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In vitro effect of essential oils on Mushroom pathogen *Mycogone perniciosa* causal agent of Wet Bubble Disease of White Button Mushroom.

KEYWORDS: *Mycogone perniciosa*, *Agaricus bisporus*, essential oils

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ABSTRACT *Clove, castor, eucalyptus, olive, citrullina oil and Cinnamic aldehyde were tested for their antifungal activity against Mycogone perniciosa causal agent of wet bubble disease of Agaricus bisporus. Cinnamic aldehyde and Eucalyptus oil were found to substantially inhibit the growth of M. perniciosa, having a fungicidal effect at a concentration of 2.5 and 5 µlL^{-1} for cinnamic aldehyde and 2.5, 7.5 µlL^{-1} for Eucalyptus oil, it was also found that at these concentration lower toxicity towards host strains was demonstrated. This clearly suggests that essential oils can be introduced in mushroom industry either for prophylactic control or to replace the use of synthetic fungicides to prevent losses due to wet bubble disease.*

Introduction
M. perniciosa (Magnus), the causal agent of Wet bubble disease of mushroom is an important fungal pathogen of white button mushroom, *Agaricus bisporus* (Umar *et al.,* 2000). Fruiting bodies infected with *M. perniciosa* become large, irregular tumorus fungal masses are formed (Dielemann-Van Zaayen, 1976). Young mushroom pinheads infected with *M. perniciosa* develop amorphic shapes, which often do not even resemble mushrooms (Sharma and Kumar, 2000).

Certain synthetic fungicides (prochloraz-Mn, carbendazim) and chemical agents (formalin, salts or Lysol) are used to get quick and satisfactory results for control of the mycopathogen (Yang Wang *et al.,* 2005; Pieterse, 2005; Gea *et al.,* 2010). The widespread use of chemicals has significant drawbacks like increased production cost, handling hazards and residual levels in food which can be a potent threat for human health. An alternative to these chemical agents is the use of certain plant derived oils with antifungal properties. Antimicrobial properties of certain essential oils have already been known for a long time (Rios and Recio, 2005). Previous *in vitro* experiments by Tanovic *et al*. (2009) showed that the volatile phase of certain essential oils such as those of Scots pine, eucalyptus, juniper, orange, rosemary, and thyme, applied at a concentration of 0.65 µl/ml of air, inhibited mycelial growth of soilborne pathogens like *Fusarium* spp., *Rhizoctonia* sp., and *Pythium* sp.

In this study, different oils -Clove, castor, eucalyptus, olive, citrullina and cinnamon (cinnamic aldehyde) were selected on the basis of available literature to find their ability to inhibit the growth of *M. perniciosa*, without affecting the development and yield of the edible mushroom, *A. bisporus.*

Materials and Methods:
Organisms and culture conditions: Reference *A. bisporus* strains were collected from Directorate of Mushroom Research (DMR), Solan, India with depository number DMRA-1, DMRA-13 and DMRA-11 representing the commercial strains S11, U3 and P1, respectively of *A. bisporus* and were subcultured on Potato Dextrose Agar medium at 25°C for two weeks and maintained on slants under refrigerated conditions at 4^{0}C.

Diseased mushrooms fruit bodies infected with wet bubble disease were collected from different mushroom farms in Punjab and pure culture of *M. perniciosa* was isolated. The mycopathogen was maintained on potato dextrose agar (PDA). The cultures were stored at 4°C and subcultured once a month (Booth, 1971).

Sensitivity of pathogen and host strains – Essential oils viz. Clove, castor, eucalyptus, olive, and cinnamic aldehyde (HiMedia) and citrullina oil (pure extract collected from Department of Agronomy, PAU, Ludhiana), were used for the present study. The oils along with tween-80 to form a stable emulsion were dissolved in sterile distilled water to make stock solution of each oil. Seven day old fungal culture grown on potato dextrose agar (PDA) was used as inoculum in the form of 5 mm disc with a sterile cork borer and was transferred aseptically to the centre of petriplates (in triplicates) containing PDA medium with the varying concentrations of the oils and incubated at 25±1˚C for 15 days. Observation on fungal colony growth was recorded and the percentage inhibition of mycelial growth was calculated (Vincent, 1947).

The selected oils were also tested for their ability to inhibit the pathogen in submerged culture (in triplicates). The stock solution of each of the oil was then added in 40 ml of potato dextrose broth to achieve range of 2.5-10µlL^{-1} and inoculated with 5 mm agar plug taken from edge of an active mycelium. Dry weight of the mycelial biomass was recorded after 15 days of incubation at 25±1˚C.

Results and Discussion
Inhibitory effect of the phytochemicals tested both in solid and liquid state against pathogen and different host strains gave different degrees of percentage inhibition (Table 1 and Fig. 1). The most effective oil in inhibiting the growth of pathogen was cinnamon oil in the form of cinnamic aldehyde (2.5 and 5 µlL^{-1}) and eucalyptus oil (2.5 and 7.5 µlL^{-1}) with negligible inhibition on the host strains. Other oils like citrullina, clove, olive and castor effectively controlled the mycelial growth of pathogen but they also elicited higher fungicidal effect against the three host strains. Cinnamic aldehyde (2.5 and 5 µlL^{-1}) and eucalyptus oil (2.5 and 7.5 µlL^{-1}) tested on solid media in PDA plates were effective in inhibiting growth of mycoptahogen. The results were further compared with positive control plate and a clear variation in the growth pattern of mycopathogen was observed. (Fig. 2).

Essential oils have been reported to control various fungal diseases related to vegetable and citrus crops (Du Plooy *et al.,* 2009). However, use of such oils in mushroom industry is of great problem as in this case both the antagonist and the crop of interest are fungi. Essential oils have been reported

Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

to have antimicrobial effects (Dzamic *et al.*, 2008). Among 40 oils tested for their ability to prevent growth of *M. peniciosa in vitro* as well as *in vivo*, it was found that Lemon verbena (*Lippia citriodora*), lemongrass (*Cymbopogon citratus*) and thyme (*Thymus vulgaris*) oils were able to substantially inhibit the growth of the pathogen (Regnier and Combrinck, 2010). Oregano oil (*Origanum marjorana*), characterized by high levels of thymol and carvacrol, has been reported to be useful in inhibiting *M. perniciosa* and *Cladobotryum sp* (Tanovic *et al.*, 2009).

Cinnamon oil has been shown to have potent anti-fungal activity against wood decaying fungi (Yang Wang *et al.*, 2005). Both white rot and brown rot fungus were completely inhibited at 100 ppm and the main constituent of this oil was cinnamaldehyde. Eucalyptus oil has also been shown to reduce growth of *Fusarium solani* f. sp. melongenae (Joseph *et al.*, 2008). The production of α-amylase, cellulases and chitinases from two pathogenic fungi *Trichoderma virens* and *Fusarium solani* was drastically affected in liquid culture (Hamedo and Shamy, 2008) resulting in inhibition of growth. The basic mechanism of the action of the essential oils against certain pathogenic fungi has not been studied in detail but it is assumed according to the available data that the high activities of oxygenated monoterpenes against fungal pathogens are the consequence of interference of the terpenoids with enzyme reactions (Zambonelli *et al.*, 1996) and/or disruption of cell membranes in the target organism (Inouye *et al.*, 2000)

Natural plant-derived oils should therefore provide a wide variety of compounds as alternatives to synthetic fungicides with added advantages of cost effectiveness and the environment (Daferera *et al.*, 2003). The present study justifies the fact that the use of essential oils can be a better alternative to the use of synthetic fungicides for fungal disease control.

Table 1- Effect of various oils on the mycelial growth of host and pathogenic strains.

Oils conc. (in µl/L)	M. perniciosa (dry wt in gms.)	A. bisporus U3 (dry wt in gms.)	A.bisporus P1 (dry wt in gms.)	A.bisporus S11 (dry wt in gms.)
Cinnamic aldehyde (2.5)	0.20 ±0.014	0.75±0.003	0.70±0.003	0.80±0.005
5	0.20±0.003	0.65±0.005	0.60±0.005	0.60±0.005
7.5	0.10±0.005	0.05±0.003	0.10±0.005	0.30±0.005
10	0.45±0.012	0.50±0.003	0.50±0.005	0.55±0.003
Eucalyptus oil (2.5)	0.05±0.003	0.50±0.003	0.50±0.003	0.50±0.003
5	0.30±0.003	0.50±0.003	-	0.40±0.005
7.5	0.05±0.003	0.45±0.005	0.70±0.003	0.50±0.003
10	0.10±0.003	0.15±0.008	0.20±0.014	0.50±0.003
Citrullina oil (2.5)	0.25±0.006	0.10±0.005	0.10±0.005	0.15±0.008
5	0.10±0.005	0.15±0.008	0.15±0.008	0.25±0.006
7.5	0.35±0.003	0.20±0.014	0.15±0.008	0.10±0.005
10	0.10±0.005	0.65±0.003	0.20±0.014	0.30±0.003
Clove oil(2.5)	0.30±0.003	0.05±0.003	0.25±0.006	0.20±0.014
5	0.30±0.003	0.15±0.008	0.40±0.005	0.15±0.008
7.5	0.35±0.003	0.15±0.003	0.35±0.005	0.20±0.003
10	0.40±0.003	-	0.40±0.005	0.15±0.003
Castor oil(2.5)	0.25±0.01	0.20±0.005	0.50±0.003	0.45±0.003
5	0.45±0.006	0.25±0	0.20±0.003	0.30±0.003
7.5	0.30±0.003	0.25±0.003	0.40±0.003	0.20±0.003
10	0.45±0.003	0.35±0.01	0.30±0.003	0.45±0.003
Olive oil(2.5)	0.70±0.003	0.40±0.003	0.20±0.005	0.50±0.005
5	0.50±0.003	0.15±0.006	0.20±0.005	0.25±0.003
7.5	0.50±0.003	0.15±0.003	0.50±0.003	0.40±0.003
10	0.50±0.003	0.15±0.003	0.40±0.003	0.25±0.006
Control	0.90	0.80	0.90	1.0
CD at 5%	A= 0.37	B=0 .30	C= 0.74	AB= 0.30	BC=0 .74	AC= 0.60

Where A is different oils, B= different concentration of oils and C=different strains of host and pathogen

(-) stands for no growth, or complete inhibition in growth.

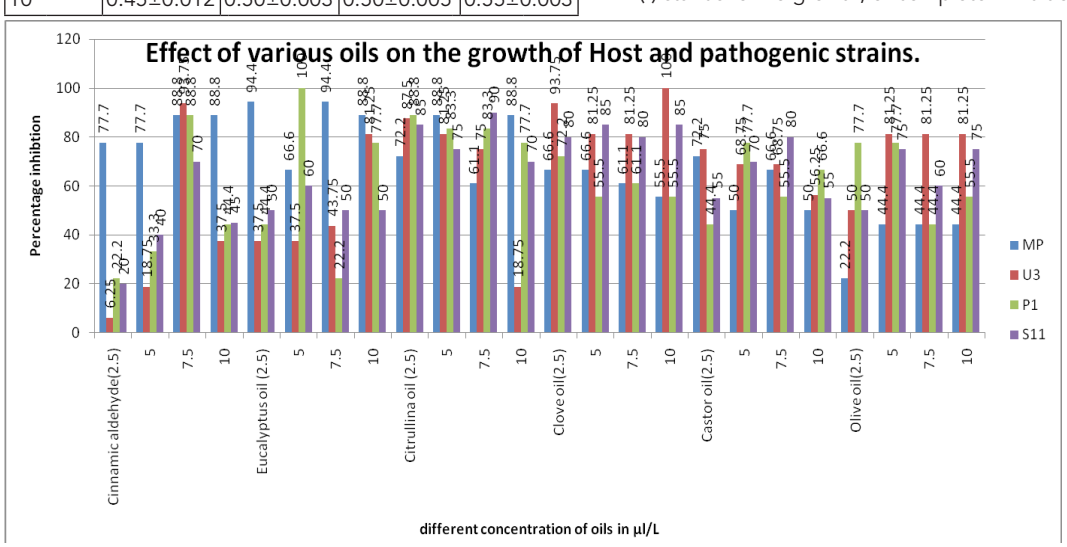

Fig 1- Effect of different oils at different concentration on the growth of *M. perniciosa* and three strains (U3, P1 and S11) strains of *A. bisporus*.

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Fig 2- Effect of different oils on the growth of *M. perniciosa* in solid state.

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