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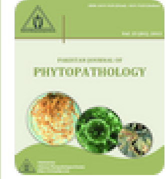

RESEARCH ARTICLE

Bio Management of Mushroom against *Aspergillus niger* Under *In Vitro* Conditions

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A B S T R A C T

Mushrooms have nutritional value as well as medicinal value. Various fungal contaminants effect the mushroom production, substrate and mushroom spawn, which ultimately leads to decreased yield, impacted spawn, and low-quality mushrooms. The current study aims to determine the in vitro assessment of *Aspergillus niger* contamination in mushroom spawn culture and its bio management using essential oils. at various concentrations (4%, 8%, 12% and 16%). Severity of various substrates was observed. After inoculation of *Aspergillous niger* in the samples and by comparing inoculated and control was done, it was observed that *Aspergillus niger* having highest prevalence of 35.71 percentage found to be most dominant pathogen in the substrates among the contaminants. The occurrences of fungal contaminants were managed through essential oils. For in vitro management of *Aspergillous niger* essential oils viz., cinnamon oil, coconut oil, Neem oil and rose oil were used, All the essential oils reduced the mycellial growth of pathogen at all concentration, results of mycellum inhibition of coconut oil was 35.23% followed by Neem oil 26.21%, Cinnomon oil 16.77% and Rose oil 16.09 % at 16% concentration control showed highest growth.

Keywords: In vitro management, essential oils, Mushroom, *Aspergillus niger*.

INTRODUCTION

The mushroom (*Pleurotus ostreatus*) belongs to the order Agricales, class Basidiomycetes, subclass Hollo basidiomycee. An edible basidiomycete with high nutritional value and flavor. Oyster mushrooms are low in calories, high in protein, and contain a variety of other nutrients, including vitamins B1 and B2. Because of this, it is highly popular among dieters (Bhandari and Mukerji, 1959).

It is thought that the history of mushroom consumption began during the time of hunting and gathering (Wani *et al.,* 2010). Nonetheless, China is currently The biggest producer and consumer in the world of mushrooms, with the first recorded cultivation of mushrooms thought to have begun there circa 600 BC (Zhang, 2014). The three main categories of the global mushroom industry are medical, edible, and wild mushrooms (Royse *et al.,* 2017). The most widely consumed eatable mushrooms worldwide are the oyster, white button, shiitake, cremini, morel, and straw mushrooms (Joseph, 2021). More than 3,000 species of mushrooms are thought to be edible out of all the species. Approximately 60 of these 3,000 edible mushroom species are grown commercially worldwide, while 100 of these species are economically cultivated (Chang and Wasser, 2017).

More than 100 countries economically cultivate mushrooms (Gupta *et al.,* 2018). The macro-fungi known as mushrooms are characterized by their characteristic fruiting bodies, which can be either hypogenous or epigenous and are large enough to be observed with the

unaided eye and harvested by hand (Chang and Miles, 1992). They have no leaves, seeds, or roots because they lack chlorophyll, and they actually don't require light to grow. These are a species of fleshy, macroscopic fungi that spread by releasing spores in the dark. They usually grow on soil above ground or on their food supply. The fructification process develops the fruiting bodies from large subterranean mycelia (hyphae). To absorb nutrients and generate enzymes that break down complex organic stuff, they need an agricultural waste substrate (Walde *et al.,* 2006).

Fruiting bodies only have an estimated lifespan of 10–14 days (Kalac, 2009). About 16,000 species of mushrooms are among the 110,000 different types of fungi (Wasser, 2010). Mushrooms have a great nutritional and therapeutic value and are being farmed more and more these days. They are said to be the main natural source of ergosterol or provitamins and are known for their high-quality amino acids, vitamin B complexes, sodium, potassium, iron, and dietary fibres (Jr, 2005).

There are currently about 2000 edible kinds of mushrooms spread over the world, making them fungi with substantial nutritional value (Rathore *et al.,* 2019). With roughly 35% of the world market for mushrooms, China is the biggest producer in Asia. Button mushrooms (*Agaricus bisporus*), shiitake mushrooms (*Lentinula edodes*), and oyster mushrooms (which were valued at US dollars in 2018) are the most common species (Elaine and Tan, 2009).

Asia accounts for up to 76% of the world's mushroom production, with Europe coming in second at 17.2% and the US at 5.9% (Sande *et al.,* 2019). For the best development and yield, mushrooms must be produced under ideal conditions. Symptoms of the disease include patchy caps, blowout (bent and/or broken stipes), and bubbles (undifferentiated spherical masses) (Fletcher and Gaze 2008; Largeteau and Savoie 2008; Berendsen *et al.,* 2010).

Fungal growth first develops on the casing, then spreads and turns greyish yellow. Light brown superficial patches eventually develop on crowns and eventually combine to form sizable brown blotches. The illness is typically spread by contaminated casing soil and compost (Kumar *et al.,* 2014).

If the infection occurs later, the stipes become deformed and the caps become slanted. Symptoms of this mold disease include a thick, cushioned white patch on packed and cased bags that has greenish fungus growth and eventually turns bluish green. Growers find it challenging to differentiate between the two because Trichoderma spp. initially generate a dense, pure white mycelium that resembles the mycelium of mushrooms (Shah *et al.,* 2013).

The spawn-run is impacted if the spawned trays are attacked by this fungus. Pin-head mushroom formation is slowed down if it grows on casing soil. Green mold is a fungus that aggressively colonizes dead mushroom tissue and organic matter. High humidity and improper phase II composting are partly to blame for the disease's proliferation. This fungus's spores are dispersed by air, water, and negligent handling (Munshi *et al.,* 2010).

The majority of these pollutants originate from substrates that have not been adequately sanitized. Alkalinization, bleaching, sterilization by steam, hot water, and chemical means are some of the sterilization methods that can be used to get rid of pre-existing impurities. Each method has a relative benefit over the others. Furthermore, botanicals and live antagonists employed in biological control can be used as therapeutic sprays or as a preventative sterilant (Ghimire *et al.,* 2021).

MATERIAL AND METHODS

The studies were carried out in the Plant Pathology laboratory at the Baluchistan Agriculture College in Quetta.

Obtaining mushroom culture

Oyster mushroom spawns were purchased from an internet marketplace. Distilled water (DW) and potato dextrose agar (PDA) were used to make the culture media. The of culture media used in this study are given in Table 1

Table. 1. Preparation of PDA with addition of malt extract

Ingredients	g/L DW	g/0.5L DW
Dextrose	20	10
Potato Extract	4	2
Agar	15	7.5
Malt Extract	6	3
PH	7.3 ± 2 at 25°C	

Formula adjusted standardized to suit performance parameters

Isolation and identification of fungi: Isolation of *Aspergillus niger,* from infected growth media, such as compost, wheat straw, and spawn, upon contamination in these substrates we picked contaminated portions for further culturing. Glassware and prepared PDA media were autoclaved for 20 minutes at 121°C. The laminar flow cabinet was cleaned using cotton soaked in ethanol. The UV light inside the laminar was then turned on to kill microorganisms and expose things to UV radiation for up to fifteen minutes.

After cooling and pouring the fluid onto Petri plates to solidify, a single colony infected through a sterile loop was moved to the laminar flow cabinet (Usman *et al.,* 2023).

Parafilm was used to seal the Petri plates, shielding the media from potential contaminants. Plates were incubated for the development of the pathogen at 27°C after inoculation. Then incubated Petri dishes were observed for growing fungal contaminants and were identified microscopically. Slide was prepared in one drop of water kept single spore's petri dishes colony rubes for few seconds then cover slip kept upon it and observed under microscope.

Isolation of pathogen from mushroom substrate *Aspergillus niger:* Mushroom substrates, including millet, sorghum, and wheat grain, were prepared, and the mushrooms were spawned in a polythene bag, tray, or other container with compost, rice bran, sawdust, and wheat straw. Black mold and other fungal pathogens were seen in bag cultures. Fungal pathogens were isolated using PDA media.

Pathogen was grown on PDA media at noted colony of this fungus pathogen to check these spores to make a slide one drop of water feel on a center of the slide took fungal pathogen through the rubber and loop, fixed it, and left the cover slip on the slide. After positioning the slide beneath the stage microscope, the lens was adjusted. They were 20x, 40x, 60x, and 100x lenses. We checked the spore size while simultaneously connecting the laptop to the microscope.

Pathogenicity Test: Three techniques with various goals were used. Polypropylene bags containing 500 g of wet substrate and 1% w/w (weight by weight) calcium carbonate (CaCo3) were used to treat the substrate in a different way. They were then combined and sprayed with three milliliters of *Aspergillus niger* conidia water suspension per bag.

Three replications per bag were used for the contamination. Subsequently, the bags were mixed with 10% w/w mushroom spawn, incubated for 10 days at room temperature, and the substrate bags were observed. Assessing the growth of Aspergillus Spp following hot water treatment was the main goal of the first technique.

Three distinct sterilized and non-sterilized substrates were used, along with two strains. (Mushroom spawn + Aspergillus spp. spray inoculum).

The purpose of the second experiment was to assess how various substrate heat treatments affected the development of black mold. Bagged substrates were steam sterilized at 60, 80, and 121 degrees Celsius for 15 minutes. The third technique was centred on assessing the growth of the contamination (Aspergillus Spp.) following a spray on the alkalinized water substrate.

Wheat straw was sprayed with an alkaline solution for five minutes, twenty-four hours, and forty-eight hours. *Agaricus bisporus* and *Pleurotus ostreatus* strains were employed in four substrates (wheat straw), including sawdust and wheat seed compost. The substrate underwent treatments (Colavolpe *et al.,* 2014).

Management of mushroom: Wheat straw, saw dust, wheat grains, compost, mixed in 12 different bags and was kept at required temperature, after that autoclaved the bags with 3 replications. The temperature was 121 °C centigrade for 15 minutes for first treatment and other, which was heated at 70°C for 15 minutes. These were incubated at the room temperature in disinfected area. After 15 days when the bags were observed. The calcium carbonate was used in straw, sawdust and wheat grain. Essential oils were used to control the pathogens *Aspergillus* spp. the following were the essential oil cinnamon oil, coconut oil, neem oil, rose oil and control.

Treatment of the substrate: Various treatments were performed, including: 1) immersion in hot water: a temperature-controlled thermal bath control was used; substrates were submerged in the bath for 30 minutes after reaching the treatment temperature (60 or 80 °C), drained, placed on absorbent papers to bring the humidity down to 70%, and then bagged: 2) steam sterilization, in which substrates were first sealed in bags, followed by the addition of tap water to reach 70% final humidity and sterilization for two hours at 120 °C and 1.2 psi of pressure; The third method we employed was immersion in alkalinized water, as suggested by

Contreras *et al.* (2004).

An alkaline solution containing 0–5% calcium oxide was used to soak the substrates for 0, 5, 12, 24, or 36 hours.

Preparation of Essential oils: The Setup The solution was made with essential oils at 4%, 8%, 12%, 16%, and 0%, respectively, in 20 millilitres. The backer Essential oil concentration of 4% 0.20 ml of ethanol, 4.80 ml of Tween20, 0.25 ml, and 4.75 ml of distal water (DH2O) the entire solution (10 ml). 0.40ml of essential oil with an 8% concentration, 4.60ml of ethanol, 0.25ml of Tween20, and 4.75ml of DH2O 10 millilitres of the entire solution 0.60 ml of essential oil with a 12 percent concentration, 4.40 ml of ethanol, 0.25 ml of Tween20, and 4.75 ml of DH2O 10 millilitres of the entire solution 0.80ml of essential oil with a 16 percent concentration, 4.20ml of ethanol, 0.25ml of Tween20, and 4.75ml of DH2O

The entire solution (10 ml) 0.00ml of essential oil at a 0% concentration, 5.00ml of ethanol, 0.25ml of Tween20, and 4.75ml of DH2O the 10ml solution.

below given in the table 2.

Table 2. Essential oils different concentration

			5% Tween 20 (Fixed)					
%	Essential oil		Ethanol	Tween 5%		DH_2O		Grand Total	
4%	0.20	Ml	4.80 Ml	0.25	Ml	4.75	ml	10	Ml
8%	0.40	Ml	4.60 Ml	0.25	Ml	4.75	ml	10	Ml
12%	0.60	Ml	4.40 Ml	0.25	Ml	4.75	ml	10	Ml
16%	0.80	Ml	4.20 Ml	0.25	Ml	4.75	ml	10	Ml
0%	0.00	Ml	5.00 Ml	0.25	Ml	4.75	ml	10	Ml

***In Vitro* Antifungal Effects of the Essential Oils:** Pteria plates containing potato dextrose agar (PDA) media are used to apply various essential oils at varying concentrations to combat *Aspergillus niger.* After placing a 5 mm disc of Aspergillus niger mycelium on the PDA medium that has been treated, the plate is incubated at 25°C. Mycelial growths is measured daily to compare with the control petri plate after 18ml of media and 1ml of essential oils are mixed at 45°C and the media are solidified (Aminifard and Mohammadi, 2013a).

STATISTICAL ANALYSIS

Together with replication, the date was applied. Data was recorded and examined using Statistics 20 for statistical tests like LSD and Analysis of Variance (Steel *et al.,* 1997).

RESULTS AND DISCUSION

Morphological identification *Aspergillus niger:* Cultures of *Aspergillus niger* grown on potato dextrose agar (PDA) media, which is white and yellow in color and later produces black conidia, were used to analyze the species' traits, including colony appearance and sporulation pattern. On isolated plates, the colonies' color became apparent after six days at 28°C.

Figure 1 Cultural Characteristics of Pathogen (*Aspergillus niger*)

The slide was prepared by placing a drop of lacto phenol at the center of slide and take a part or spores rubes on drops put cover slip on drop portion then check in microscope and observed spores' structure.

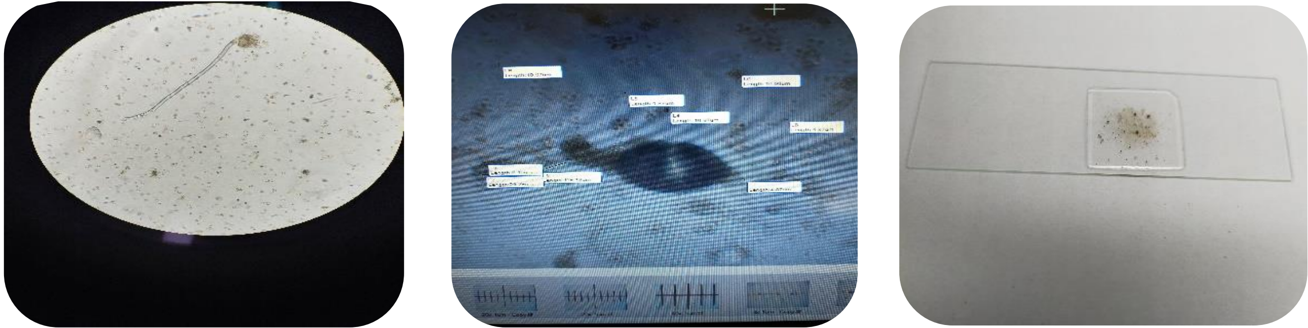

Figure 2. Microscopic study of black mold (*Aspergillus niger*)

Isolation of pathogen from mushroom substrate *Aspergillus niger*: *Aspergillus,* spp. and spore forms were 10.97, 4.87, 5.45, 16.98, micrometer respectively. displays the drag force acting on a spore that has been isolated as a variable that depends on the blowing speed. Because our optical microscope measurements showed that Since the spore diameter was approximately 5.0 µm (based on five separate measurements), it was assumed that the *A. niger* spores were 5 µm in diameter.

The estimated spore diameter was consistent with a prior study28 that found that the diameter of *A. niger* spores varied between 3.0 and 5.4 µm, depending on the growth conditions. Using the 4-day-old colony's threshold air speed of 3.50 m/s, the estimated drag force was 3.31 nN, which was marginally higher than the average force (3.27 nN) that the AFM measured to separate a spore. Similarly, the average force measured by the AFM was 1.98 nN, whereas the drag force for the 10-day-old colony was estimated to be 2.53 nN. Accordingly, the results of the AFM measurements and the aerosolization tests were similar (Zhang *et al.,* 2018).

Pathogenicity Test: Three techniques that had various goals were completed. The substrate was treated differently using polypropylene bags that contained 500 g of wet substrate and 1% w/w (weight by weight) calcium carbonate (CaCo3).

After they were spray with 3 ml water suspension of conidia of fungi like *Aspergillus niger* per bags and mixed. Three treatments were tested *Aspergillus* spp plus (+) mushroom spawn. After 15 days of incubation at room temperature in a dark environment, controls without inoculation were observed in all technique bags. Assessing the visual level of Aspergillus spp growth and colonization allowed for a qualitative expression of the findings. The following icons show the various stages of the black mold disease's development in the bags: (+): Substrate colonization is less than 20%; (+ +): Substrate colonization is between 20 and 50 percent; (+++): Substrate colonization is more than 50 percent; and (-): no growth.

Severity of different substrates: Severity was highest in substrate of seed as it showed total 72 percent severity. Total number of bags infected were18 from 25 bags in which 5 bags (20 %) showed poor growth, 6 bags (24 %) with mediated growth and 7 bags (28 %) showed abundant growth. In straw it appeared with total 64 percent of severity. Total number of bags infected were 16 from 25 bags in which 7 bags (28 %) appeared with poor growth, 4 bags (16 %) appeared with mediated growth and 5 bags (20 %) appeared with abundant growth. In compost it represented at the most 56 percent of severity. Total number of infected bags were 14 out of 25 bags in which 6 bags (24%) represented poor growth, 4 bags (16%) represented mediated growth and 4 bags (16%) represented abundant growth. Sawdust with least severity having total number of 13 infected bags out of 25 bags appearing with 52 percent of severity in which 4 bags (16 %) manifested with poor growth, 6 bags (24%) manifested with mediated growth and 3 bags (12%) manifested with abundant growth. It was further noticed that substrate prepared with sawdust was more approving with least appearance of disease severity along with maximum rate for non-growth represented as the highest among other substrates. This appeared with 12 numbers of bags representing 48 % of non-growth follow by compost appearing in 11 bags depicting 44 % of non-growth. The straw appeared 36 % of non-growth for 9 bags and seed was least effective manifesting 28% of non-growth out of total 7 bags. (Colavolpe *et al.,* 2014) was with related results.

Table. 3 severity comparison of various substrate

Substrate	Infected bags			Non growth (%)
	Poor growth (%)	Mediated growth (%)	Abundant growth (%)	
Compost	24	16	16	44
Straw	28	16	20	36
Seed	20	24	28	28
Sawdust	16	24	12	48

In vitro* evaluation of Cinnamon oil on mycelial growth *Aspergillus niger: Treatment of *Aspergillus niger* through different Essential oils utilized in food poisoning methods. In the center of each PDA plate, 18 ml of PDA culture medium at 45 °C was mixed with 1 ml of each oil solution concentration. single spore was placed to check culture growth.

Data was gathered at intervals of every two days. Table 4's data was compared to control (C), which had the highest growth rates (46.90 mm) because essential oils were not applied and colony growth was not decreased.

The maximum pathogen growth rate in a petri plate treated with 4% cinnamon oil was 46.02 mm, and the least amount of colony growth percent reduction was 1.88. The second concentration, 8%, showed 45.32 mm of growth, and the colony growth percentage decreased by 3.38.

The third concentration at 12% showed a reduction in the colony growth percentage of 5.54 and a total colony growth of 44.30 mm. The pathogen colony grew the smallest overall at a dosage of 16% concentration, measuring 39.03 mm, while the largest reduction in colony growth percentage was 16.77.

While ginger oils demonstrated inhibition zones of 13–16 mm and cinnamon essential oil demonstrated inhibition zones of 23–30 mm against Aspergillus spp., camphor oil provided inhibition zones of 14–27 mm.

Whatever lavender oil provided (14–15 mm) against isolates of Aspergillus spp. without an inhibition zone with pepper oil While ginger and cinnamon essential oils produced 28 mm and 10 mm at a concentration of 1:10 v/v, respectively, the tested oil concentrations varied from 1:11; 1:3; 1:5; and 1:10 v/v. However, there was no inhibition of Aspergillus isolates by camphor, pepper, or lavender oils at a concentration of 1:10 v/v. As a negative control, DMSO was employed (Shouny *et al.,* 2017).

Table. 4 *In-vitro* efficacy of Cinnamon on colony growth of *Aspergillus niger*.

Treatment	Dose	Linear Colony Growth (mm)			Total Growth	Reduction in Colony Growth (RCG)	Reduction Percentage
		2nd Day	4th Day	6th Day		RCG=C-TCG	RCG(100)/C
Cinnamon oil	4%	23.80	35.53	46.02	46.02	0.88	1.88
	8%	23.43	33.57	45.32	45.32	1.58	3.38
	12%	22.30	29.43	44.30	44.30	2.60	5.54
	16%	11.20	22.00	39.03	39.03	7.87	16.77
Control (C)		26.38	35.70	46.90	46.90	0.00	0.00
LSD							

In vitro* evaluation of Coconut oil on mycelial growth *Aspergillus niger: The information was gathered two days later. All of the information in Table 5 was contrasted with control (C), which had the highest growth rates (61.45 mm) because no essential oils were applied and the colony growth was not decreased.

The maximum pathogen growth rate in a petri plate treated with 4% coconut oil was 45.72 mm, and the least amount of colony growth was reduced to 25.60 percent. The colony growth percentage decreased by 27.61 percent at the second concentration of 8%, which showed 44.48 mm of growth. Colony growth was 40.80 mm overall at the third concentration of 12%, and the colony growth percentage decreased to 33.60.

The greatest reduction in the colony growth percentage was 35.23, while the smallest total growth of the pathogen colony measured at a 16% concentration dosage was 39.80 mm.

The antifungal properties of coconut milk kefir were demonstrated against Aspergillus niger and Saccharomyces cerevisiae. At a concentration of 175 µl, the zone of inhibition of coconut milk kefir against Saccharomyces cerevisiae was 7 mm. For Aspergillus niger, the 200 µl concentration of coconut milk kefir showed a 6 mm zone of inhibition.

This is in line with the results of (Rihakova *et al.,* 2002), who found that coconut is an effective antifungal agent because it can inhibit radial growth and prevent spore germination. This may be because monolaurin, the active ingredient in coconut, is involved (Lakshmi, 2017).

Table. 5 *In-vitro* efficacy of Coconut oil on colony growth of *Aspergillus niger*

Treatment	Dose	Linerar Colony Growth (mm)			Total Growth	Reduction in Colony Growth (RCG)	Reduction Percentage
		2nd Day	4th Day	6th Day		RCG=C-TCG	RCG(100)/C
Coconut oil	4%	36.80	42.02	45.72	45.72	15.73	25.60
	8%	31.25	38.08	44.48	44.48	16.97	27.61
	12%	29.57	36.40	40.80	40.80	20.65	33.60
	16%	11.78	17.00	39.80	39.80	21.65	35.23
Control (C)		37.37	53.20	61.45	61.45	0.00	0.00
LSD							

In vitro* evaluation of Neem oil on mycelial growth *Aspergillus niger: The information was gathered two days later. Table 6's data were all compared to control (C), which had the highest growth rates (54.05 mm) because no essential oils were applied and colony growth was not decreased.

In a petri plate treated with 4% neem oil, the pathogen's maximum total growth rate was 69.13 mm, and the colony growth percent decreased by the least amount, 5.62. The colony growth percentage decreased by 11.77 percent at the second concentration of 8%, which showed 64.63 mm of growth.

Total colony growth was 63.13 mm at the third concentration of 12%, and the colony growth percent was reduced by 13.81. At a dosage of 16% concentration, the pathogen colony's total growth was 54.05 mm, with the largest reduction in colony growth percentage being 26.21.

Dill and neem seed oils significantly decreased The concentration of tested oils in the medium was directly correlated with the growth rate of inhibition. *D. rostrata* and *D. hawaiiensis* demonstrated more suppression at 1.0% concentration, while D. specifera and D. papendorfii were more vulnerable to the neem seed oil at 0.1% and 1.0% concentration.

A dose of 0.001% had a moderate effect on all fungi. *D. papendorfii* was the most sensitive of the four Drechslera species to all neem seed oil concentrations (Fig. 1b).

According to Govindachari *et al.* (1998), neem seed oil has a fungicidal effect on *Alternaria alternata, Fusarium oxysporum*, and *D. oryzae.* They also discovered that *D. oryzae* was more vulnerable to neem oil. Additionally, Additionally, Vir & Sharma (1985) found that 100% inhibition of *D. rostrata, Aspergillus niger*, and *Macrophomina phaseolina* was achieved at 10% neem oil concentration. [Niaz and others, 2008].

Table. 6 *In-vitro* efficacy of Neem on colony growth of *Aspergillus niger*.

Treatment	Dose	Linerar Colony Growth (mm)			Total Growth	Reduction in Colony Growth (RCG)	Reduction Percentage
		2nd Day	4th Day	6th Day		RCG=C-TCG	RCG(100)/C
Neem oil	4%	54.07	62.29	69.13	69.13	4.12	5.62
	8%	50.60	57.13	64.63	64.63	8.62	11.77
	12%	50.57	56.37	63.13	63.13	10.12	13.81
	16%	43.03	48.38	54.05	54.05	19.20	26.21
Control (C)		54.93	68.43	73.25	73.25	0.00	0.00
LSD							

In vitro* evaluation of Rose oil on mycelial growth *Aspergillus niger: The information was gathered two days later. All of the information in Table 7 was contrasted with control (C), which had the highest growth rates (52.20

mm) because no essential oils were applied and the colony growth was not slowed down.

The maximum pathogen growth rate in a petri plate treated with 4% concentration of rose oil was 50.43 mm, and the smallest colony growth percent reduction was 3.38. With a second concentration of 8%, the colony growth percentage decreased by 10.41 and showed 46.77 mm of growth. The colony grew 45.75 mm overall at the third concentration of 12%, and the colony growth percentage decreased by 12.36.

At a dosage of 16% concentration, the pathogen colony's total growth was at its lowest, measuring 43.80 mm, and at its highest, it decreased by 16.09 percent.

Three essential oils were tested in vitro for their ability to inhibit fungal radial growth at concentrations of 0.25, 0.5, and 1.0%. Essential oils of lemongrass and thyme at 0.5% were found to completely inhibit fungal growth.

More tolerance to rose essential oil was demonstrated by *A. niger* mycelial growth, which decreased by 68.9% at the maximum concentration of 1.0%. There have also been similar findings regarding the effectiveness of essential oils as antifungal inhibitors (Abdel-Kader *et al.,* 2013).

Table. 7 *In-vitro* efficacy of Rose on colony growth of *Aspergillus niger*.

Treatment	Dose	Linear Colony Growth (mm)			Total Growth	Reduction in Colony Growth (RCG)	Reduction Percentage
		2nd Day	4th Day	6th Day		RCG=C-TCG	RCG(100)/C
Rose oil	4%	37.90	41.75	50.43	50.43	1.77	3.38
	8%	33.05	39.18	46.77	46.77	5.43	10.41
	12%	32.27	38.82	45.75	45.75	6.45	12.36
	16%	25.23	34.07	43.80	43.80	8.40	16.09
Control (C)		44.40	50.58	52.20	52.20	0.00	0.00
LSD							

CONCLUSION

It was determined that *Aspergillous niger* mushrooms have an impact on the substrate, which ultimately leads to low-quality mushrooms and less yield-effected spawn. Coconut oil had the greatest impact on mycelium inhibition of *Aspergillus niger*, followed by neem, cinnamon, and rose oils. The current study aims to assess the in vitro evaluation of *Aspergillus niger* contamination in mushroom spawn lifestyle and its biomanagement through essential oils.

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