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Antifungal Activity of Clove, Neem and Peppermint Oils against *Fusarium graminearum*

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ABSTRACT

Wheat is the most commonly cultivated cereal crop in the world. It is infested by an economically important wheat disease worldwide viz. Fusarium Head Blight (FHB). This disease causes a huge loss in both yield and grain quality. Though there are many chemical pesticides to control it, there is a dire need for a biological control method, to achieve a sustainable agriculture system. Therefore, the present study was conducted to compare the antifungal activity of Clove oil (*Syzygium aromaticum*L. Merrill and Perry), Neem oil (*Azadirachta indica*), and Peppermint oil (*Mentha piperita*) against the pathogenic fungus *Fusarium graminearum*. The results of this study revealed that among all the herbal oils used, Clove oil is considered to be the most effective oil. The complete growth inhibition of *F. graminearum* was observed at the concentration of 1µLml^{-1}.

Key words: *Fusarium graminearum*, Clove, Neem, Peppermint oil, Antifungal activity

India is an agricultural country. The Indian economy is dependent on agriculture. Despite economic expansion and industrialization, agriculture still forms the backbone of the Indian economy. Approximately two-thirds of India's population depends on agriculture as their source of income. Even though large areas in India are under cultivation, still the productivity of agriculture is very low. In most parts, especially the northern regions of the country, wheat is used as one of the staple food grains. The sowing and harvesting time of wheat varies in different regions of the country due to different climatic conditions. During sowing, less temperature is required but for proper ripening of the crop, the temperature should be high. A sudden decrease in temperature at the time of maturity, frost at flowering time, and hailstorm at the time of ripening can cause heavy damage to the wheat crop. It is widely accepted that several diseases caused by fungi, bacteria, and viruses result in even higher yield losses. Loss of crops from fungal, bacterial, or viral plant pathogens may result in hunger and starvation, especially in developing countries where the disease-control methods are limited and annual losses for major crops are common.

Humidity during the blooming stage of the wheat causes the fungal infection by *Fusarium graminearum. F. graminearum* (also known as *Gibberellazeae*) causes Fusarium Head Blight of wheat (FHB), also called head scab. This disease causes considerable loss in yield and also reduces the grain quality. *F. graminearum* produces mycotoxins, especially deoxynivalenol (DON), also known as vomitoxin that is toxic to humans and livestock [1-3]. The distribution and severity of Fusarium Head Blight depend on the two important environmental factors, that is, temperature and moisture [4-5]. Seeds infected with *F. graminearum* show reduction in germination, resulting in slow emergence, and are also affected by seedling blight disease. Diseased kernels are often of pink or white discolouration with a shriveled coat. Affected grains might have detrimental effects on both animal and human health because of the mycotoxins they carry [6].

To prevent these damages to food crops, growers often rely on chemical insecticides and pesticides. These chemicals, no doubt has helped in increasing yields per hectare as well as total production, but it has also resulted in environmental pollution, ill-health to the biotic community, and is hazardous to the ecological systems. Therefore, the biological method of plant disease management seems to be a better alternative against these chemicals. The purpose of using microorganisms as biological control methods has proved to be successful for controlling various plant diseases. Biological control promises to be a useful alternative approach in the control of plant pathogens in the sustainable agriculture system. These

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biological pesticides have proved to be a potential source of eco-friendly and safe antimicrobial agents useful in plant protection. Literature indicates that there are several herbal oils/essential oils (Peppermint oil, Mint oil, Eucalyptus oil, Basil oil, Chenopodium oil, Clove oil, Neem oil) which show antifungal activity [7]. These oils are also environmentally safe, so they are more easily acceptable and also less hazardous to plants and animals [8-9]. The demand for these essential oils has been increased in recent years and these are considered a valuable source of biocontrol products [10]. Keeping this in mind, the present investigation aims to study the effect of herbal oils (Clove oil, Neem oil, Peppermint oil) against Head blight, a common disease found in the North-Western Plain Zone.

MATERIALS AND METHODS

The strain of *Fusarium graminearum* was obtained from the Indian Type Culture Collection (ITCC), Indian Agriculture Research Institute, New Delhi, India.

The Agar diffusion plate method was used to assess the effect of Clove, Neem, and Peppermint essential oil. Varied concentrations were obtained by adding different volumes of the crude extracts and these were incorporated into the PDA medium before pouring in sterilized petri plates. The different essential oil of these concentrations (0.1, 0.2, 0.4, 0.5, 1, 2 and 5 µl/ml) were prepared. From the margins of 7 days old culture, mycelial discs of 5 mm were taken and kept in the center of a PDA plate; followed by their sealing with parafilm. For each treatment three replicates were taken and was incubated at 28±2°C for one week. The radial growth of colonies has been observed and measured. Based on these values the percentage of inhibition of colony growth has been calculated by the formula given by Djordjevic *et al*. [10].

$$\text{Percent inhibition (I\%)} = \frac{gc - gt}{gc} \times 100$$

Where;
gc = mycelium growth in control plates
gt = mycelium growth in treated plates

Statistical analysis

The significance of correlation (P-value) was calculated using the Microsoft Excel data analysis tool.

RESULTS AND DISCUSSION

It was observed that when *Fusarium graminearum* was treated with 0.1 µl/ml of Clove, Neem, and Peppermint oil, growth inhibition percentage was 24.5%, 13.4%, and 7.8% respectively. The growth of the colony in Clove, Neem, and Peppermint was 6.8 cm, 7.8 cm, and 8.3 cm respectively.

When the *F. graminearum* was treated with 0.2µl/ml Clove, Neem, and Peppermint, growth inhibition was 47.8%, 32.3%, and 3.4% respectively. The growth of the colony was 4.7cm, 6.1 cm, and 6.9 cm respectively (Fig 1-6). At the concentration 0.5µl/ml, growth inhibition was 88.9%, 64.5% and 50% respectively. The growth of the colony was 1cm, 3.2cm, and 4.5cm respectively (Fig 2-6). At the concentration of 1µl/ml, no growth of *Fusarium* was observed in the petri plate treated with Clove oil, whereas in other petri plates treated with Neem and Peppermint growth inhibition was 86.7% and 56.7% respectively. The growth of the colony was 1.2cm and 3.9cm respectively (Fig 3-6). So, we can conclude that 1µl/ml is the minimum inhibitory concentration (plate with the lowest concentration of oil, showing no visible growth was regarded as MIC) of the Clove oil. Similarly, 2µl/ml is a minimum inhibitory concentration of the Neem oil as no growth of *Fusarium* was observed at this concentration (Fig 4-6). In Peppermint treated petri plate growth inhibition was 75.6% and the diameter of the colony was 2.2 cm (Fig 5-6).

The result indicated that different herbal oils have different efficacies. Different oils possess various components which may be active against different fungal pathogens. Clove oil showed the minimum inhibitory concentration at 1µl/ml, Neem at 2µl/ml, and Peppermint at 5µl/ml. Among all herbal oils, Clove oil is one of the best oils which can be used as an alternative against toxic chemicals to prevent the disease. So, Clove oil can be used as a natural biocontrol product. The principal component in Clove oil is eugenol (80-95%), and therefore its strong antifungal activity may be attributed to eugenol [11]. In Neem, the active constituent is azadirachtin [12] whereas in Peppermint it is menthol which is active against the fungal pathogen. Similar work has been reported by Djordjevic *et al*. [13] and Gali [14] where they observed the antifungal effect of various essential oils against *Fusarium oxysporum*f.sp. *lycopersici*) and *Penicillium digitatum*, respectively.

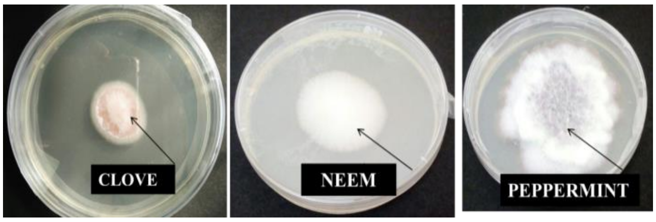

Fig 1 Petri plates showing the growth of *F. graminearum* at the concentration of 0.2 µl/ml of clove, neem, and peppermint oil

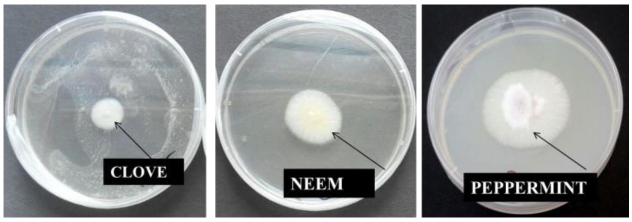

Fig 2 Petri plates showing the growth of *F. graminearum* at the concentration of 0.5 µl/ml of clove, neem, and peppermint oil

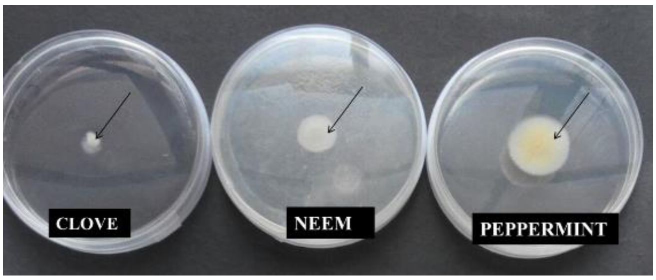

Fig 3 Petri plates showing the growth of *F. graminearum* at the concentration of 1µl/ml of clove, neem, and peppermint oil

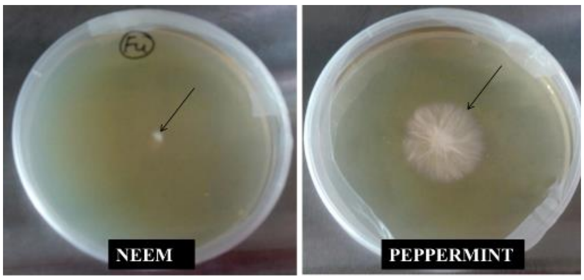

Fig 4 Petri plates showing the growth of *Fusarium graminearum* at the concentration of 2 µl/ml of neem and peppermint oil

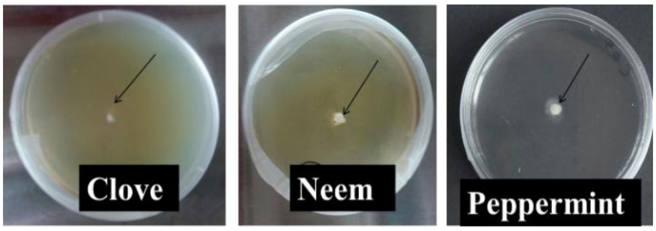

Fig 5 Petri plates showing the complete inhibition of *Fusarium graminearum* at the concentration of 5 µl/ml of clove, neem, and peppermint oil

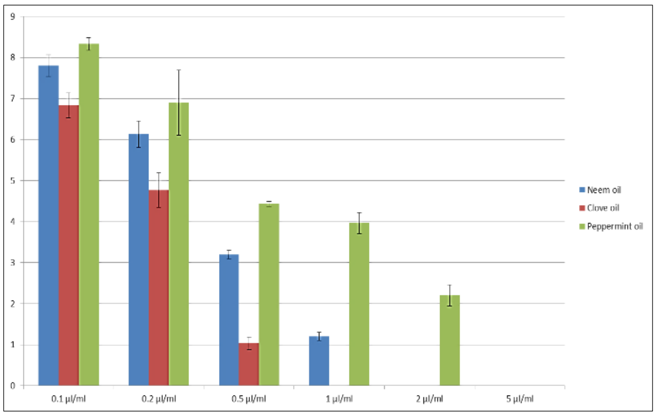

Fig 6 Graph showing the antifungal activity of clove, neem, and peppermint oil against *Fusarium gramineaum*

Phytopathogenic fungi are responsible for various crop diseases and causes negative effect on yield and productivity. These fungi not only damage the host plant but also produce mycotoxins, containing carcinogenic compounds and therefore causing severe health effects on human beings [15].

The control of fungal pathogens by chemical fungicides has been of significant help in increasing crop yield. However, the practice of these toxic chemicals is being discouraged as it leaves contaminated residues in soil, water, and food. Some chemicals pose a serious threat to the non-target organisms, which leads to ecological imbalance, and sometimes it may also develop the various resistant strains of pathogens. All these limitations necessitate an environmentally friendly plant disease management strategy i.e., biological control [16]. Biological control is considered to be a safe strategy as ithas no side effects and is an eco-friendly option to eradicate or control these plant pathogens.

Clove (*Syzygium aromaticum* L. Merrill and Perry) is one of the most valuable spices, belongs to the family Myrtaceae. The dried flower buds have been used for since long as a food preservative and in various medicines because of their antimicrobial, antifungal, stimulating, and anesthetic properties. Eugenol extracted from powdered cloves inhibited the growth of *Aspergillus flavus*, *A. fumigates*, *A. acculeatus*, and *A. versicolor* [17]. Clove oil rich in eugenol (approx. 90%) has also been reported to inhibit the growth of *Aspergillus niger* [18-19]. Clove oil exhibits the complete inhibition of mycelial growth of *Botrytis cinerea* also, which causes a great loss to our wine industry [20-21]. It was reported that if Clove oil and Cinnamon oil are mixed in a proper ratio, grapes can also be protected against postharvest decaying fungi such as *Aspergillus niger*, *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodiatheobromae*, *Phomopsis viticola* and *Rhizopus stolonifer* [22]. Similarly, the combination of Clove oil and Cinnamon oils at 3.0% was capable of providing complete protection in rubber wood particle boards against the growth of *Aspergillus* sp. and *Trichothecium* sp. for 9 weeks at 25°C and 100% RH [23]. It was also reported that anthracnose caused by *Colletotrichum gloeosporioides* can also be controlled by Clove oil [24]. The clove essential oil is significant against many spoilage bacteria, pathogens, and fungi as they show antibacterial and antifungal activities [25-27]. The clove oil in water nanoemulsion was found to be highly effective in regulating *Fusarium* growth and production of mycotoxin during the malting process [28].

CONCLUSION

Plant pathogens are biotic agents, causing serious damage to plant products. Throughout the world, these plant diseases should be regulated to maintain the quality and quantity of food and food products, as several developing countries are still facing starvation and malnourishment. Several techniques have been used to control plant diseases. Despite good agronomic and horticultural practices, growers are still dependent on chemical fertilizers and pesticides. These chemicals, no doubt have contributed extensively to crop improvement and crop productivity, but on the other hand, the excessive use of these chemicals is also responsible for environmental pollution and several diseases in human beings. Nowadays there are strict rules and policies on the use of chemical pesticides, and these hazardous chemicals should be removed from the market. Due to the extensive use of chemicals, severe environmental threat and health issues have been created, so keeping in mind, a search for alternative safe methods is inevitable. From our study, we can conclude that Clove, Neem, and Peppermint oil can be used as a biological control measure of the *Fusarium* disease of wheat. The result indicated that different herbal oils have different efficacy. Different oil owns various components which may be active against different fungal pathogens. The minimum inhibitory concentration of Clove oil is 1%, Neem it is 2% and Peppermint is 5%. (Plate with the lowest concentration of oil, showing no visible growth was regarded as MIC). Among all herbal oils, Clove oil is one of the best oils which can be used as an alternative against synthetic chemicals to prevent the disease. The principal component in Clove oil is eugenol (80-95%), and therefore its strong antifungal activity may be attributed to the presence of this component and can act as a fungicide. Treatments containing herbal oils seem to be having no side effects and are capable of providing effective control of fungal pathogen (*Fusarium*). It is a cheap, environmentally friendly, and safer method for wheat management. The application of these essential oils against food pathogens is an important step towards food safety and therefore human health also.

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