Garlic Leaves (*Allium Sativa*) as Control of Black Molds (*Rhizopus Stolonifer*)

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Abstract: This research was undertaken to help mushroom farmers in SitioPactil in BaukoMonamon Sur Mt. Province, Philippines to control Black molds in their farm and to tap the beneficial use of Garlic leaves. Black mold tends to lower the production of spawns in Mushroom Farm as nutrient competitor. Experimental research method with four treatments was utilized. Black molds were cultured under standard laboratory condition. Dried garlic leaves was macerated in 70% ethanol and concentrated in Rotary evaporator. Three concentrations (30%, 45% and 60%) served as experimental groupsand were used to inhibit the growth of Black molds by Poison Technique Method. Data was analyzed using ANOVA single factorial and Fisher Least Significant Difference Test as post hoc test. Findings of the study revealed that least mycelial growth, was obtained from treatments with 30%, 45% and 60% ethanolic dried garlic leaf extracts. The zone of inhibition was also high on the said treatments which implie that any of the three concentrations inhibited the growth of Black Mold.

Key words: Allium sativa, doratomycesspp, pleurotus ostreatus.

1. Introduction

Research becomes meaningful and useful if its result can augment problems in the livelihood of people in the community. This study was conceptualized due to the urgent need of mushroom growers in *SitioPactil in BaukoMonamon Sur* Mt. Province, Philippines. *SitioPactil* is located in mountainous slope range of 15 to 18 degrees. It is approximately 2300 meters above sea level [1]. Located in the highlands of the northern part of the country, the place is very much favorable for growing cold loving vegetables such as carrots. Pactil is one of the partner communities of San Beda College, Manila Philippines. It served as one of the venues of community outreach activities of San Beda College [2]. Although the village is known to grow vegetable as source of livelihood, some families operate and grow mushroom in their farm. It was observed by mushroom farmers/growers that their production decreases once they saw Black fungus in their fruiting bags. The result of this study will be used to help mushroom growers in Pactil Mountain Province which is one of the adopted communities of San Beda Institutional Community Involvement Center.

The culture of mushroom in the Philippines was introduced by Professors from the University of the Philippines and the Department of Science and Technology together with Bureau of Plant Industry. The said organizations spearheaded the technology dissemination in rural areas. In Pactil Mountain Province, mushroom growing started in 2012.Aside from vegetable growing it became a source of livelihood to

farmers in the said area. For three years it was observed that the harvest tends to decrease, which alarms the mushroom growers. With the research benchmarking done by the authors in the area it was found out that their mushrooms are affected by Black Whiskers Mold.

Mushroom growing, involves several phases. Phase I, composting is initiated by mixing and wetting the ingredients as they are stacked in a rectangular pile with tight sides and a loose center. Normally, the bulk ingredients are put through a compost turner. Water is sprayed onto the synthetic compost as these materials move through the turner. Nitrogen supplements and gypsum are spread over the top of the bulk ingredients and are thoroughly mixed by the turner. Once the pile is wetted and formed, aerobic fermentation (composting) commences as a result of the growth and reproduction of microorganisms, which occur naturally in the bulk ingredients. Heat, ammonia, and carbon dioxide are released as by-products during this process [3]. Black whisker mold occur in mushroom fruiting bag and produces black powdery spores that appear as smoke when disturbed. This mold indicates the presence of certain carbohydrates in the compost at spawning time. It also indicates that the straw has been incompletely caramelized or under heated in Phase I in composting, therefore, carbohydrates are in a form easily utilized. The proportion of carbohydrates, particularly cellulose, may be too high. The black whisker mold is also present in compost that overheated during spawn run. Simple carbohydrates are utilized by this fungus but it can also utilize lignin. *Doratomyces*, *Aspergillus*, and *Penicillium* produce copious numbers of spores and may cause respiratory problems nasal and throat irritation, chest congestion, breathing difficulty, etc [4]. The trend in the control of pest in any food source is through the use of organic method or natural products, wherein any biodegradable material will be used to reduce and stop the population of any pest. The Philippines was endowed with indigenous herbal plants. Many of these plants have not yet been explored and some are neglected and remain underutilized There are numerous studies on the use of garlic bulb/cloves against microorganisms/pathogens. However the leaves which are left in the field after harvest, has not been tapped for its potential use against pathogens, hence this study.

Garlic is a wonderful plant having the properties of empowering immune system, anti-tumour and antioxidant.Itis effectively used in cardiovascular diseases as regulator of blood pressure and with dropper effects of it on glycaemia and cholesterol, against bacterial, viral, mycosis and parasitic infections [5]. Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *Escherichia coli;* antifungal activity, particularly against Candida albicans; antiparasitic activity, including some major human intestinal protozoan parasites such as Entamoebahistolytica and Giardia lamblia; and antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of E. histolytica [6]. A study on the use of garlic extract was found to be fungicidal against a broad range of soil borne fungal organisms, but the concentration required to kill the organisms varied depending on root substrate [7].

1.1. Objectives of the Study

This study determines the mycelial growth after five days inoculation of black molds with different concentration of garlic leaves ethanolic extract and thezone of inhibition of Black molds in different concentration of garlic leaves.

2. Materials and Methods

2.1. Materials and Equipment

In the preparation of garlic leaves ethanolic extract, the following materials were used; dried garlicleaves, 70% ethanol, storage bottles, electric blender, beaker, cheesecloth, Whatman paper no. 1 and rotary evaporator.

For incubation of black molds, the following materials were used: inoculating loops and needles, weighing scale, petri dishes. Erlenmeyer flask, graduated cylinder, beaker, alcohol lamp, hot plate, stirring rod, Sabouraud Dextrose Agar, Autoclave and incubator.

2.2. Methods

2.2.1. Isolation of pathogen

Isolation of pathogen was patterned from the methods used by Vincent in 1974 [8]. Samples of pathogen (exactly similar to the Black Whiskers Mold in Mushroom Farm in Pactil) werebought from FilAna-Serve, a company that sells microbial and fungal cultures to culture uncontaminated black molds in the Philippines. Spores of mycelium were swab on the Sabourad Dextrose Agar (SDA) using a sterilized cotton swab included in the mycelial swab that was purchased from Fil Ana-Serve. The plates were incubated at temperature of 27°Cuntil fungal growth was visible. The fungi were then sub-cultured on fresh SDA medium for identification. The fungus that were obtained was stored in refrigerator at 4°C and was maintained by periodic sub-culturing in SDA slants after every 15 days. (See Fig. 1 below).

Fig. 1. Isolation of pathogens.

2.2.2. Preparation of dried garlic leaf extract

Two thousand grams (2000) of dried garlic leaves was used for the control of the molds. Dried garlic leaves were turned into small pieces using electric blender and macerated for 72 hours in 70% ethanol with 1:4 ratio (1 part ethanol to 4 parts of grounded dried garlic leaves).The mixture was frequently agitated by gently shaking the storage jar thrice a day to facilitate the extraction of the active constituents contained in the plant material. After seventy two hours of maceration, the mixture was filtrated using cheese cloth and Whatman paper no.1. It was concentrated in rotary evaporator to further remove the ethanol (See Figs. 2a, 2b, 2c).

a). Crushing dried garlic leaves in a blender.

b). Macerated dried garlic leaves in storage jars.

Fig. 2 a). Crushing dried garlic leaves in a blender.; b). Macerated dried garlic leaves in storage jars; c). Ethanolic extract in rotary evaporator.

2.2.3. In vitro evaluation

The effectiveness of garlic extracts was evaluated in vitro through Poison food technique [9]. Test concentrations of 30%, 45% and 60% was obtained by adding appropriate amount of sterile distilled water to the standard solution (100%). Five (5) mL of each garlic extract (30%, 45% and 60%) was dispensed in petriplates (9 cm) and then 20 mL of molten SDA was poured gently in petri plates containing the extract solution. After solidification, inoculations was done with 5 mm diameter mycelial cut from 6 days old cultures of black mold. The media without the garlic extract served as the control (T1) as comparison to check the mycelia growth. The plates were incubated at 27±1°C for 7 days (see Fig. 3a). Using a Vernier Caliper the mycelial growth was measured and tabulated for computation. Percent inhibition in growth was calculated in relation to growth in control using the following formula of Vincent 1947 [10]. The effect of the extract was based on the diameter it produces on the molds (Fig. 3b). The Table 1 below shows the basis of the of the effectivity of the extract

$$\text{Mycelial Inhibition} = \frac{\text{Radial growth in control} - \text{Radial growth in treatment}}{\text{Radial growth in control}} \times 100$$

Table 1. Scale of Susceptibility of Fungi to Fungicide (Herbal Extract)

	Diameter of zone of inhibition (mm)
Resistant	10mm or less
Intermediate	11mm-15mm
Susceptible	16mm or more

Fig. 3 a). Incubation/culture of fungus in laboratory incubator.

Fig. 3 b). Measurement of mycelial growth.

2.2.4. Experimental layout

The experimental groupings were treatments 1, 2, 3 and 4. Treatment 1 was the control group while treatment 2 has 30% ethanolic garlic leaf extract, T3 has 45% ethanolic garlic leaf extract and T4 has 60% ethanolic leaf extract. Each treatment has five sample petri dishes as shown in Table 2.

Table 2. Experimental Layout

T1 (No treatment)	T2 (30% Treatment)	T3 (45% Treatment)	T4 (60% Treatment)
T1S1	T2S1	T3S1	T4S1
T1S2	T2S2	T3S2	T4S2
T1S3	T2S3	T3S3	T4S3
T1S4	T2S4	T3S4	T4S4
T1S5	T2S5	T3S5	T4S5

2.2.5. Data gathered and statistical analysis

Mycelial growth per treatment after Five days and zone of inhibition per treatment were the data gathered. It was analyzed using single factorial Analysis of variance in Microsoft excel version 2013. Significant difference among treatments was further determined by Fisher Least significant difference test.

3. Results and Discussion

3.1. Mycelial Growth after Five Days (in Millimeters)

Table 3 shows the mycelial growth per treatment. Black molds in the control (T1) had the highest mean mycelial gowth after five days (84.9 mm,), followed by blackmolds in T3 with 17.3 mm mean mycelial growth. Next is T2 with mean mycelial gowth of 10.92mm. The least mean mycelial growth was measured in

T4 with 10 mm mycelia growth. Analysis of Variance showed a significant difference among the treatments, which implies that ethanolic dried garlic leaf extract inhibited/reduced the growth of mycelia of black molds. Fisher Least Significant Difference test revealed significant result between T1 and treatments 2, 3 and 4. Treatment 2 and treatment 3. No significant difference between treatment2 and treatment 4 as implied in similarity ofsuperscripts between paired treatment means in Table 3. The result signifies that a mushroom grower whose farm is infected with black molds can use concentration of 30% and 60% ethanolic dried garlic leaf extract to eliminate black molds in his farm. The result can be supported by the result of study onAntimicrobial Activity of Allicin-Containing Garlic Extracts against *Burkholderiacepacia*. It is stated that "Allicin and allicin-containing garlic extracts possess inhibitory and bactericidal activities against the Bcc. Present therapeutic options against these life-threatening pathogens are limited; thus, allicin-containing compounds merit investigation as adjuncts to existing antibiotics [11]." Similarly it is revealed in the study of Ankrion **t**he effect of a garlic extract and root substrate on soil borne fungal pathogens, concluded that the garlic extract was found to be fungicidal against a broad range of soil borne fungal organisms.

Table 3. Mycelial growth after Five Days (mm)

Treatment	Petri Dish Number					Mean
	1	2	3	4	5	
T1	84.9	84.9	84.9	84.9	84.9	84.9[a]
T2	11.73	10.76	7.04	9.54	17.06	10.92[b e]
T3	10.45	22.13	14.59	16.78	24.27	17.3[c]
T4	16.11	6.63	8.37	10.65	7.61	10[d e]

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	19603.61	3	6534.6	439.09*	1.44	3.24
Within Groups	238.1098	16	14.9			
Total	19841.72	19				

Legend: * significant at 5% level of significance

3.2. Zone of Inhibition

Zone of inhibition was computed by subtracting the radial growth of the treated group from the radial growth of the control group. The difference is then divided to the radial growth in the control and multiplied by 100. Table 4 shows that Black molds in treatment 4 had the highest zone of inhibition (65.11), followed by Blackmolds in T2 with 60.31 zone of inhibition. Treatment 3 follows with 49.42 and the least was observed in T1 (control) with 28.3 zone of inhibition. Based from the Scale of Susceptibility of Fungi to Fungicide/herbal extract (Table 1) treatments 2, 3 and 4 are susceptible to the different concentrations, which imply that the fungus in the treatments was inhibited. Analysis of. Variance showed a significant difference among the treatments, which implies that ethanolic dried garlic leaf extract inhibit the growth of mycelia of black molds. Fisher Least Significant Difference test revealed significant result between T1 and treatments 2, 3 and 4. Treatment 2 and treatment 3. Treatment 3 and treatment 4. No significant difference among treatments with ethanolic dried garlic leaf extracts (T2, T3 and T4) as implied by similar superscripts between pairedtreatments means in Table 4. The result signifies that a mushroom grower whose farm is infected with black molds can use any of the concentrations (30%, 45% and 60%) with ethanolic dried garlic leaf extract to eliminate black molds in his farm. The result of this claims can be supported by the studies as follows; Spices such as garlic (*Allium sativum)*, ginger (*Zingiberofficinale*), clove (*Eugenia caryophyllata*), and cinnamon (*Cinnamomumzeylanicum*) possess antimicrobial effect and has

been used traditionally. The spices showed inhibitory effect and garlic showed maximum inhibition on Escherichia coli and Bacillus subtilis. On treating the extracts of garlic and ginger at both room temperature (26°C) and at higher temperature (100°C) it was seen by Ankri in 1999 that garlic has significantly greater zone of inhibition than ginger as revealed by Ankri (1999). Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit i) antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *Escherichia coli*; ii) antifungalactivity, particularly against *Candida albicans*; iii) antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoebahistolytica* and *Giardia lamblia*; and iv) antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcoholdehydrogenase, thioredoxin reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of E. *histolytica*.

Table 4. Zone of Inhibition of Black Wishers Mold

Treatment	Petri dish No.					Mean
	1	2	3	4	5	
1 Control	28.3	28.3	28.3	28.3	28.3	28.3[a]
2 30%	58.58	61.94	75.12	66.21	39.72	60.31[bef]
3 45%	63.07	21.8	48.41	40.71	73.11	49.42[ceg]
4 60%	43.07	76.57	70.42	62.37	73.11	65.11[dfg]

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4016.94	3	1338.98	7.17*	0.00288	3.24
Within Groups	2987.83	16	186.74			
Total	7004.771	19				

Legend: * significant at 5%

4. Conclusion and Recommendation

Findings of this study revealed the leaves can be used as antimicrobial agents, black molds or fungus in particular. Instead of throwing the leaves after harvest of the bulb it can now be used by mushroom growers to inhibited the growth of the pest known as Black molds that greatly lowers the spawn production. The ethanolic dried leaves extract has antifungal property. With the result implicated in this study it can help mushroom growers minimize the use of commercial insecticide which is quite expensive, thus increase spawn production with less expense.

Further studies should be explored on the possibilities of using dried garlic leaves as pest control.

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