Research article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Antifungal Efficacy of Essential Oils against common bean Anthracnose caused by *Colletotrichum lindemuthianum*

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ABSTRACT

Fifteen essential oils were evaluated to determine the fungitoxicity against anthracnose of common bean caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara. For screening of antifungal susceptibility in each essential oil, healthy control and fungicide control were set to verify growth inhibition zone diameters. After bioassay, oils of lemon grass and peppermint were found to be most effective with complete inhibition followed by oils of winter green and geranium against test fungus. If anthracnose is found in the field, spread of disease can be limited by the use of fungicides. To protect agricultural crops enormous amount of synthetic pesticides are used worldwide resulting ill-effects on the life and life supporting systems. Efforts are thus being made world over to replace these synthetic chemicals with safer and eco -friendly alternatives. The plant world comprises of a rich storehouse of biochemical that could be tapped for use as fungicide. Given the abundance of flora in India, botanical fungicides have a great potential in integrated disease management in the context of eco-friendliness and sustainable agro-ecosystem.

KEYWORDS: Bean anthracnose, *Colletotrichum lindemuthianum*, essential oil, antifungal activity

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INTRODUCTION

A number of economically significant pests, pathogens are seed-borne and seed transmitted[1]. A list of more than 1500 fungi belonging to 287 genera reported to be seed-borne in 534 crops of cereals, millets, vegetables and ornamental etc [2]. Seed transmission of pathogens is the most important mode of long distance dissemination and carry over from season to season. World over, pests (especially weeds, pathogens and insects) are the largest competitor of agricultural crops and severely reduce the crop production in the range of 25-50% [3]. Seed-borne /transmitted fungi thus play a crucial role in the development of disease in the field and also in the trans-boundary movement of pathogens along with the exchange of plant genetic resources (PGR) to be utilized in crop improvement programmes in the country.

About one third crop losses worldwide are attributed to plant pathogenic micro-organisms including fungi, bacteria and viruses. Bean anthracnose, caused by the fungus *Colletotrichum lindemuthianum, is* a major disease of beans (*Phaseolus vulgaris* L.), causing serious crop loss in many parts of the world. In 1921, M. F. Barrus of Cornell University demonstrated that bean anthracnose is seed borne. This information resulted in the widespread use of plant treated, diseased free seed to minimize the risk of anthracnose infection.

The use of plant-derived pesticides against crop pests both on the field and during post-harvest is now emerging as one of the important means to be used in crop protection under an Integrated Pest Management framework following the multiple global challenges created by synthetic chemical pesticides. Interest in the antifungal activity of essential oils has increased markedly in recent years also because of drug resistance in microbes. The volatile (vapor) components of several essential oils have been demonstrated to have potent antifungal activity. Essential oils from various aromatic plants are known to show a wide spectrum of anti-microbial activity. Antibacterial properties in the floral petals of many higher plants have been reported[4] .Efficacy of essential oils for control of seed-borne pathogens has been evaluated[5].

Essential oils of some higher plants have recently been proved successful in providing effective control of storage losses and post harvest fungal diseases of plant origin[6, 7, 8]. Very little work has been done in case of *Colletotrichum* spp.[9, 10,11,12,13] and need to explore the scope of utilization of biopesticides to control anthracnose of common bean. Effective management of a plant disease is a key to save plants from diseases caused from microbes, since plants are significant as they are both economical and aesthetic.

MATERIAL AND METHODS

Collection of samples

Essential oils were procured from Jain super store, Palika Bazar, Connaught place, New Delhi for bio-assay. Seed material from different districts (Chamba, Shimla and Hamirpur) of Himachal Pradesh was collected to get a pure culture of *Colletotrichum lindemuthianum*.

Isolation and maintenance of pathogen culture

Seeds of *Phaseolus vulgris* were examined for seed health status. After visual and stereo-binocular microsocpical detection seeds with disease symptoms like discoloration, pigmentation, spores/conidia and fruiting body fruiting bodies (acervuli) and subjected to blotter test by placing them on 4 layers of moistened blotter discs of 110 mm. Plastic Petri plates were incubated for 7 days at 21^{O} (±1) C under alternate cycle of light and darkness (12h each) [14]. Mycelium were picked up with the help of sterilized needle and cultured on PDA in sterilized Petri plates. Pure culture were prepared and maintained for inhibition studies.

Screening of extracts (antifungal assay)

15 essential oils from 11plant families were bio –assayed by poisoned food technique [15]. Appropriate quantity of the desired essential oil was added to a pre-autoclaved adequately cooled PDA. Tween 20 was also added to the media to get better suspension. The medium was then dispensed into sterilized petripates. PDA without any extract served as control, bavistin (0.02%), mancozeb (0.25%) and a binary mixture of bavistin & mancozeb, (1:1 w/w)(B+M) served as fungicide control for comparison. Five replicates were kept for each treatment and incubated for seven days at 21^{O} (±1) C at alternate cycle of light and darkness. Radial growth of colonies was measured at two points along the diameter of the fungal colony. The growth of the colonies in control sets was compared with that of various treatments and calculated the percent inhibition by using this formula:-

Percent Inhibition= $\frac{C-T}{C} \times 100$

Where, C= radial diameter of the colony in control, T= radial diameter of the colony in treatment

Completely randomized design was used in carrying out ANOVA from which LSD at 5% level was computed for each test concentration.

RESULT AND DISCUSSION

Table No.1: *In-vitro* effect of essential oils with radial growth (mm) and percent inhibition of *Colletotrichum lindemuthianum* on eighth day of incubation

Botanical name/Family	Common name	Concentration (PPM)	Radial growth (mm)	Percent Inhibition
Pimpinella anisum (Apiaceae)	Anethloe Oil	500 1000	72.75 66.40	19.00 26.22
Cymbopogon winterianus (Poaceae)	Citronella Oil	500 1000	74.60 67.40	17.00 25.11
Tagetes erecta (Asteraceae)	Tagetes Oil	500 1000	75.40 71.20	16.00 20.89
Cymbopogon martini (Poaceae)	Palma Rosa Oil	500 1000	75.00 66.60	16.67 26.00
Juniperus virginiana (Pinaceae)	Cedar Wood Oil	500 1000	74.20 68.80	17.56 23.56
Laurus nobilis (Lauraceae)	Kranj Oil	500 1000	75.40 66.20	16.22 26.44
Ocimum basilicum (Lamiaceae)	Basil Oil	500 1000	69.20 74.80	23.11 16.89
Gossypium.hirsutum (Malvaceae)	Cotton seed Oil	500 1000	72.00 67.00	20.00 25.56
Cymbopogon citratus (Poaceae)	Lemon Grass Oil	500 1000	0.00 0.00	100.00 100.00
Gaultheria procumbens (Ericaceae)	Winter Green Oil	500 1000	26.50 0.00	70.56 100.00
Pogostemon cablin (Lamiaceae)	Patchauli Oil	500 1000 1500 2000	76.00 59.60 36.33 13.67	15.56 33.78 59.63 84.81
Pelargonium graveolens (Geraniaceae)	Geranium Oil	500 1000 1500 2000	77.00 53.60 0.00 12.00	14.44 40.44 100.00 86.67
Eucalyptus globulus (Myrtaceae)	Eucalyptus Oil	500 1000 1500 2000	57.60 44.20 36.67 21.00	36.00 50.89 59.26 76.67
Mentha piperita (Lamiaceae)	Peppermint Oil	500 1000	0.00 0.00	100.00 100.00
Azadirachta indica (Meliaceae)	Neem Oil	500 1000 1500 2000	55.00 48.60 29.00 71.00	38.89 46.00 67.78 21.11
Bavistin	-	0.02%	0.00	100.00
Mancozeb	-	0.25%	0.00	100.00
Bavistin+mancozeb (B+M)	-	(1:1)	0.00	100.00
Control	-	-	90.00	0.00
LSD (5%)		500 1000	1.9 2.8	-

The average radial growth of pathogen on eight day of incubation and their percent inhibition with various essential oils are mentioned in Table 1. On the basis of percent inhibition of the fungi at different concentrations of the essential oils, the degree of fungi toxicity was clustered in three broad group *viz;* high active (71-100%). active (51-70%) moderately active (31-50%) and least active (0-30%).

We started the dose dependent bio-assay with 500 & 1000 PPM and found oils of Lemongrass and peppermint were giving complete inhibition on 500 PPM than we proceeded further with lower concentrations (100-500) and found both the oils were exhibiting highest efficacy as that of reference fungicides (Fig.1). Zambonelli *et al.* also found *Mentha piperata* fungicidal against test fungus. Other oils with at least moderately activity on 1000 PPM were selected and screened with higher concentrations (1000, 1500 & 2000) and rest with least activity were rejected for screening. Oils of winter green and geranium both ranked in first category with complete inhibition at 1000 & 1500 PPM.

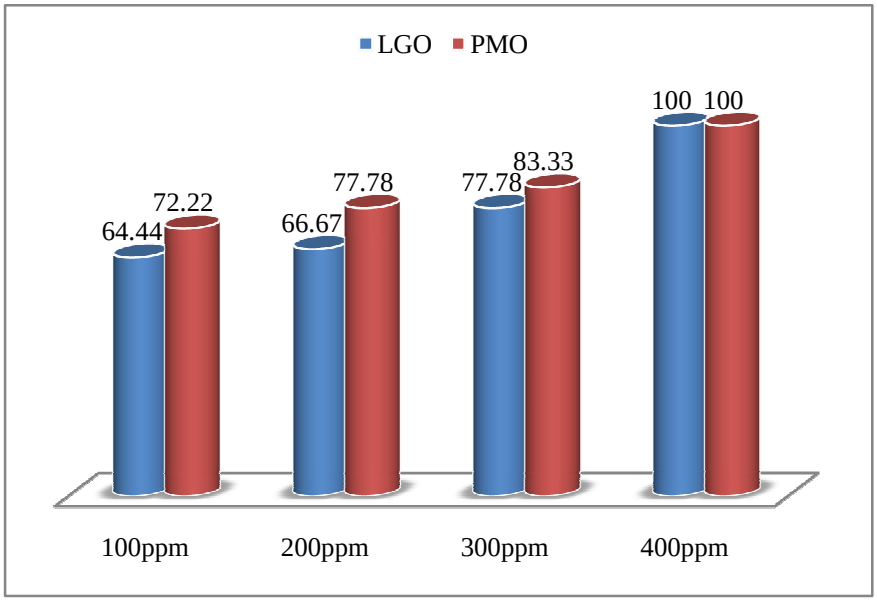

Fig.1 Percent Inhibition of oils of Lemon grass and Pepper mint

In poaceae family only lemon grass oil is effective with highest fungitoxicity while oils of palma rosa & citronella belonged to fourth category respectively with least inhibition of 26 & 25.11%. At 2000 PPM oils of patchouli & eucalyptus showed highest category with percent inhibition of 84.41&76.67 respectively.

Eucalyptus species not only provide fuel mass and reduce atmospheric carbon dioxide levels directly but also used as pesticidal agents. Eucalyptus oil showed its inhibitory action against *C. lindemuthianum*. In fact eucalyptus oil has been known for hundreds of years as antibacterial, antifungal and antiseptic in nature[16.] Whilst eucalyptus oils have a sturdy toxicity in the vapor form against a wide range of microbes and insects, and they could be commercially exploited as a fumigant for stored products.

Neem, basil and geranium oils after getting their maximum inhibitory action at specific concentration, whereas accelerated the growth of fungal mycelium too. We observed the rest of oils were least fungitoxic with lowest percent inhibition against test fungi.

LD50 determination

The purpose of estimating different concentrations is to determine the antifungal activity with its LD50 value which is sufficient to kill fungus and to use the estimate to make comparisons. It is generally easiest to estimate the median (50%) response level of the population. Lethal dose (LD50) of the test essential oils were determined by using GW-Bascis LD50 programme.

Table No. 2. LD 50 value of promising essential oils

Essential oil	**LD 50(ug/ml)**
Cymbopogon citratus	380.27
Gaultheria procumbens	1157.85
Pogostemon cablin	951.23
Pelargonium graveolens	951.23
Eucalyptus globulus	438.72
Mentha piperita	110.61
Azadirachta indica	292.23

CONCLUSION

Present investigation revealed that oils of lemongrass, peppermint, along with wintergreen and geranium were found to be most fungicidal with complete inhibition but lemongrass & peppermint were considered to be more effective and promising to be continue with for *in-vivo* study because they gave complete inhibition at lowest concentration as compared to others. Implications caused by fungi mean there is a constant striving to produce safer food crops and to develop new antifungal agents. It is clear from above discussion that essential oils posses a wide spectrum of antifungal activity and provide a simple, inexpensive, and environmental friendly alternative pest control. The oil of lemon grass and pepper mint also be exploited for seed treatment and under field conditions in ensuring effective disease management. These are the findings with possible potential application in augmenting the crop yields.

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