (2004). Antifungal activity of essential oils and their importance as biocontroling agent in Agriculture is explained by many authors. (Regnault-Roger *et al*., 2012; Johnson and Lee, 2022; Smith *et al*., 2023). Soylu *et al*. (2006) showed that the essential oils of lavender (*Lavandula angustifolia*) and peppermint (*Mentha piperita*) had strong antifungal effects against *Botrytis cinerea* and *Rhizoctonia solani*. Amini *et al*. (2012) reported that eucalyptus (*Eucalyptus globulus*) essential oil was effective in reducing the incidence of *Alternaria* and *Fusarium* on wheat and barley seeds. Kumar and Patel (2022) confirmed that Thyme Oil reduced fungal contamination in wheat seeds to 38% (filter paper method) and 75% (PDA method), indicating significant antifungal activity. Pinto *et al*. (2020) highlighted the antifungal activities of thyme and oregano essential oils, noting their effectiveness against common pathogens like *Fusarium* and *Aspergillus* while Daferera *et al*. (2003) found that oregano and thyme oils were particularly effective against *Penicillium* and *Aspergillus* species, common contaminants in stored seeds.

This study aimed to evaluate essential oils' influence on the growth of seed-borne fungi to investigate the long-term effects and sustainability of essential oils.

MATERIALS AND METHODS:

The seeds understudy, Rice and wheat, were collected from Vikrampur village in Madhya Pradesh. Maize seeds were collected from a local market in Mumbai, representing a wide array of corn varieties. Essential oils used in the experiment, thyme and clove, were collected from the local Market at Dadar, Mumbai. Blotter method and PDA method were used to determine the potential antifungal effect of essential oils.

In Blotter method, filter paper was placed in Petri plates. Each plate was moistened with distilled water. All the plates were sterilized in an autoclave at 120^0C. The plates were cooled and then 2 ml of clove oil and 2 ml of thyme oil was applied separately to the paper under aseptic conditions. The control plates were prepared in a similar way without adding any essential oil.

In PDA method, the Potato Dextrose Agar was prepared and sterilized in Autoclave for 20 minutes at 120^0 C and 15 lbs pressure. The PDA medium was allowed to cool to about 50-60°C. Subsequently, 4 mL of thyme and 4 ml clove essential oil was added separately to 200 mL of the PDA medium, creating a consistent concentration of essential oil throughout. The medium was then thoroughly mixed and poured into sterile petri plates. Plates poured with PDA without addition of essential oil were kept as control.

16 surface-sterilized seed samples were arranged separately in a square pattern, consisting of four rows and four columns on each Petri dish of both blotter method and PDA method (Figure 1). All petri plates were incubated at 37°C in the dark for 7 days.

Fig.1: Sample

RESULTS AND DISCUSSION:

In this study, PDA method and blotter method were used to evaluate the fungal contamination on the seeds under the influence of different essential oils. Fungal species belonging to *Aspergillus niger and Aspergillus flavus,* were isolated from the untreated seeds. These fungi were previously reported as the most frequent seed borne fungi.

PDA Method:

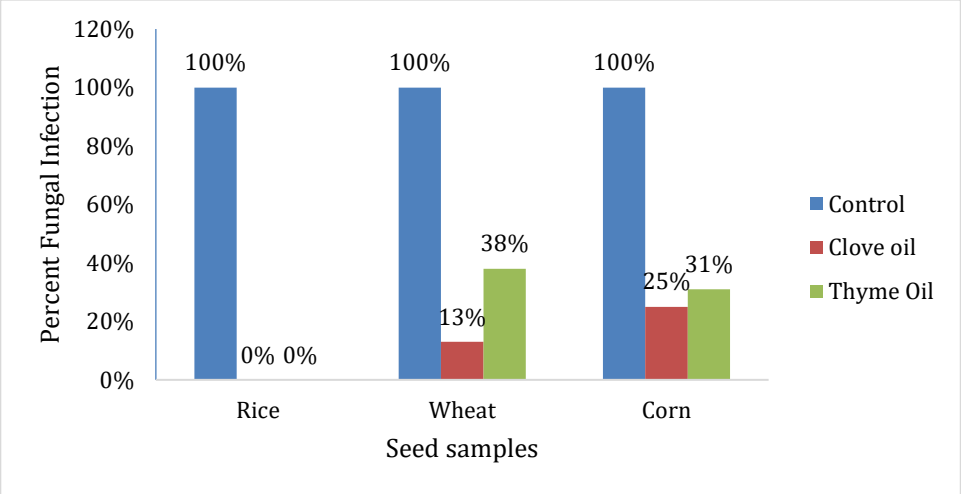
The result recorded in Table no. 1 recorded the effect of Clove oil and Thyme oil on fungal contaminatio by PDA method. On control plates, all seeds showed 100% contamination. In clove oil treated PDA, rice and wheat seeds showed absence of any fungal species. However, Corn seeds show 5 out of 16 seeds (31%) showed presence of fungal contaminants. The Thyme oil was effective in controlling these contaminants in Rice seeds equally. But the former did not effective in wheat seeds and Corn seeds as well. The contamination was reduced to 75% and 81% in Wheat and Corn respectively.

Thus, clove essential oil had a great control over fungal contaminants. Compared to clove oil, thyme oil was not as effective. It may be due to use of low dose of Thyme oil. According to Karaca et al. (2017) lower doses of essential oils failed to inhibit fungal growth. However, the volume of the contamination was reduced compared to control.

Table no. 1: Effect of clove and Thyme oil on fungal contaminants of seeds

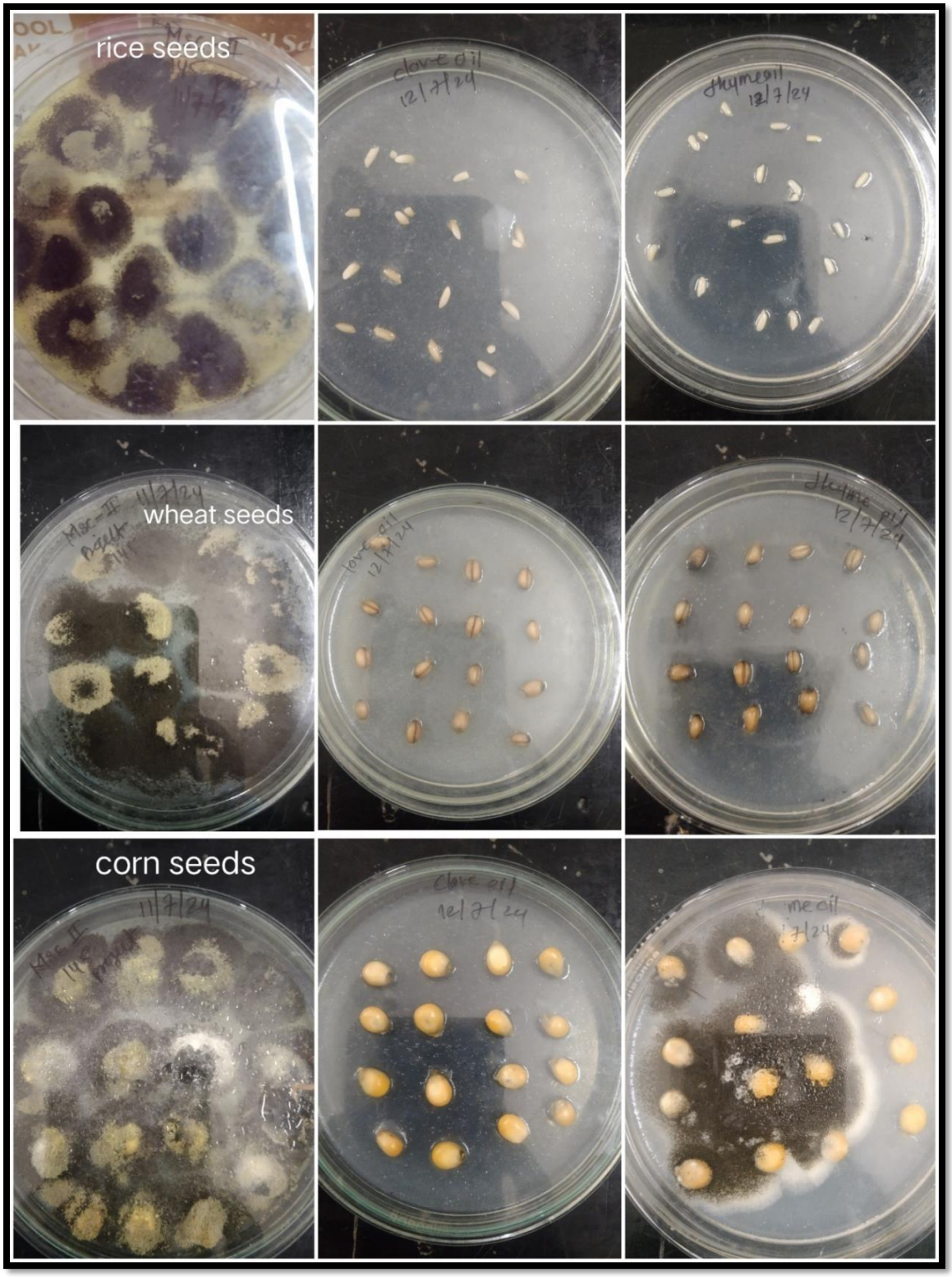
Seed sample	% of seeds contamination		
	Control	Treated with clove oil	Thyme Oil
Rice	100%	0%	0%
Wheat	100%	0%	75%
Corn	100%	31%	81%

Sharma *et al*. (2018) reported on the broad-spectrum antifungal properties of clove essential oil, which is attributed to its high eugenol content. Silva *et al*. (2019) examined the antifungal activity of essential oils from several plants, emphasizing that clove oil was particularly effective against *Fusarium* species due to its ability to disrupt fungal cell membranes.



Graph No. 1: Effect of Clove oil and Thyme oil on Fungal contaminants by PDA Method

Untreated seeds **Treatment with clove oil** **Treatment with thyme oil**



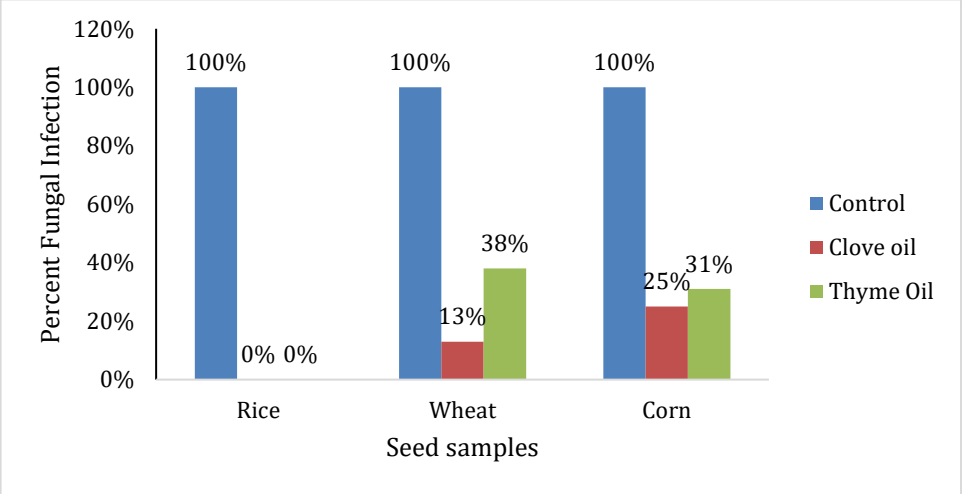
Blotter Method:

The result recorded in table no. 2 revealed that clove essential oil had a great control over fungal contaminants.

Table no. 2: Effect of clove oil on fungal contaminants of seeds by blotter method

Seed sample	% of seeds contamination		
	Control	Clove oil	Thyme oil
Rice	100%	0%	0%
Wheat	100%	13%	38%
Corn	100%	25%	31%

In blotter method, clove oil as well Thyme oil was equally effective and reduced the contamination to 0% in Rice. For Wheat and Corn, the contamination reduced considerably due to both Clove oil and Thyme oil. However, Clove oil was more effective.

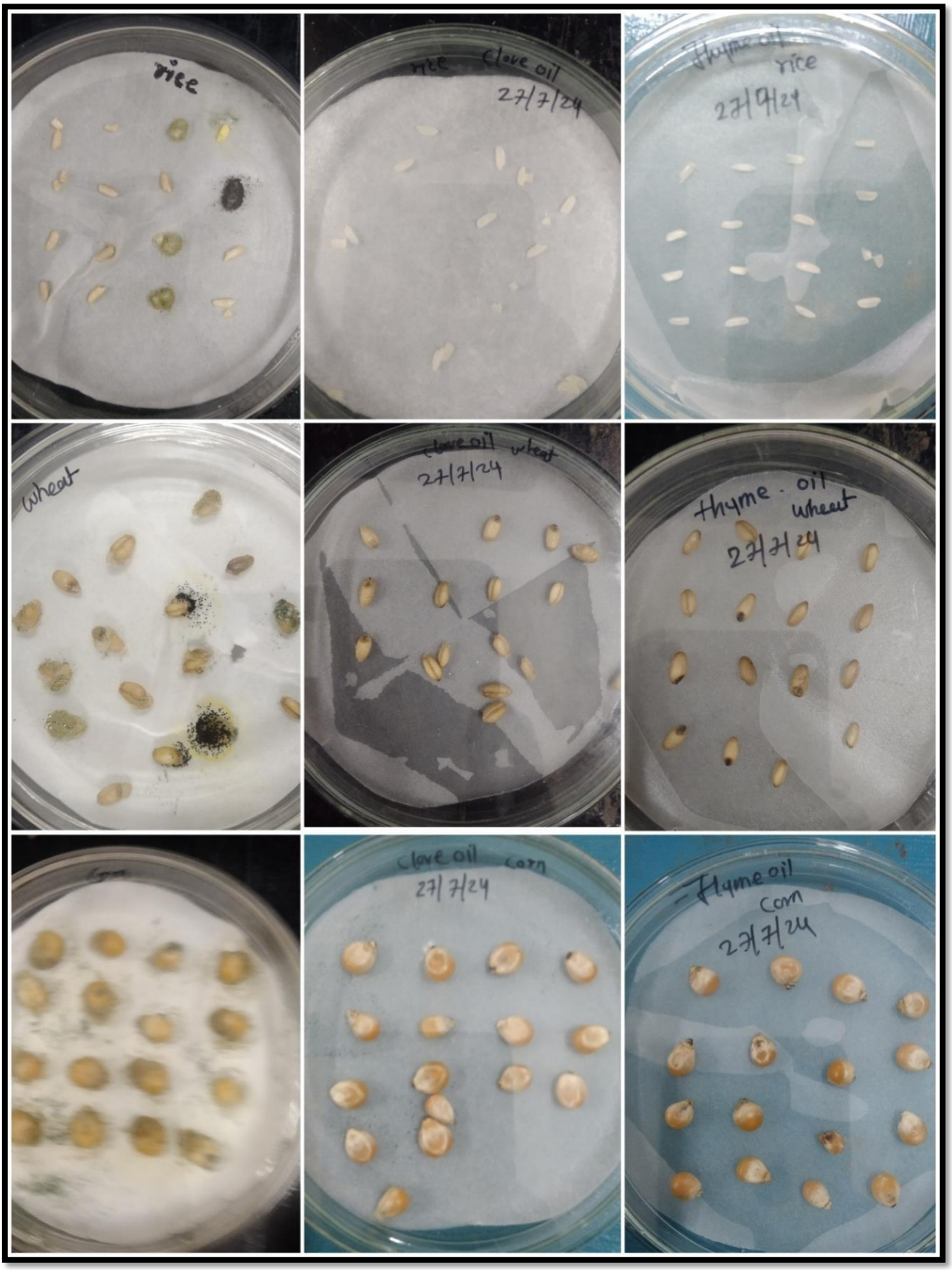


Graph No. 2: Effect of Clove oil and Thyme oil on Fungal contaminants by blotter Method

Bakkali *et al*. (2008) reviewed the biological effects of essential oils, explaining that their antifungal activity is primarily due to the disruption of cell membranes and inhibition of enzymatic pathways. Isman (2020) elaborated on the insecticidal and antifungal mechanisms of essential oils, focusing on their ability to cause oxidative stress in fungal cells. Hammer *et al*. (2018) provided evidence that essential oils can inhibit spore germination and mycelial growth by altering cell wall integrity.

Although Thyme Oil was not as effective as Clove Oil, it still offered a notable reduction in fungal contamination, underscoring its utility in corn seed treatment. The less effectiveness of Thyme oil may be attributed to low concentration and at high concentration Thyme oil may be as effective as clove oil in reducing the fungal contaminants. Karaca *et al*. (2017) also got similar findings where mint oil was less effective at when used in low concentration, however, the higher dose of mint oil totally inhibited fungal growth. Also, Chemical composition of plant essential oils differ among species; it is affected by factors including the geographical location, environment, the stage of maturity and method of extraction. This chemical difference is directly correlated to differences in biological activities (Nazzaro et al., 2013).

Untreated seeds **Treated with clove oil** **Treated with thyme oil**



CONCLUSION:

The observations demonstrate the effectiveness of essential oils, specifically Clove Oil and Thyme Oil, in controlling seed-borne fungi in rice, wheat, and corn seeds. It is evident that essential oils, specifically Clove Oil and Thyme Oil, exhibit significant role in controlling seed-borne fungi in rice, wheat, and corn seeds. The present data demonstrate that Clove Oil is exceptionally potent, achieving complete elimination of fungal contamination in rice seeds and markedly reducing contamination in wheat and corn seeds across different testing methods. Thyme Oil also displayed considerable antifungal properties, though it was slightly less effective compared to Clove Oil in wheat and corn seeds.

Given these compelling results, it is recommended to integrate Clove Oil and Thyme Oil as natural alternatives for managing seed-borne fungi in agricultural practices to enhance seed health and improve crop yields sustainably.

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