International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(3): 325-328
www.biochemjournal.com
Received: 06-01-2024
Accepted: 11-02-2024

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In vitro management of white plaster mould (*Scopulariopsis fimicola*) using different botanicals

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DOI: https://doi.org/10.33545/26174693.2024.v8.i3d.736

Abstract
The *Calocybe indica* (Milky Mushroom) affected by large number of biotic and abiotic factors during cultivation. Among them, white plaster mould caused by *Scopulariopsis fimicola* encounter the compost and casing soil during cultivation and adversely affect the spawn run, growth and yield of *C. indica.* The present research work on various aspects of milky mushroom cultivation was conducted during 2019-21. June and August months (29.2^0- 35.8 °C temperature and 73.00- 85% percent relative humidity) are most suitable for milky mushroom cultivation. Maximum percent inhibition of *S. fimicola* (white plaster mould) was recorded in Tulsi leaf extract (78.65%) followed by Neem (75.35%), Garlic (72.50%), Ginger (70.45%), *Aloe vera* (69.37%) and Kalmegh (68.21%) at 20 percent concentration *in vitro* condition. All the treatments were significantly superior over control. Same trends were also observed in 10 and 15 percent concentration of plant extracts.

Keywords: Mushroom, mould, *Calocybe indica, Scopulariopsis fimicola,* neem, Tulsi

Introduction
Mushroom are fleshy macrofungi with large fruiting bodies usually growing on dead and decaying material in the form of thread-like structures called hyphae that form a network (mycelium), after the mycelium has grown profusely and absorbed sufficient food material, it forms the reproductive structures, which generally come out of the substrate and form large fruiting bodies, commonly referred as mushroom. The history of mushroom is very old as the origin of man himself. These have existed for millions of years and mankind has used their large fruiting bodies, both for flavour and nutrition. Theophrastus (372-287 BC), the great Greek Philosopher wrote about the food value of mushrooms during Middle Ages, when they comprised royal dishes for the Greek and Roman Emperors. However, their commercial cultivation has become established as an industry only during the turn of the present century and few other mushrooms like *Flammulina velutipes* (enokitake), *Pholiota nameko* (nameko), *Auricularia* spp. (black ear), *Tremella fuciformis* (jelly fungus), *Stropharia rugoso- annulata* (giant fungus) and *Tuber melonosporum* (truffle) have been exploited only in the present century (Sharma, 1990) [8].

According to a conservative estimate, 1.5 million species of fungi actually exist and of these the number of known fungal species is about 69,000 (Hawksworth, 1991) [10]. Among these known and described fungal species, about 14,000 are fleshy macrofungi and of these approximately half are edible (Chang and Miles, 1993) [3] about 3000 species belonging to more than 30 genera are edible fungi. Among all reported edible fungi only 10 are commercial grown and 4-5 are produced on an industrial scale (Tewari, 2005) [9].

Mushroom are good source of high-quality protein, fat and other important vitamins. In addition, they contain vitamin B1, B2, C, E, K, nicotinic acid, pantothenic acid and biotin, apart from being recognized as nutritious foods, certain mushrooms are also important sources of biologically active compounds with potential pharmacological effects, such as, antitumor, antioxidant, antiviral, hypocholesterolemic and hypoglycemic effects (Cheung, 2010) [3]. In India, wide range of agro-climatic conditions exist, which favour cultivation of a large variety of agricultural and horticultural crops. Therefore, a major output of most agricultural and food processing operations is the unusable lignocellulosic waste material, whose predominant components are cellulose (35-50%),

hemicelluloses (20- 30%), and lignin (10-25%). In addition to these, some minor components, such as, proteins, oils, and ash make up the remaining fraction (Rashad *et al*., 2009) [6]. Lignocellulosic waste materials are one of the most abundant naturally available and renewable complex organic carbons. The annual production of lignocellulosic waste is 150 billion tonnes worldwide (Saber *et al*., 2010) [7].

Food and Agriculture Organisation (FAO) has recommended mushroom as a food item that can contribute significantly to protein nutrition of people especially in the developing countries like India. India produces about billions of tonnes of agricultural- residues and waste annually even if 1% of it is utilized to produce mushrooms, India can emerge as the major mushroom producer of the world in future (Chadha, 1994) [1] However, the mushroom production can be developed into a big industry in India. One such promising mushroom of the tropical region is the milky mushroom (*Calocybe indica* P & C), whose wild forms were reported first time by Purkayastha and Chandra in 1974 from Calcutta markets, and thereafter, tried to make it domesticate.

The first attempt towards induction of fruiting bodies in milky mushroom was made by Purkayastha and Chandra (1976a) [5]. However, there is limitation in cultivation and productivity. Therefore, a need was felt to standardize its cultivation methodology on locally available lignocellulosic wastes in North India and make special efforts to increase its yield. North India has tropical to sub-tropical type of climate with optimum temperature of 25-35 °C existing for most period of the year. In addition, a wide range of agro-wastes, garden-wastes and forest wastes are available in this region, many of which have not been utilized so far for the cultivation of *Calocybe indica*.

Materials and Methods

The present investigation entitled "Management of plaster mould in *Calocybe indica* using botanical" was conducted at the Plant Pathology Research Laboratory of Acharya Narendra Deva University of Agriculture & Technology, Narendra Nagar, Kumarganj, Ayodhya (U.P.).

Media: Before preparation of culture, the preparation of medium is essential where the vegetative growth of mushroom is being occurred *in vitro* at Potato Dextrose Agar (PDA).

Preparation of Potato Dextrose Agar (PDA) medium:
Peeled and sliced Potato: 200 g
Dextrose: 20 g
Agar (powder): 20 g
Water: 1000 ml

Potatoes were peeled, washed and sliced into small pieces and boiled in tap water (500 ml) in a conical flask 20-25 minutes then, it was strained by a strainer and the decoction was kept into a beaker. Agar powder is boiled with 500 m1 water in a conical flask with continuous stirring with a glass rod for 2-3 minutes now, both agar solution and potato decoction were mixed together. The previously measured was also added to the mixed solution with stirring and made them homogenous. The volume was raised up to l000 ml. The prepared media was then sterilized in autoclave at l5lbs pressure (120 °C) 20 minutes. The medium during hot condition was poured in culture tubes up to l/3 height, and kept the culture tubes in culture tube's rack. The culture tubes were then plugged with non- absorbent cotton plug and wrapped 5-6 tubes by brown paper/two folds of used news-paper and rubber band then wrapped tubes were kept into a wire net basket few clean petriplate pairs were also wrapped with brown paper and sterilized along with medium in autoclave.

Efficacy of different plant extracts against plaster mould *in vitro*

Efficacy of locally available five plants were selected for this study which have antifungal properties in the form of the leaf/bulb extract of the plants at 10, 15 and 20 percent concentration against plaster mould fungus following Poison Food Technique. These plants were selected because they were associated with disease management practices in several part of India. To obtain extracts, these plant parts were thoroughly washed under running clean tap water to remove dust and other foreign matter from the surface. Then plant material were weighed individually (100 gm each). Materials were surface sterilized with 0.1% mercuric chloride solution and washed thrice with sterilized water each plant part was ground individually in Pestle & Mortar by adding equal amount (100 ml) of sterilized water (1:1 w/v) and boiled at 80 °C for 10 mm. The resultant slurry was drained through double layer muslin cloth to remove the pellets of fibrous tissue of plants.

All plant extracts were tested at 10, 15 and 20% concentration in vitro condition by using Poison Food Technique to study the inhibitory effect of these botanicals on mycelial growth of plaster mould causing agents. 10, 15 and 20 ml plant extract of each stock solution were added to the 90.0, 85.0 and 80.0 ml of sterilized MEA medium. This extract mixed MEA poured into sterilized Petridish in three replications for each concentration after solidification of the medium each plate was centrally inoculated with 5 mm disc cut of the edge of the culture Plaster mould causing agent using sterilized cork borer. All the inoculated petri-plates were incubated at 24±2 °C. Colony diameter was measured after 7, 14 and 21 days of inoculation ercent inhibition over control was calculated by applying the following formula Vincent, 1927.

$$I = \frac{C - T}{C} X100$$

Where,
I = Percent inhibition
C = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

Statistical Analysis of data:

Data in different experiments were analyzed statistically following standard procedure to draw the conclusion.

Result

The investigation was carried out at Plant Pathology Laboratory of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.) during 2019-2021. Therefore, keeping in view the importance of milky mushroom and seriousness of the plaster mould, it was worthwhile to investigate the plaster mould with the suitable methods of eco-friendly management through botanicals as outlined in the introductory chapter with the objective and the results obtained are discussed in the light of available literature.

***In vitro* management of plaster mould (*Scopulariopsis fimicola*) with application of botanicals:**
The five plant extracts were used in the present study were evaluated *in vitro* against plaster mould (*Scopulariopsis fimicola*) by Poison Food Technique at 10, 15 and 20 percent concentration after 21 days of inoculation.

At 10 percent concentration
In 10 percent concentration, the minimum radial growth was obtained in Tulsi (34.06 mm) followed by Neem (35.88 mm), Garlic (37.56 mm), Kalmegh (41.95 mm), Aleo vera (42.65 mm) and Ginger (43.12 mm) as compared to control (54.00 mm) each treatment was significantly superior to control.
In 10 percent concentration the maximum percent inhibition on mycelial growth of *Scopulariopsis fimicola* was recorded in Tulsi (33.24%) followed by Neem (31.03%), Garlic (28.65%), Ginger (16.15%), Kalmegh (15.09%) and *Aloe vera* (14.80%). All the treatments were significantly superior over control.

At 15 percent concentration
In 15 percent concentration, the minimum radial growth was obtained in Tulsi (28.76 mm) followed by Neem (31.09 mm), Garlic (38.84 mm), Kalmegh (40.54 mm), *Aloe vera* (41.54 mm) and Ginger (42.67 mm) as compared to control (56.09) each treatment was significantly superior to control. In 15 percent concentration the maximum percent inhibition on mycelial growth of *Scopulariopsis fimicola* was recorded in Tulsi (49.65%) followed by Neem (47.34%), Garlic (32.98%), Kalmegh (27.43%), *Aloe vera* (26.09%) and Ginger (25.87%). All the treatments were significantly superior over control.

At 20 percent concentration
In 20 percent concentration, the minimum radial growth was obtained in Tulsi (15.09 mm), Neem (17.45 mm), Kalmegh (19.56 mm), Garlic (19.76 mm), *Aloe vera* (20.11 mm) and Ginger (21.68 mm) as compared to control (57.19 mm). All treatments was significantly superior to control. In 20 percent concentration the maximum percent inhibition on mycelial growth of *Scopulariopsis fimicola* was recorded in Tulsi (78.65%) followed by Neem (75.35%), Garlic (72.50%), Ginger (70.45%), *Aloe vera* (69.37%) and Kalmegh (68.21%). All the treatments were significantly superior over control.

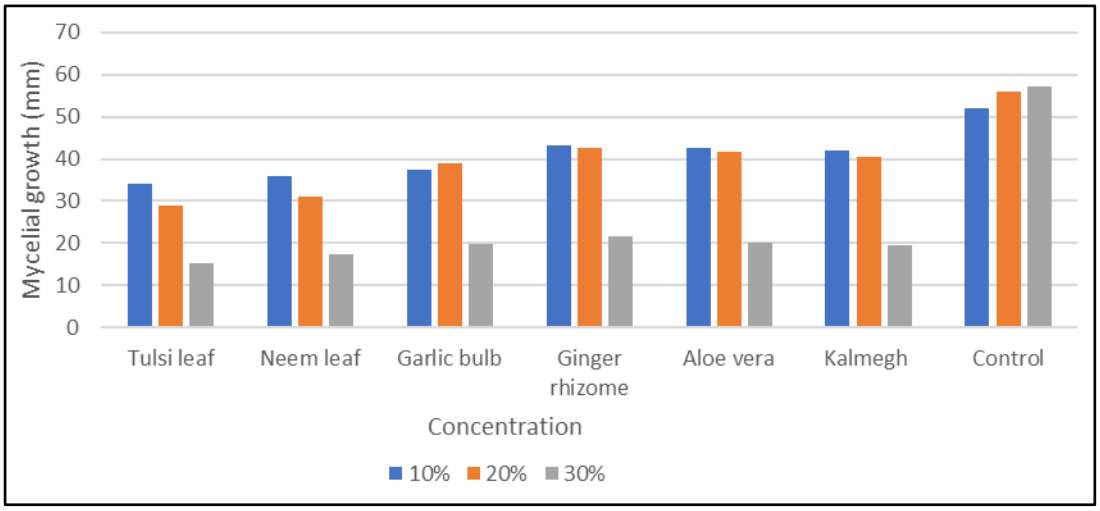

Fig 1: Effect of plant extract at different concentrations against *Scopulariopsis fimicola* on mycelial growth and percent inhibition, *in-vitro* at 21 days.

Conclusion: It may be concluded from the study that June and August month was most suitable for milky mushroom (*Calocybe indica*) cultivation. Tulsi leaf extract was most effectively controlling the *Scopulariopsis fimicola (*white plaster mould) *in vitro* condition at 20 percent concentration. The re-casing combined with surface treatment with Neem leaf extract was effective in management of white plaster mould of milky mushroom.

Funding: This work is supported by the Acharya Narendra Deva University of Agriculture & Technology, Kumarganj, Ayodhya. U.P. India. Authors have no financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

Declarations
Conflict of interest: All authors declare that they have no conflict of interest.

Ethical approval: Not applicable.

Acknowledgements
First and foremost, I want to express my gratitude to Dr. S. K. Singh, my primary supervisor, for his advice, encouragement, and support during the entire process. Their advice and knowledge were really helpful to me in guiding the course of my research and developing new concepts.

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