Effect of Essential Oils on Seed Borne Fusarium sp. and Seed Quality of Sesame (Sesamum indicum L.)

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ABSTRACT

Sesame (Sesamum indicum L.) is as an important oilseed crop and seed borne fungi are reducing the yield as well as quality of the seeds produced. The present study aimed to evaluate the effect of essential oils for control of seed borne fungi as well as enhancement of seed quality of sesame. Fusarium sp., was isolated from sesame seeds by agar plate method. Four essential oils such as camphor oil, cinnamon oil, clove oil and rose oil were screened for their antifungal potential against the isolated Fusarium sp., by poisoned food method. Selected essential oils were tested at different concentrations of 100, 200, 300, 400 and 500 ppm concentrations for their ability to inhibit the mycelial growth of test fungi. All the oils completely inhibited the mycelial growth of test fungi at 500 ppm concentration. The effect of selected essential oils on sesame seed quality was analyzed by standard blotter method. All the four oils increased the germination percentage and seed vigour while the fungal infection was completely inhibited without any phytotoxic effects. Comparatively, clove oil showed the best activity followed by camphor oil, cinnamon oil and rose oil. These results observed of the essential oils indicate the possible usage of them for the sesame seed treatment following further investigations.

Keywords: Antifungal potential, Eco-friendly management, Fungitoxicity, Seed health testing, Seed germination, Phytotoxicity

Introduction

India is one of the largest producers of oilseeds in the world and this sector occupies an important position in the Indian agricultural economy. India being the fourth largest edible oil economy in the world contributes about 10 per cent of the world oilseeds production [1]. Sesame seed (Sesamum indicum L.), commonly known as til in India, is largely produced for its oil and is also used as a flavoring agent. Sesame seeds have high nutritive value and are used in numerous cuisines. The seeds have high oil content around 55%. In the world market for sesame, India has a reputation of being one of the largest exporters of sesame exporting between 5 to 6 lakh metric tons of sesame annually [2].

Plant diseases, especially caused by seed borne fungi, are among the main factors reducing yield and quality of oil seeds including sesame seeds. From seed germination to harvest, soil-borne and seed-borne diseases reduce the vigour and yield of plants [3] and also infected seeds represent a primary source of infection in the field [4]. Furthermore, seed borne fungi produce mycotoxins that cause diseases to humans or animals fed with these seeds or food made by them [5]. Fusarium is an important pathogen of sesame. Fusarium moniliforme causes root rot and seedling blight [6] and sesame Fusarium wilt is caused by Fusarium oxysporum f.sp. sesamum [7]. Other fungi viz., Aspergillus flavus, A. niger, A. viridus, A. alba, Alternaria redicina, A. brassicola, Drechslera sp., Curvularia sp., Cephalosporium sp. and Penicillium sp. have also been isolated from sesame [8].

Seeds are generally treated with synthetic fungicides to manage the seed borne fungi. But the use of synthetic fungicides is associated with problems such as pollution, phytotoxicity and development of resistant pathogenic strains [9]. Post-harvest treatment of stored seeds with synthetic
fungicides is also not preferable as it influences the quality of seeds and causes serious health hazards for the consumers [10]. Therefore considerable interest has developed in the recent years for using more consumer- and nature-friendly protectants in the seed treatment [11]. Plants contain a broad spectrum of antimicrobial compounds that are effective antifungal agents and among them essential oils have become important seed-decontamination alternatives to synthetic seed preservatives [11]. Essential oils, being strong fungicidal compounds, are also biodegradable and show low toxicity to humans and animals [12, 13]. Essential oils are also reported to improve seed germination without cytotoxicity even at high concentrations [14, 15]. Seed coating of wheat with thyme oil could cause stimulation of seed germination and promotion of seedling growth by transient acidification of seed endosperms and embryos. The acidification of seed endosperm and embryo induced multiple effects such as reversing the inhibitory effect of abscisic acid, acidifying the cytosol and stimulating the electrogenic proton pump leading to increased germination, cell enlargement, and elongation of seedling. Treatment also increased the uptake of water by seeds causing interruption of dormancy, and promotion of germination and growth [15].

Many studies have documented the antifungal activities of essential oils against seed borne fungi [16, 17, 18, 19]. In the current study, effect of cinnamon, camphor, clove and rose essential oils on the Fusarium sp., isolated from sesame seeds was determined. In addition, effect of these essential oils on the seed germination and seed health was also investigated.

**Material and Methods**

**Isolation of seed-borne fungi from sesame seeds**

Random samples of sesame oilseeds were collected from local market. The standard method namely Agar plate method [20] was used for the isolation of fungi from oilseeds. The selected seeds were first surface sterilized with distilled water and then immersed in 0.2% sodium hypochlorite solution for 1-2 minutes, and again rinsed with distilled water and dried for almost 1 minute. 20 mL of sterilized Potato Dextrose Agar medium (PDA, Himedia) was poured into to the sterilized Petriplates of 9 cm diameter. After solidification, 25 sterilized sesame seeds were placed onto the PDA medium. In total 100 seeds were plated. The inoculated plates were incubated for a week at 26 ± 2°C under 12 h, alternating cycle of artificial day light and darkness. After incubation period, the seeds were observed for infection and the fungi occurring on each and every seed in the plates were identified preliminary on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic binocular microscope. The identification and further confirmation of seed borne fungi was made by observing them under compound microscope and with the help of standard manual [21]. After identification Fusarium sp., was sub-cultured onto PDA plates and maintained as a pure culture at 4°C for further studies.

**Effect of essential oils on the growth of Fusarium sp.**

Four essential oils such as cinnamon oil, camphor oil, clove oil and rose oil were studied for their effect on Fusarium sp., by poisoned-foood technique [22]. Stock solution of 10,000 ppm concentration of each essential oil was prepared by dissolving in acetone. Stock solutions were added at different concentrations separately to sterile PDA to obtain concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm and 500 ppm. The PDA medium with only acetone (500 ppm) served as control. The oil amended medium was poured into sterile 9 cm diameter Petriplates (20 mL/plate). Five mm mycelial agar disc of Fusarium sp., obtained from the margin of seven-day-old culture was inoculated at the centre of both control and essential oil amended PDA plates. All the plates were incubated at 26 ± 2°C with 12 h photoperiod. After 10 days of incubation the colony diameter was measured. The percentage mycelial growth inhibition (PI) with respect to the control was computed using formula PI = (C-T / C) × 100 where C is the colony diameter of the control and T is that of the treated combinations. The experiment was repeated three times.

**Effect of essential oils on seed quality (seed germination and infection)**

The surface sterilized sesame seeds were treated with different concentrations of essential oils such as 100, 200, 300, 400 and 500 ppm. The seeds were dipped into oil dilutions for one hour. The seeds treated with 500 ppm of acetone served as control. The treated and control seeds were blotted dry and subjected to germination test by standard blotter method [20]. One hundred treated and control sesame seeds were drawn randomly and were allowed to germinate between two layers of autoclaved blotter paper at 26 ± 2°C for 10 days to investigate the influence of essential oils on seed germination and seed health. After incubation, number of germinated and non-germinated (inclu-
Results and Discussions

Isolation of seed-borne fungi from sesame seeds

Most of the sesame seeds inoculated on to PDA plates gave rise to fungal colonies. A number of different seed borne fungi were isolated. Aspergillus ochraceus was most predominant followed by A. niger, Fusarium sp., Cladosporium sp., Mucor sp. and Rhizopus sp. (Figure 1). Fusarium sp., was identified based on cultural characteristics and sporulation and sub-cultured onto PDA medium. The pure cultures obtained were preserved at 4°C for further studies (Figure 2 and 3).

Effect of essential oils on the growth of Fusarium sp.

All the four essential oils tested (camphor oil, cinnamon oil, clove oil and rose oil) showed significant antifungal activity on Fusarium sp. All these oils completely inhibited the mycelial growth of the pathogen at 500 ppm. Clove oil showed comparatively better activity than other oils (Figure 4-7; Table 1).

Effect of essential oils on seed quality (seed germination and infection)

Essential oils significantly inhibited the fungal growth including the Fusarium sp., in the treated sesame seeds and also enhanced the percentage of germination. At 500 ppm concentration all the oils completely inhibited the seed borne fungi and showed better germination of seeds in comparison to control. The percentage germination was found to be proportional with the concentrations of oils while the fungal infection was inversely proportional to the concentrations of oils. Seed vigour of sesame seeds was positively influenced by the treatment with essential oils. Phytotoxicity of essential oils on sesame seeds was not observed even at the highest concentration of 500 ppm tested. Overall a significant improvement in the quality of sesame seeds was observed with the treatment of essential oils in comparison to the control (Figure 8 and 9; Table 2).

Good quality seed is an essential input in any agricultural production system. In addition to high germination and purity, good seeds should be free from pathogens [23]. Seed microflora brings about quality loss in seed by reducing germination and depletion of major seed constituents, along with contamination of the substrate by mycotoxins.
In this study, sesame seeds procured from the local market were found to be highly contaminated with fungi and definitely needed treatment. Seed treatment with synthetic fungicides reduces the seed borne infections and subsequently protects the seed from deterioration [25, 26]. However, excess application of synthetic fungicides for seed treatment causes phytotoxicity to the seedlings as well as death of soil beneficial flora and fauna [27]. Consumption of seeds contaminated with fungicides could harm human as well as animal health. The use of botanicals in seed treatment for the control of plants pathogens is an alternative technique to the conventional synthetic fungicides. Biologically active essential oils are potential source of an alternative, environmentally acceptable management strategy against seed borne fungi [11, 28].

Owing to these aspects the present research was undertaken to isolate the Fusarium sp., associated with sesame seeds, develop control measure using essential oils as well as determine the influence of essential oils on the seed quality of sesame. Several fungi including Fusarium sp., were isolated from sesame seeds by agar plate method. Many fungi, in addition to Fusarium sp., have been reported to be associated with sesame seeds [8]. Agar plate method is regularly used to isolate seed borne fungi and is a better method than standard blotter method as this yield a greater number of fungal species than the latter one [29].

All the four essential oils significantly inhibited Fusarium sp., and complete inhibition was observed at 500 ppm concentration. Essential oils are

### Table 1. Effect of essential oils on Fusarium sp.

| Sl. No. | Concentrations | Camphor oil | Cinnamon oil | Clove oil | Rose Oil |
|---------|----------------|-------------|--------------|-----------|----------|
| 1.      | Control        | 8.3 ± 0.16  | 8.3 ± 0.16   | 8.3 ± 0.21| 8.3 ± 0.16|
| 2.      | 100 ppm        | 3.6 ± 0.12  | 4.3 ± 0.12   | 2.3 ± 0.08| 3.6 ± 0.12|
| 3.      | 200 ppm        | 3.2 ± 0.08  | 3.4 ± 0.16   | 1.4 ± 0.09| 2.9 ± 0.10|
| 4.      | 300 ppm        | 1.7 ± 0.12  | 2.8 ± 0.08   | 0.9 ± 0.09| 2.6 ± 0.16|
| 5.      | 400 ppm        | 1.2 ± 0.08  | 1.6 ± 0.12   | 0.8 ± 0.10| 1.9 ± 0.08|
| 6.      | 500 ppm        | 0.0 ± 0.0   | 0.0 ± 0.0    | 0.0 ± 0.0 | 0.0 ± 0.0 |

Values given are mean of three replicates ± standard error

Figure 4. The antifungal activity of camphor oil on Fusarium sp., at concentrations of 100, 200, 300 and 400 ppm
Figure 5. The antifungal activity of cinnamon oil on Fusarium sp., at concentrations of 100, 200, 300 and 400 ppm.

Figure 6. The antifungal activity of clove oil on Fusarium sp., at concentrations of 100, 200, 300 and 400 ppm.
well known as antifungal agents and have been successfully employed for seed treatment by many researchers [11, 16, 28, 30]. Inhibition of seed borne phytopathogenic Fusarium sp., by essential oils has been reported [18, 31]. The essential oils were capable to exhibit antifungal activity against Fusarium moniliforme, F. solani, and other fungi through the damage of their cell wall and cell membrane to various degrees, cytoplasm leakage and by partial inhibition of DNA, RNA, and protein [32]. The essential oils have the ability to penetrate and disrupt the fungal cell wall and cytoplasmic membranes through a permeabilization process. They bring a change in the fluidity of membranes that cause electrolyte leakage and hinder cytochrome C pathways, proteins metabolism, and calcium ion concentrations. The permeabilization of inner and outer mitochondrial membranes

Figure 7. The antifungal activity of rose oil on Fusarium sp., at concentrations of 100, 200, 300 and 400 ppm

Figure 8. Effect of essential oils on seed germination at 500 ppm concentration

Figure 9. Effect of essential oils on seed vigour at 500 ppm concentration
may result in the cell apoptosis or necrosis leading to cell death [33]. Essential oils may also act against fungi by the inhibition of ergosterol biosynthesis [34]. Essential oils can also cause inhibition of ATPase that leads to intracellular acidification and fungal cell death as well as mitochondrial dysfunction-induced ROS accumulation that can lead to the death of fungal cell [35]. Concentration-dependent antifungal activity was observed in poisoned food bioassay wherein the colony diameters decreased with increase in the concentration of essential oils. Essential oils were reported to show such concentration-dependent antifungal activity [31]. Seed health testing studies by standard blotter method revealed the efficacy of tested essential oils in improving the seed quality of sesame without any phytotoxicity. Standard blotter method is a recommended method for routine seed health diagnosis as the method is simple, sensitive and reproducible [36]. Effect on seed germination is generally studied as a bioassay for phytotoxicity [37]. Seed coating of wheat with thyme oil increased germination rate and enhanced seedling growth development [15]. Essential oils of higher plants were not phytotoxic on seed germination and seedling growth of guar, Cyamopsis tetragonoloba L. (Taub.) even at 2000 ppm [14]. The germination of tomato seeds [38] and soybean seeds [39] were not negatively affected by treatments with essential oils.

**Conclusion**

Results obtained from this study confirm the effective fungitoxicity of four essential oils (camphor oil, cinnamon oil, clove oil and rose oil) on *Fusarium* sp., associated with sesame seeds. They also, in addition to inhibiting the fungal mycoflora of sesame seeds, increased the seed germination and vigour while phytotoxic effects on seeds were not observed. Although significant activities were observed by all the four oils, clove oil demonstrated better activity in all parameters studied than camphor oil, cinnamon oil or rose oil. These essential oils could be recommended for sesame seed treatment to get higher germination and healthy seedling that will eventually increase sesame production as well as to maintain seed quality during storage. However, it is imperative that further studies should be carried out to better understand the mode of action of these oils against the pathogenic fungi as well as the effects observed on seed quality.

**Acknowledgment**
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