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Analysis on Inhibiting Pathogenic Activity of Fungi *Curvularia lunata* by Essential Oils

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ABSTRACT

Essential oils play a major role in the management of phytopathogenic fungal diseases and come forward as a replacement of chemical fungicides. Many of the medicinal and aromatic plants essential oils are used for its preservative, and pesticidal properties. The study of how essential oils affect the phytopathogenic fungal diseases is not widely studied by many researchers, specifically, the essential oils which we have taken. This compelled us to conduct an original research for *Ageratum conyzoides*, *Zanthoxylum armatum*, and *Mentha arvensis* and fungi *Curvularia lunata*. The essential oils of *Ageratum conyzoides*, *Zanthoxylum armatum*, and *Mentha arvensis*, were procured from fresh herbage sampled from the Lucknow region of India. However, *A. conyzoides* was also sampled from the Lakhimpur, Pantnagar, and Kanpur region of India. These plant-based essential oils were extracted by the hydrodistillation method and were stored for further studies. The antifungal activity was examined by the use of poison food techniques. Hundred percent of fungal (*Curvularia lunata*) growth inhibition showed by the use of 0.2 mg/ml and 8 mg/ml concentration of *A. conyzoides* and *Z. armatum* essential oils respectively. *C. lunata* is the major cause of leaf spot/leaf blight disease on economically important crops and causing heavy loss of economy. *M. arvensis* essential oil showed greater antifungal activity as compared to its major compound menthol and DMO via poison food technique. Overall, the study revealed the antifungal activity of essential oils against *C. lunata*. Moreover, the essential oil of *M. arvensis* shows the higher inhibition as compare to the menthol/DMO against the *C. lunata*.

KEY WORDS: AGERATUM CONYZOIDES; ZANTHOXYLUM ARMATUM; MENTHA ARVENSIS; CURVULARIA LUNATA; ESSENTIAL OIL.

INTRODUCTION

Foliar region of plant provides the favourable habitat for epiphytic microorganisms, many of which are capable to influence the growth of pathogens. More than 12% reduction of world crop yield reported due to pre- and post-harvest diseases and FAO proposed that about 20 to 40 percent of global crop yields losses per year by the plant pest and diseases (Agrios, 1997; FAO, 2020). The fungal disease causes major yield reduction and economic losses in agriculture and forestry of the world.

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Among them, the foliar disease is one of the major constraints. Medicinal and aromatic plants (MAPs) have been commercially cultivated as a source of important medicinal constituents and essential oil in various parts of the world but adversely affected by many fungal diseases in the humid season. Fungal pathogens infect MAPs under the favorable condition and produce a serious threat to their commercial cultivators. In the present scenario, the infection of *Curvularia* spp. (Singh et al. 2020). on the commercial fields of MAPs increases day by day which adversely affected the economic yield of the crops. *Curvularia* belongs to family Pleosporaceae, the largest family in the Pleosporales (Zhang et al., 2012; Hyde et al., 2013; Wijayawardene et al., 2014).

Leaf blight and leaf spot are the most common foliar diseases caused by the *Curvularia* spp., and posed to potential threats in many important crops. *Curvularia lunata* seems to infect a wide range of medicinal and aromatic crops such leaf spot of *Zatropha curcus* in Mexico, leaf blight of *Ocimum basilicum*, Leaf spot of *Mentha arvensis*, and leaf spot of *Acorus calamus* in India (Thakur et al., 1974; Cisnero-Lopez et al., 2012; Srivastava et al., 2015; Srivastava et al., 2019). The Indian trade of medicinal and aromatic products is approximately 120 million US$ per annum. India is the biggest producers of MAPs and potential supplier of essential oils to the world market (Tripathi et al., 2016).

The foliar diseases (leaf spot and leaf blight) cause the economic losses and reduced the shelf life and market values of the food commodities. Thus, it is essential to manage the foliar disease with biological features without any harm the quality of MAPs. *Ageratum conyzoides* L., is an annual herb belong to the family Asteraceae, also known as billygoat-weed, chick weed and white weed. It used as medication in Asia, Africa, and South America (Vera, 1993; Okunade et al., 2002; Singh et al., 2013; Moore et al., 2020).

The essential oil of *A. conyzoides* has the potential antimicrobial, antiaflatoxigenic and antioxidant activity (Patil et al., 2010; Osho et al., 2011). *Zanthoxylum armatum* DC. (Rutaceae) is a shrub, and widely distributed in Asian region. The leaf, seed and fruit essential oil of *Z. armatum* has antibacterial, antifungal and anti-inflammatory activity (Mehta et al., 1981; Guo et al., 2010; Sati et al., 2011). *Mentha arvensis* L. belongs to the family Lamiaceae, native to temperate regions of Europe, western and central Asia, east to the Himalaya and eastern Siberia and North America. A lot of antimicrobial activity recorded of *M. arvensis*. The major bioactive compounds of *Mentha* are menthol, menthone, eugenol and pulegone (Luca et al., 2011; Moghtader, 2013; Akbar et al., 2014).

Herbal-based approaches come forward for development of new strategies to manage the plant diseases. A lot of plants essential oil used in the management of plant diseases and reduced the fungal as well as bacterial growth. Some plants like *Origanum vulgare*, *Cuminum cyminum*, *Eucalyptus citriodora*, *Thymus vulgaris*, *Cymbopogon citrates*, *Lavandula officinalis*, and *Zingiber officnalae* have essential oils that are reported for its antifungal activity (Lee et al., 2007; Cosic et al., 2010; Saroj et al., 2018; Raveau et al., 2020). In this study, *A. conyzoides*, *Z. armatum*, and *M. arvensis* essential oil used to the management of fungal pathogen *C. lunata*, cause leaf spot/leaf blight disease. The comparative antifungal activity of Menthol, Dementhol (DMO), and *M. arvensis* essential oil against *C. lunata* also studied in this paper.

MATERIAL AND METHODS

Plants and essential oils: The fresh *A. conyzoides* plant sample was collected in the plastic bag from the Lakhimpur, Pantnagar, and Kanpur, Uttar Pradesh, India. Whereas *Z. armatum*, and *M. arvensis* plants were sampled from the CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India. Sampled plants were taken quickly to laboratory and the fresh herbages were processed for essential oil extraction by the hydrodistillation method in the clevenger type apparatus (Maji et al., 2013). Extracted oils were purified and collected in to glass vial for further use.

***In vitro* screening of essential oils:** Potato dextrose agar (PDA) plates have been prepared by adding different concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 mg/mL) of essential oil {(A. conizoides (code L1, P1 and K2)}at 40- 45°C. To ensure the proper mixing of essential oil, 0.05% Tween-80 was added. A 6 mm disc of *C. lunata* was placed on the PDA filled Petri plates. All Petri-plates were incubated at 27±1°C. Plates without essential oil served as a control. The effect of essential oil on the growth of *C. lunata* was measured after five days of incubation. Minimum inhibitory concentration (MIC) was calculated using poison food technique (Grove and Moore, 1962; Knobloch et al., 1989; Nene et al., 1993). Percentage of the growth inhibition was calculated as follows:

$$\% \text{ of growth inhibition} = \frac{\text{Growth in control} - (\text{Growth in treatment})}{(\text{Growth in control})} \times 100$$

***In vitro* screening of chemical fungicides:** By using the poison food technique, the fungicides Ridomil Gold and Bavistin are also tested for antagonistic activity against *C. lunata* in the concentration of 0.2, 0.4, 0.6, 0.8mg/mL. This study helps in the comparative study of chemical fungicide against selective essential oils.

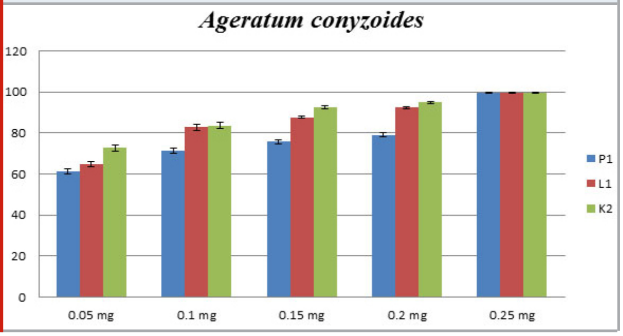
RESULTS AND DSICUSSION

Management of fungal diseases on plants using synthetic chemicals is more in practice. However, they possessed hazardous effect on plant, environment and human health. Thus, an alternate approach, employing of natural products viz Singh et al. (2019). Essential oil and chemical substances of plant origin, gained attention for the *in vitro* management of diseases caused by a plant pathogen. Here *Z. armatum*, *A. conyzoides*, and *M. arvensis* screen for the antifungal activity against the growth of *C. lunata* by Poison food technique.

Screening of essential oils against the growth of *Curvularia lunata*: Our results on in vitro screening showed that essential oils of *Ageratum conyzoides*, *Zanthoxylum armatum*, and *Mentha arvensis* proved highest antagonistic activity against the *Curvularia lunata* cause leaf spot/leaf blight diseases.

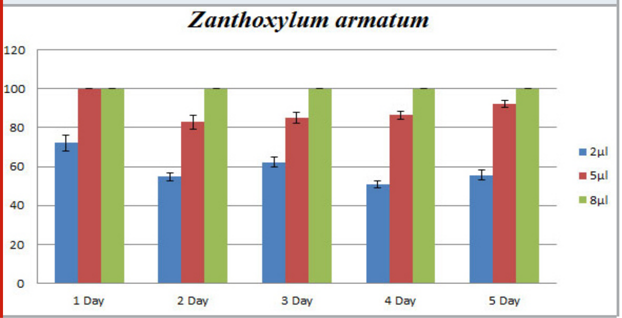
Ageratum conyzoides: Antifungal activity of *Ageratum conyzoides*, isolated from the different geographical location (Lakhimpur (L1), Pantnagar (P1), and Kanpur (K1) district of Uttar Pradesh) showed a similar type of growth inhibition. The concentration of 0.2mg has revealed a higher percentage (more than 90%) of inhibition as compared to others three concentrations of essential oils against *C. lunata* (Fig.1). The MIC was measured as 0.25 mg/mL, showed 100% of inhibition.

Figure 1: Effect of P1, L1 and K2 essential oils on phytopathogenic *C. lunata*. Percentage of growth inhibition measured using poison food technique. (P1, L1, and K2 shows that *Ageratum conyzoides* get from the different geographical location as Pantnagar, Lakhimpur and Kanpur respectively).



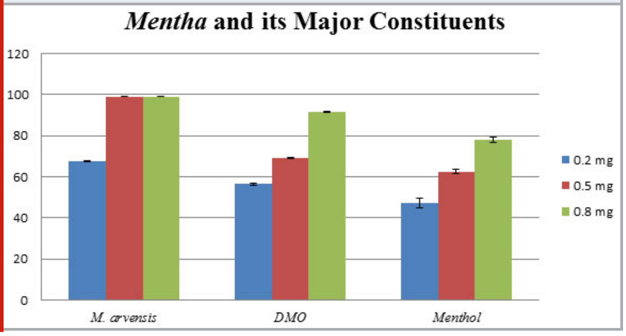
Zanthoxylum armatum: *Z. armatum* (8µl) showed 100% of inhibition of *C. lunata* by applying poison food technique while 5µl oil resulted in 80-100% of inhibition. Bar diagram revealed the percent of inhibition by applying 2, 5, and 8µl of essential oil continuously for five days (Fig.2).

Figure 2: Effect of *Z. armatum* essential oil on phytopathogenic *C. lunata*. Percentage of growth inhibition studied using poison food technique.



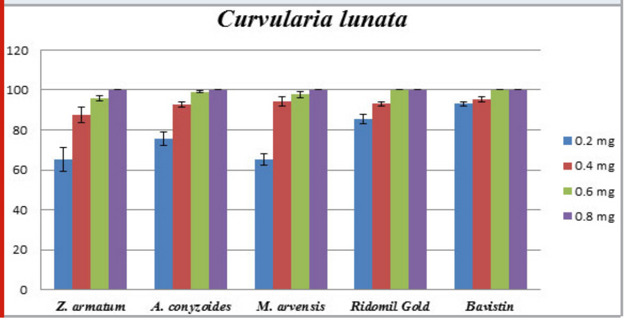
Mentha arvensis: Menthol is the major constituent of *M. arvensis* essential oil, discussed and proved its potential by various researchers as described in the review of literature section. Removal of menthol from *M. arvensis* essential oil, Dementhol or DMO remains present in the oil. Comparative analysis among menthol, DMO and *M. arvensis* oil; *M. arvensis* oils show greater antifungal activity against *C. lunata* as compared to menthol and DMO as denoted in the bar diagram (Fig.3).

Figure 3: Comparative effects of *M. arvensis* EO, DMO, and Menthol against phytopathogenic *C. lunata*. The mean of three independent experiments shows the percentage of growth inhibition using poison food technique.



Comparative study of Essential oil vs. specific fungicide: From above result, it shows that *Z. armatum* (8mg/mL), *A. conyzoides* (0.25mg/mL) and *M. arvensis* (0.8mg/mL) showed 100% of inhibition against *C. lunata* by applying poison food technique. Results in the case of selective chemical fungicides such as Ridomil Gold and Bavistin demonstrated 100% of inhibition when applied with a concentration of approx 0.5 mg/mL against *C. lunata*. Therefore, selective essential oils showed results to be at par as selected chemical fungicides (Fig.4).

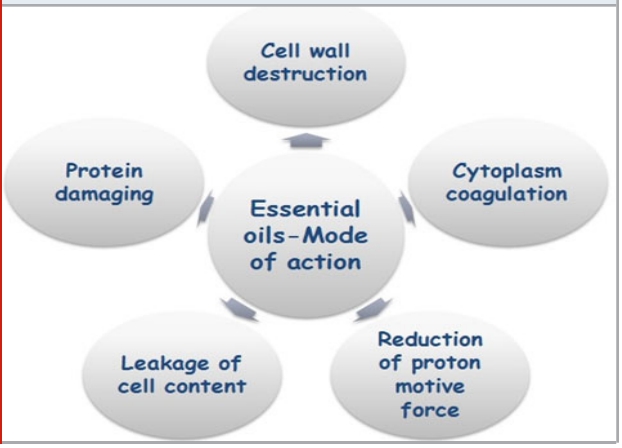
Figure 4: Comparative effect of *Z. armatum, A. conyzoides, M. arvensis* oils, Reidomil Gold, and Bavistin on phytopathogenic *C. lunata*. Percentage of growth inhibition established using poison food technique.



Many researchers have studied the antagonistic activity of essential oils and its compound against bacteria and fungus. Essential oils are the mixture of compounds so, it is most likely to show antimicrobial activity is not due to a single compound therefore it may be a synergistic/ synchronous effect (Skandamis et al., 2001; Carson et al., 2002).

Proposed Mechanism of fungal inhibition by essential oil: The antifungal activity of essential oils and their components is based on the feature of essential oils that plays a critical role in antagonistic activity is hydrophobicity interaction with the cell membrane. It enables them to penetrate in the lipids of the cell membrane and mitochondria; disturbing the cell structure and causing cell lysis/cell bursting resulting into the leakage of ions and other cell contents (Knobloch et al., 1989; Sikkema et al., 1994; Oosterhave et al., 1995; Helander et al., 1998; cox et al., 2000; Lambert et al., 2001; Skandamis et al., 2001; Ultee et al., 2002; Carson et al., 2002; Pragadheesh et al., 2013). Figure 5 shows the mode of action of essential oils on the fungal cell.

Figure 5: Diagrammatic representation of essential oil actions on fungal cell



Expansion in population and food demand lead the development of new strategies for enhancing the agricultural crops resistant to any constraint. To avoid phytopathogenic diseases conventional pesticides applied by the farmers on the agricultural crop. However, conventional pesticides are cheap and lead to loss of nutrients in the soil and agricultural products as well (Singh et al., 2020). Thus, biological pesticides come in light, which is effective on plant diseases and sustain the quality of soil and plant products. Essential oil is the most effective biological pesticides used in the management of plant diseases. However chemical compositions of the essential oils are very complex because of the huge number of different groups of chemical constituents present in it. Thus, it is most likely that their antimicrobial activity is not due to one compound alone or by single mechanism but might be there are several targets in the cell (Skandamis et al., 2001; Carson et al., 2002; Raveau et al., 2020).

CONCLUSION

Fungi are the major causative organism of plant diseases on the MAPs as well as agricultural crops. The incidence of *C.lunata* fungi seems to be higher on the MAPs field shown in the recent years. Thus, the management of leaf spot/leaf blight diseases on sustainable basis should be necessary for save the economy of our country. In the present study, *M. arvensis* essential oil shows greater antifungal activity as compared to its major compound menthol and DMO via poison food technique. It means that there is a synergistic effect of menthol and another compound present in the oil to show greater antifungal activity while menthol or DMO show lesser activity. Along with *M. arvensis, A. conyzoides* and *Z. armatum* also exhibited significant pharmacological and biocidal activity.

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