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Biorationals in the Suppression of Sooty Mold Fungus, *Capnodium* spp. (Camille Montagne) established on the coconut whitefly honeydew

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Abstract

Sooty mould, *Capnodium* spp., a saprophytic fungus from the phylum Ascomycota, produces dark-coloured colonies on plant surfaces smeared with honeydew of coconut whitefly. This study explains eco-friendly biorationals, comprising plant extracts, organic materials, and microbial agents, to control the growth of *Capnodium* spp. Morphological analysis revealed distinct hyphal septation and conical black spores, providing insights into *Capnodium* fungal development. Experiments were conducted using 35 plant extracts, of which 12 showed significant antifungal properties. Inorganic pesticides cannot be sprayed on coconut to control whitefly, as the spray may reach the households, especially waterbodies at households. Extracts of *Acorus calamus* (Sweet flag) exhibited the highest inhibition (98.61% at 50% concentration), followed by *Allium sativum* (Garlic, 84.06%) and *Asparagus racemosus* (Shatavari, 84.72%). In contrast, extracts of vermicompost tea showed no inhibitory effects, while fermented cow urine and distillery spent wash reached 100% inhibition of fungal growth. In microbial agents, *Trichoderma* spp. completely inhibited fungal growth, whereas *Pseudomonas* fluorescens exhibits no effect. The results explained that plant extracts with organic compounds or microbial agents provide solutions as an alternative to chemical fungicides. Combining extracts could provide an eco-friendly and sustainable strategy for managing *Capnodium* spp., reducing crop losses and increasing agricultural sustainability. These findings contribute to the growing body of research supporting biorational approaches as viable, environmentally safe options for fungal management. Field-scale applications are to be tested, and the effect of the biorationals on coconut whitefly also has to be determined.

Keywords- *Acorus calamus, biorational control, Capnodium, Sooty mould, Trichoderma spp.*

Introduction

Sooty mould are saprophytic fungi from Ascomycota, typically with dark-coloured hyphae, resulting in seemingly brown to black colonies on live plants. These types of moulds are frequently connected to whitefly and other producers of honeydew, albeit they can also happen in their absence. Sooty moulds can develop into a pseudoparenchymatous crust, a pellicle, a velutinous growth, or a thin network of hyphae on leaves and smaller twigs. Many sooty mould hyphae have a distinctly mucilaginous outer wall that serves as an adhesive, and quickly absorbs water surely keeping the leaf surface moist for a longer amount of time. Upon bigger branches and trunks the growths are typically stronger and could appear as spongy or lumpy pseudoparenchymatous stromata (Hughes, 2018).

The most frequent genera of fungi that cause sooty mould on garden and landscape plants are *Fumago, Scorias* and *Capnodium*. A few less frequent genera are *Antennariella, Limacinula, Fumiglobus and Aureobasidium.* The kind of presence of sooty mould-causing fungi depends on several factors, including the host, habitat and insect species. A few certain plants or insects are preferred by specific species of sooty mould. Whereas other mould species might grow on a variety of surfaces and make use of honeydew made by several insect species (Ipm 2006). Sooty moulds decrease plants' photosynthetic potential by large mycelial networks, resulting in chlorosis under the mycelia. Hence, sooty moulds hurt crop output and quality (Haituk et al., 2022) especially it over grow on the fruits that cause low market value.

To lessen the negative effects of chemical fungicides on the environment, which can damage non-target organisms, contaminate soil and water, and cause pesticide resistance, eco-friendly management of sooty mould is crucial. Sustainable substitutes that support ecological balance and agricultural sustainability include plant extracts, organic materials, and microbiological agents. They also provide longer-lasting, safer solutions.

The following objectives were developed for the study

i. To conduct a morphological study of the sooty mould fungus colony for a better understanding of its growth characteristics.
ii. To explore eco-friendly alternatives to chemical fungicides for the management of sooty mould.
iii. To assess the antifungal potential of microbial agents in controlling sooty mould fungus.

Materials and methods

These experiments were carried out at the JICA - Plant Protection Laboratory of the Department of Agricultural Biology, Faculty of Agriculture, University of Jaffna.

Isolation and identification of the sooty mould fungus

Isolation and inoculation of sooty mould

Whitefly infested leaves of coconut were collected from a home garden at Iranaimadu, Kilinochchi. The leaves were cut into small pieces and rinsed in distilled water. Then the sample was inoculated into 9 cm Petri dishes containing PDA. Plates were incubated at room temperature (30ºC) for five days. The pure culture of the fungal isolate was maintained in PDA plates and stored in the refrigerator at 4°C for further use. Mycelial growth rate, colour and spore characters of the colony were measured after 3 days of inoculation.

Screening of plant botanicals against sooty mould fungus

For this experiment, thirty-five kinds of plant extracts were tested on sooty mould as a preliminary experiment, however only 09 extracts were found to prominently inhibit mycelial growth higher than 50%.

Selection of plant extracts

Based on the literature research, thirty-five medicinal plants (Table 1)bthat show antifungal properties were identified and selected for *in-vitro* screening against sooty mould fungus.

Table 1: Screening of botanicals to determine their suppressive effect on Sooty mould fungus

Treatment	Botanicals	Family	Plant part used
T1	*Acorus calamus*	Acoraceae	Leaves
T2	*Punica granatum*	Punicaceae	Leaves
T3	*Asparagus racemosus*	Asparagaceae	Rhizome
T4	*Azadirachta indica*	Meliaceae	Leaves
T5	*Aloe barbadensis miller*	Asphodelaceae	Leaves
T6	*Curcuma longa*	Zingiberaceae	Rhizome
T7	*Curcuma aromatica*	Zingiberaceae	Rhizome
T8	*Zingiber officinale*	Zingiberaceae	Rhizome
T9	*Allium sativum*	Amaryllidaceae	Bulb
T10	*Alpinia officinarum*	Zingiberaceae	Rhizome
T11	*Moringa oleifera*	Moringaceae	Leaves
T12	*Ricinus communis*	Euphorbiaceae	Leaves
T13	*Ocimum sanctum*	Lamiaceae	Leaves
T14	*Glycyrhiza glabra*	Liquorice	Root
T15	*Withania somnifera*	Solanaceae	Root
T16`	*Terminalia catappa*	Combretaceae	Leaf
T17	*Tectona grandis*	Lamiaceae	Leaf
T18	*Syzygium cumini*	Myrtaceae	Leaf
T19	*Cynodon dactylon*	Poaceae	Leaf
T20	*Lantana camara*	Verbenaceae	Leaf
T21	*Gliricidia sepium*	Fabaceae	Leaf
T22	*Eucalyptus globulus*	Myrtaceae	Leaf
T23	*Andrographis paniculata*	Acanthaceae	Leaf
T24	*Artemisia vulgaris*	Asteraceae	Leaf
T25	*Ocimum basilicum*	Lamiaceae	Leaf
T26	*Cassia alata*	Fabaceae	Leaf
T27	*Cassia tora*	Fabaceae	Leaf
T28	*Piper nigrum*	Piperaceae	Leaf
T29	*Colocasia esculenta*	Araceae	Tuber
T30	*Chrysopogon zizanioides*	Poaceae	Leaf
T31	*Calotropis gigantea*	Apocynaceae	Leaf
T32	*Bauhinia racemosa*	Fabaceae	Leaf
T33	*Centella asiatica*	Apiaceae	Leaf
T34	*Thespesia populnea*	Malvaceae	Leaf
T35	*Solanum trilobatum*	Solanaceae	Leaf

Preparation of plant extracts

Plant parts which are having higher antifungal properties were collected from different field locations based on the literature (Table 2) and washed with running tap water to remove adhered impurities and rinsed with distilled water, and the surface was sterilised using 3% NaOCl for five mins and thereafter rinsed with distilled water.

The plant parts of the collected plants were sun-dried and made into powder separately using a sterilized grinder. The aqueous extracts were prepared by mixing 10g plant powder with 100 ml of water, if it had the suppression effect then the extracts were prepared into different concentrations to check the suppression effect depending on the concentration of the extracts. The mixture was kept overnight in the shaker to mix well the sample. Then the mixture was filtered through the membrane filter. The supernatant was kept separately. 3.9 g of PDA powder was added with 20 ml of water and sterilized. The autoclaved PDA was mixed with plant extract.

(a) (b)

Plate 1: Procedures for getting plant extract to inhibit the growth of Sooty mould (a): Shaking the powdered plant parts with a shaker (b): centrifuged plant extract

Antifungal activity of plant extracts

The food poisoning technique (Sah et al., 2021) was used as a method to check the inhibitory effect of plant extracts on sooty mould. 15ml of PDA with plant extract was poured into the Petri plate and allowed to solidify. 5mm diameter discs were obtained from a 140-day-old pure culture of sooty mould using a cork borer. The mycelial segment was transferred to plant extracts or organic matter mixed PDA medium. Petri dishes containing PDA without plant extracts were used as a control. The treatments were replicated four times. The inoculated Petri dishes were sealed by parafilm and incubated at room temperature (30ºC). The diameter of growing colonies of the fungus was recorded from 24 hours after inoculation to the time taken for the fungus to fully cover the 9 cm Petri dish.

The percentage growth inhibition (I) was calculated using the formula given below (Sivakumar, 2002)

I%=(C-T) / C

I= Percentage of inhibition of fungus by plant extracts

C=Radial growth in control

T=Radial growth in the treatment

Collection and Preparation of Distillery spent wash, Fermented cow urine and Vermi tea

Distillery Spent Wash (DSW)

One litre of distillery spent wash was obtained from the Araly distillery unit, Jaffna. The collected distillery spent wash was kept in two conical flasks. One is maintained as unsterilized. Another conical flask with distillery spent wash was sterilized with the help of an autoclave at 121° C for 15 minutes. 10%,30%, and 50% of the concentration of the distillery spent wash was taken from sterilized and unsterilized ones, with each concentration having four replicates. and these measured DSW were added to PDA.

Fermented urine

One litre of fermented urine was obtained from the farm of the Faculty of Agriculture, University of Jaffna. The collected fermented cow urine was kept in two conical flasks, from which one was sterilized with an autoclave at 121ºC, 15 psi for 15 mins. The other one was kept as unsterilized. Different concentration (10%, 20%, 50%) of fermented cow urine was taken from sterilized and unsterilized conical flasks. and added into the petri dish with 15ml of sterilized PDA. Each treatments with different concentrations was maintained with four replicates.

Preparation of Vermi tea

Vermicompost was made with bedding material made up of cow dung. Then the vermicompost was sieved and 500 grams of fresh vermicompost was weighed by the electrical balance. That was soaked in one litre of non-chlorinated water and blended thoroughly to create the vermi tea. An aquarium pump was used to aerate the

solution during a 1–2-hour brewing period. Following that, the suspension was filtered through muslin cloth and stored in a sterile glass for use as a treatment to control the sooty mould fungus.

The prepared vermi tea was kept in two conical flasks one was maintained as sterilized with the help of an autoclave. Other is maintained as unsterilized. Different concentration of the vermi tea (10%, 30%,50%) were poured into the 15ml of PDA with four replicates in each concentration.

Effect of different biocontrol agents *Trichoderma* sp. and *Pseudomonas* spp on mycelial growth of sooty mould

Effect of *Trichoderma* spp on Sooty mould

Under *in-vitro* conditions "Dual Culture Technique" was used as described by (Morton and stroube,1955). For the experiment 15ml of sterilized PDA medium were poured aseptically into sterilized petri dishes under laminar flow. 5mm disc of antagonist and test organism were cut from 14 days old culture by sterilized cork-borer and was placed opposite in Petri dishes containing PDA medium.

The disc was placed in a straight line at one centimetre from the corner of the Petri dishes. Potato Dextrose Agar plates inoculated with sooty mould alone were treated as a control. Four replications were maintained. The inoculated Petri dishes were maintained at room temperature and radial growth of *Trichoderma* and sooty mould was recorded for five days from inoculation. The inhibition zone was estimated based on the formula given by Vincent (1947), as the percentage of inhibition

I%=(C-T)/C x 100

I=Inhibition of mycelial growth

C = Growth of pathogen in control plate (cm)

T= Growth of the pathogen in dual cultures (cm)

Plate 2:Pure culture of *Trichoderma*

Effect of *Pseudomonas* sp. on Sooty mould

Suspension of *Pseudomonas* was streaked in the nutrient agar medium. After that the plates were incubated for 24 hours at 30^{O}c.

For dual culture, 20 ml of sterilized PDA medium was poured into the sterilized Petri dishes under the laminar flow condition. The antagonistic bacteria were streaked one cm from the edge of PDA in a 9 cm diameter Petri dish as shown in the Plate 3. After 24 hours of incubation at 28˚C, a 5 mm disc of an actively growing 14 days old culture of sooty mould was inoculated at the centre. Plates inoculated with alone sooty mould were maintained as a control. Plates were incubated for three days at 28°C and the percentage of inhibition of radial growth (PIRG) was recorded using the following formula (Sariah, 1994).

PIRG = (R1-R2)/R1 ×100

R1= Radial growth of sooty mould

R2=interacting with antagonistic bacteria

Plate 3: Pure culture of *Pseudomonas*

Analysis

Statistical Analysis

Each experiments were arranged according to the Complete randomized design (CRD) in two factor factorial and the data were statistically analyzed using SAS package 3.81 (Enterprise edition). Post hoc test was performed using the Duncan Multiple Range Test (DMRT) at 95% of the confidence level**.**

Correlation Analysis

Correlation analysis was performed to understand the relationships between different plant extracts and organic materials based on their inhibitory effects against sooty mould. This analysis aimed to determine if there were any associations between the inhibitory patterns of various treatments, which could suggest common bioactive compounds or similar modes of action.

The Pearson correlation coefficient (r) was calculated to measure the linear relationship between inhibition rates of different treatments at varying concentrations. The value of r ranges from -1 to +1:

- +1 indicates a perfect positive linear relationship.
- -1 indicates a perfect negative linear relationship.
- 0 indicates no linear relationship.

The correlation coefficients were calculated separately for each concentration (10%, 30%, and 50%) to observe how the relationships changed with varying levels of the treatment. A heatmap was created to visualize the correlation matrix, using color gradients to represent the strength and direction of the correlations. Darker shades indicated higher positive or negative correlations, while lighter shades indicated weaker correlations. Correlations with p-values less than 0.05 were considered statistically significant, indicating that the observed relationships were not due to random chance.

Based on these correlation patterns, treatments with r values close to +1 were identified as having similar inhibitory patterns, while treatments with low or negative correlations suggested distinct inhibitory properties.

Results and Discussion

This chapter describes the findings of the experiments conducted to determine the objectives proposed to find a solution and discusses the literature obtained from the published research papers and sources which are documented in Chapter 3.

Colony morphological characters of sooty mould culture

Sooty mould grown in PDA is shown in Plate 1. It had a full growth in a 9cm petridish within 3 days, specific growth rate of this fungus is not reported in any reports because the growth rate may be affected by environmental conditions (Smith et al., 1980). It first created white colour mycelium but after 7 days it turned into black colour. The texture of the colonies was primarily in velvety but some of the areas displayed a powdery appearance. The surface of the colonies was smooth with slight radial grooves visible under magnification. Circular-shaped colonies were observed but after one-week irregular edges were also observed in the colony similar report byAguirre-hudson et al. (2014). After 3 days of incubation at 30ºC, 9cm of growth is observed. The pigmentation was higher in the center compared to the periphery of the colony. Spore production was started after 10 days of inoculation. The spores were observed as scattered small black dots on the upper surface of the colony.

The morphological characters were also observed in the microscope by doing the hanging drop technique. The septation was observed in the hyphae of the fungus. The spores was in black with a conical shape, these microscopic characters are alinged with (Hughes 2018) observed characters.


(a) (b)

Plate 4 : Colony of Sooty mould (a): In 3 days after inoculation (b): In ten days after Inoculation

Evaluation of plant extracts with different concentrations against Sooty mould fungus

Table 2: Inhibition percentage of different plant extracts at different concentrations against sooty mould fungus (*Capnodium* spp) under *in vitro* condition

Plant extract	Inhibition percentage (%)		
	10%	30%	50%
1. Sweet flag	79.69±0.73^{a}	83.14±0.77^{a}	98.61±1.67^{b}
2. Khus	0^{d}	46.08±0.36^{c}	64.86±2.37^{a}
3. Pepper	0^{d}	0^{d}	4.38±1.35^{d}
4. Turmeric	40.22±2.98^{c}	53.39±2.69^{e}	75.83±2.62^{a}
5. Wild turmeric	41.86±2.9^{c}	52.92±2.28^{e}	81.25±0.52^{a}
6. Asparagus	30.86±2.25^{c}	52.92±2.82^{e}	84.72±1.66^{a}
7. Lesser galangal	0^{d}	22.61^{f}	42.22^{c}
8. Garlic	45.83±6.11c	65.47±1.39^{g}	84.06±1.75^{a}
9. Indian winter cherry	0^{d}	50.83±4.19^{e}	63.97±3.29^{g}
10. sickle senna	28.81±3.27^{c}	32.64±1.38^{c}	43.89±1.92^{c}
11. Gliricidia	0^{d}	16.75±2.04^{f}	51.94±2.62^{e}
12 .Mugwort	3.75±3.18^{d}	42.22±1.92^{c}	52.22±2.46^{e}

Values having the same letters in a column are not significantly different according to DMRT at 0.05 α

The inhibition percentage of different plant extracts with different concentrations is shown in Table 3 From the 35-plant extracts only 12 plant extracts showed inhibitory effects against the Sooty mould fungus, those 12 plant extracts performance are described in Table 3. From twelve plants, only nine plant extracts shown an inhibitory percentage of more than 50%. The highest inhibition (98.61±1.66%) was observed in sweet flag plant extract .at the concentration of 50% as of inhibition. And 10% concentration of this plant extract show 79.69±0.733% inhibition and 30% of this plant extract shows 83.14±0.766 % inhibition. These results revealed that chemical compounds which are present in the sweet flag plant have high antifungal properties against the sooty mould fungus compared to other tested plant extracts. Next to the sweet flag significant inhibition percentages found in the asparagus (84.72%) and garlic (84.06%). turmeric (75.83%) wild turmeric (81.25%), turmeric (75.83%).

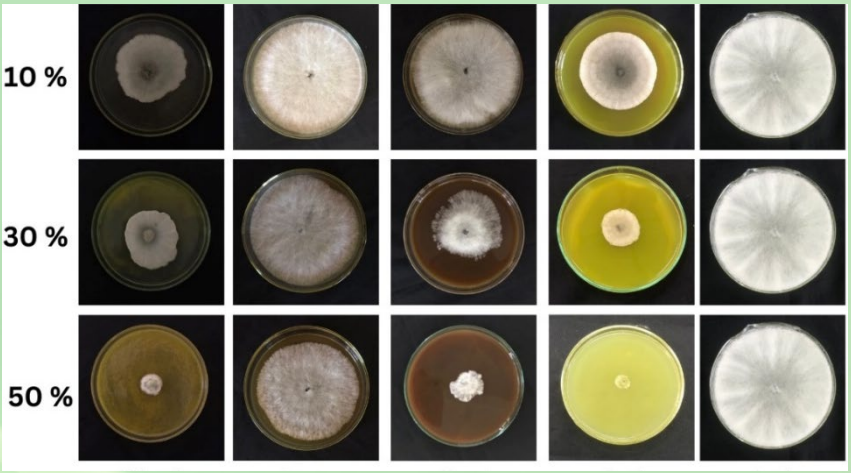
Other than this plant extracts like *Colocasia*, neem, liquorice, country almond, castor, Bermuda grass, *Centella*, ginger, teak, pomegranate, eucalyptus, bidi leaf tree, milkweed, jamun, moringa, holy basil is didn't contribute to the suppression of the sooty mould. Which means there is no significance in these treatments. So chemical compounds which are present in these plants are not suitable for the control of sooty mould or the concentration is may not be enough for the growth of the inhibition of the growth of the fungus. So the further study need to optimize the concentrations wants to see the efficacy of the chemical compounds to suppress the fungus.the chemical compounds that show antifungal properties against sooty mould fungus depend on the concentrations.

When the concentration increases in higher percentages their inhibitory property also will increase in higher levels, which is also reported by (Dellavalle et al. 2011). For example, the wild turmeric which shows 41.86% as its

inhibition percentage, changes to 65.47% when the concentration is increased from 10% to 30% and then reaches a higher inhibition percentage of 81.25% at 50% concentration. Indian winter cherry had 0% inhibition in 10% concentration of the plant extract suddenly reaches the inhibition percentage of 50.83% when the concentration at 30%. The results revealed that inhibition efficacy of the plant extract correlate with the plant extract concentration.

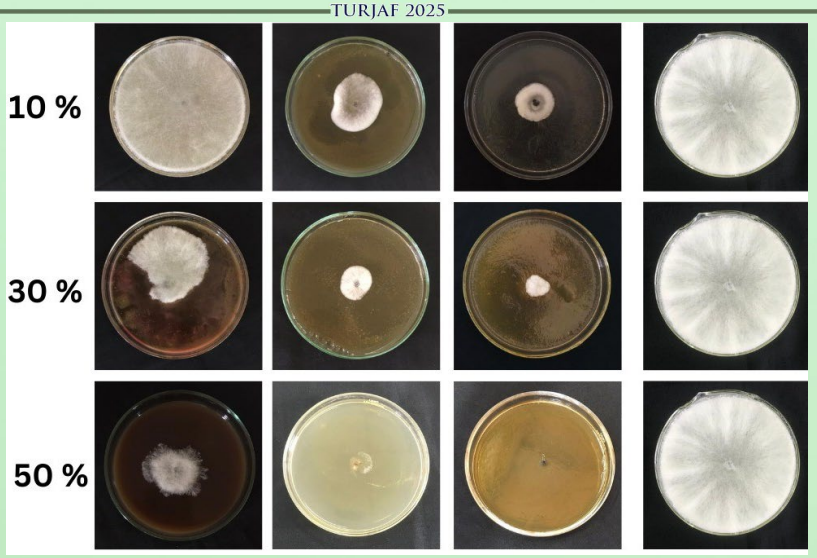
(a) (b) (c) (d) (e)

Plate 5: Comparison of growth of different plant extracts with control (a): Indian winter cherry, (b): Asparagus, (c): Lesser galangal, (d): Garlic, (e): Control



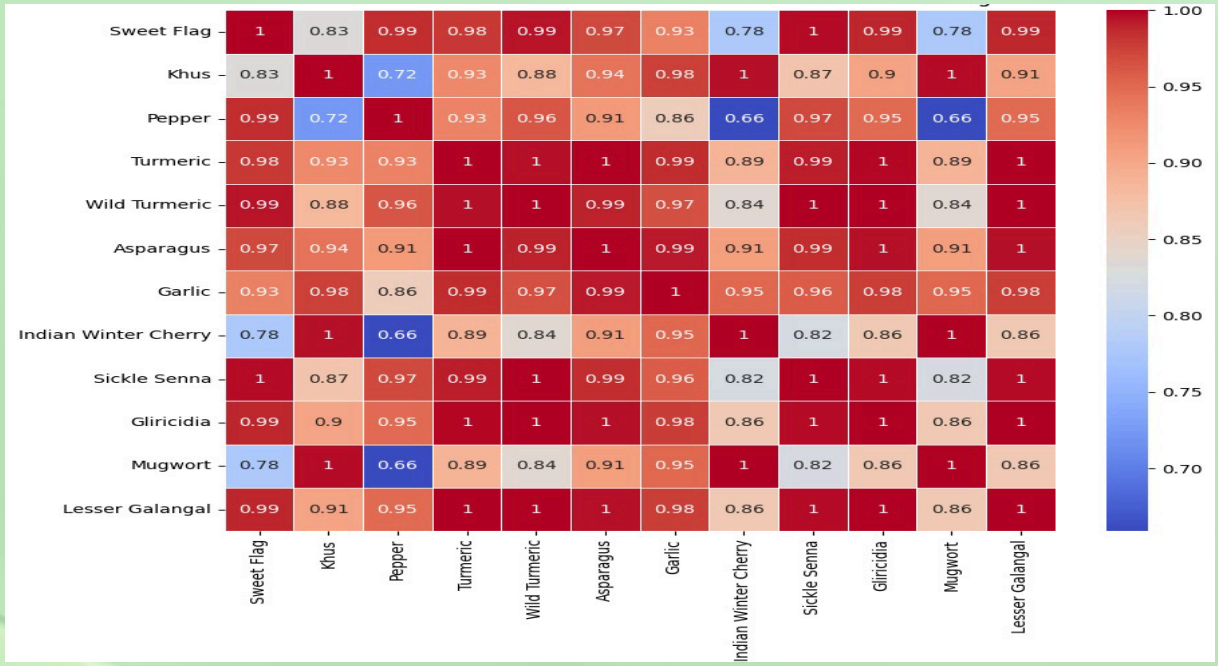
(a) (b) (c) (d) (e)

Plate 6: Comparison of growth of Sooty mould in different plant extract and in control(a): Wild turmeric (b): Pepper (c): Mugwort (d): Turmeric (e): Control



(a) (b) (c) (d)

Plate 7: Comparison of growth of Sooty mould in different plant extract and in control (a):Khus (b):Sickle senna (c) Sweet flag (d):Control



Correlation Between Plant Extract Based on Inhibition Percentage

The primary purpose of conducting correlation analysis on the antifungal activity of various plant extracts against sooty mould is to identify potential synergistic effects and relationships among the extracts. By examining correlation patterns, researchers can determine whether certain plant extracts share similar inhibitory properties, which may indicate the presence of complementary or overlapping bioactive compounds.

This analysis helps in understanding the interactions between different extracts and can provide insights into developing effective botanical mixtures with enhanced antifungal activity. Additionally, the correlation analysis can reveal unique properties of specific plant extracts that show weaker or distinct inhibition patterns, suggesting that these extracts might require different concentrations or combinations for optimal effectiveness. The findings from such an analysis are essential for guiding future research on optimizing the use of plant-based bio-fungicides in eco-friendly agricultural practices.

The idea of utilizing correlation analysis in evaluating the effects of plant extracts is supported by the research conducted by Dellavalle et al. (2011) and Singh et al. (2023). Studies have shown that synergy among bioactive compounds in plant extracts can significantly enhance their antifungal properties compared to their individual effects. The concept of synergistic effects and correlation analysis in plant extracts has been previously reviewed in other phytochemical studies that aim to understand plant–fungus interactions and to improve the efficacy of natural fungicide (Díaz Dellavalle et al. 2011);(Singh et al. 2023)

The high correlation coefficients (0.99 or 1) observed between Sweet Flag, Turmeric, Wild Turmeric, Asparagus, and Garlic indicate that these extracts exhibit highly similar inhibitory patterns against sooty mould. However, it is important to note that similar inhibition percentages do not necessarily imply similar chemical compositions or modes of action. For instance, different sets of bioactive compounds could produce comparable inhibition results due to varying mechanisms, such as disrupting cell walls or inhibiting fungal enzyme activity.

However, based on the work of Dellavalle et al. (2011) it can be intuitively expected that the bioactive chemicals contributing to the inhibitory potential of these various extracts could be complementary or overlapping. There aren't many relationships between Pepper and Indian Winter Cherry and other plant extracts. Pepper exhibits poorer correlations than the other extracts, particularly with Khus (0.72) and Indian Winter Cherry (0.66). This could mean that Pepper has a distinct mode of action or less potent inhibition than the other extracts. Indian Winter Cherry also correlates less with others, suggesting unique properties of its inhibitory action. These results suggest that these extracts might not be as effective overall or that they might require other chemicals or amounts to act as an inhibitor.

While Khus's correlations with most extracts are moderate (0.72 with Pepper, 0.88 with Wild Turmeric, and 0.87 with Sickle Senna), which suggests that its inhibition potential is not as strong as extracts like Sweet Flag, Turmeric, or Garlic, it still shows notable inhibition, particularly in specific concentrations. Gliricidia and Mugwort show moderate correlations (0.9–0.95) with most extracts, indicating that their overall inhibitory potential is slightly lower than that of other extracts like Garlic and Sickle Senna, with slightly weaker inhibition potential overall. When compared to highly correlated plant extracts like Sweet Flag and Turmeric, Pepper and Indian Winter Cherry stand out in terms of their correlation patterns. These outliers show that Pepper and Indian Winter Cherry may have unique inhibitory profiles and may not follow the same trends as the other more correlated extracts.

To make definitive conclusions regarding the similarity of bioactive chemicals, further phytochemical analysis and studies comparing the chemical profiles of these extracts would be necessary. Without such evidence, it is not possible to conclusively state that these extracts contain overlapping or complementary compounds. Thus, while the inhibition patterns observed are noteworthy, further research and references are required to confirm any claims regarding chemical similarity or mode of action.

Evaluation of inhibition of organic materials in the growth of sooty mould fungus.

Table 3: Inhibition percentage of organic materials at different concentrations against sooty mould under *in vitro* condition

Organic material	**Inhibition percentage**		
	10%	**30%**	**50%**
Distillery spent wash (Sterilized)	100[a]	100[a]	100[a]
Distillery spent wash (Unsterilized)	100[a]	100[a]	100[a]
Fermented urine (Sterilized)	100[a]	100[a]	100[a]
Fermented urine (Unsterilized)	100[a]	100[a]	100[a]
Vermi tea (Sterilized)	0[b]	0[b]	0[b]
Vermi tea (Unsterilized)	0[b]	0[b]	0[b]

All the values in each column having the same are not significantly different according to DMRT at 0.05.

Table 4: Evaluation of different parameters of fermented cow urine, distillery spent wash, vermi tea

Organic material	**TDS (mg/l)**	**PH**
Distillery spent wash (Sterilized)	1741	6.4
Distillery spent wash (Unsterilized)	1734	6.2
Fermented cow urine (Sterilized)	1600	5.8
Fermented cow urine (Unsterilized)	1587	5.6
Vermi tea (sterilized)	500	7.0
Vermi tea (unsterilized)	400	6.8

Fermented cow urine both sterilized and unsterilized was shown a 100% inhibition rate against the sooty mould fungus, it indicates strong antifungal properties. This effect may be due to the presence of bioactive compounds like phenols, ammonia, and other antimicrobial compounds which are present in the cow urine and also nutrient in a rich condition due to higher TDS (Singh Rawat et al. 2020).

Distillery spent wash also contribute to the complete inhibition of the fungus. This also due to the high organic compound content (higher TDS) and also presence of organic acids and alcohols that possess antifungal property (Kaushik et al. 2018). In contrast vermi tea's inhibition percentage is 0. It may be due to the absence of specific chemical compound/ nutrient which is required to inhibit the sooty mould fungus. The results suggest that the antifungal activity is influenced by both the chemical composition and the pH of the organic materials.

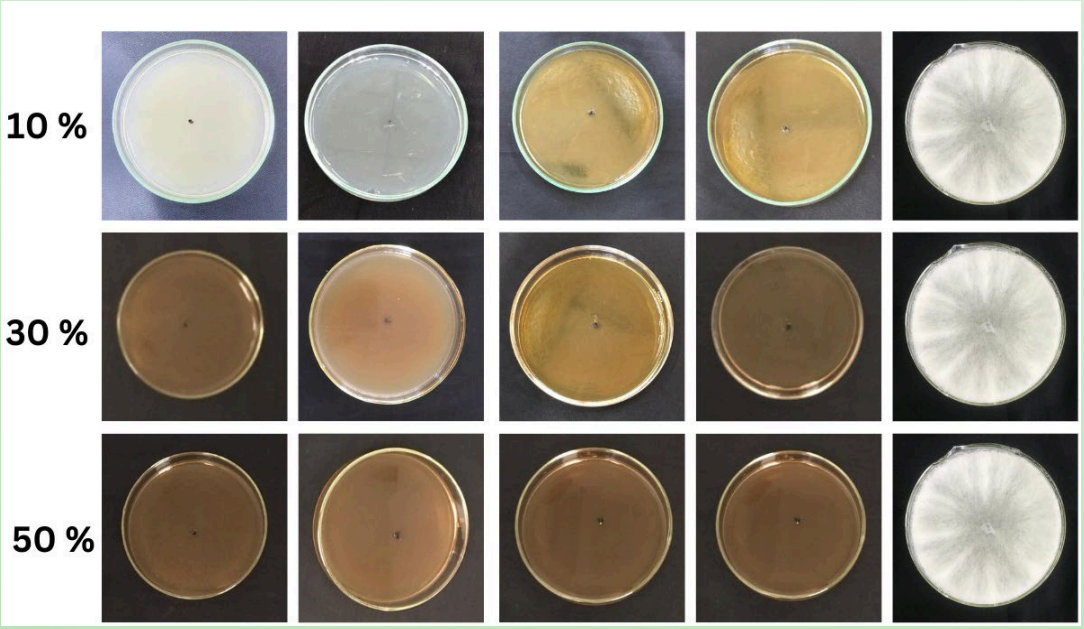


Plate 8: Comparison of growth of sooty mould in different plant extract and in control (a) Distillery spent wash(sterilized) (b) Distillery spent wash (unsterilized) (c) Fermented cow urine (Sterilized) (d) Fermented cow urine (unsterilized) (e) Control

These images indicates in plate 7 ,that there is no growth of Sooty mould in the treatments. Zero growth rate is observed.

Figure *1*:Inhibition percentage of Sooty mould in organic material at different concentration

Figure 3 shows the inhibition of Sooty mould using the fermented cow urine, distillery spent wash with different concentration of inhibition but the inhibition percentage remain as 100% even though the concentration is reduced.

Evaluation of Antifungal activity of *Trichoderma* species against Sooty mould fungus

Table *5*:Day by day growth of *Trichoderma* against Sooty mould

Organism	**Growth (cm)**				
	1st DAC	**2nd DAC**	**3rd DAC**	**4th DAC**	**5th DAC**
Trichoderma sp	1.5	2.6	3	4.7	9
Sooty mould	2.5	5.2	6	4.4	0

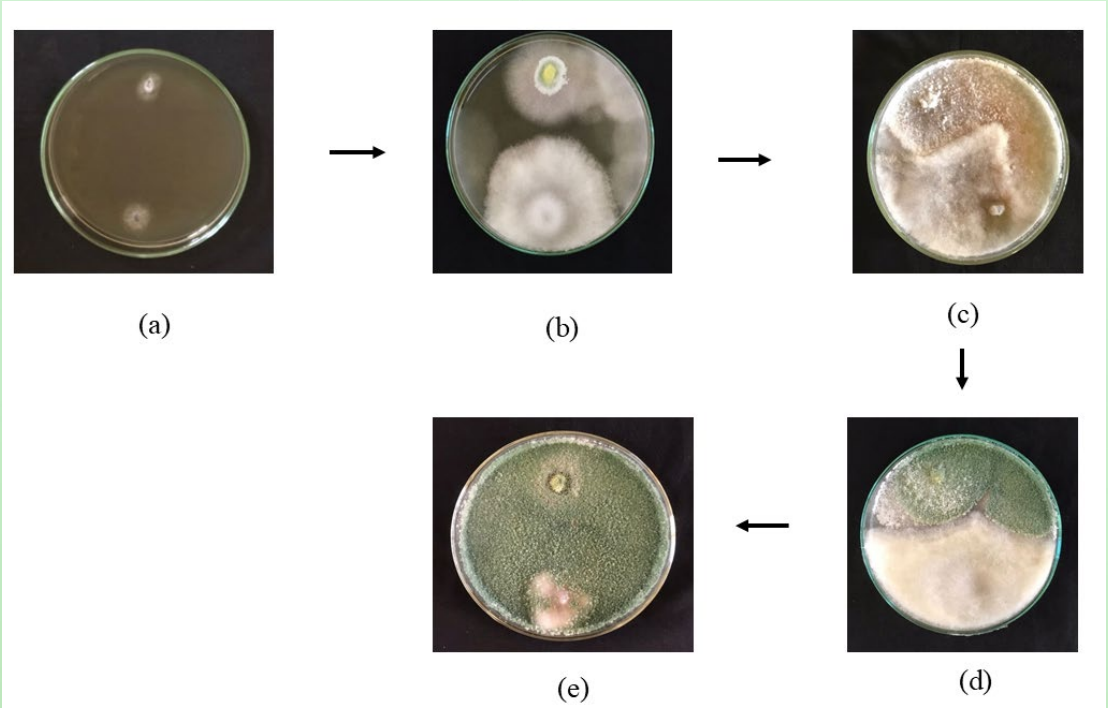


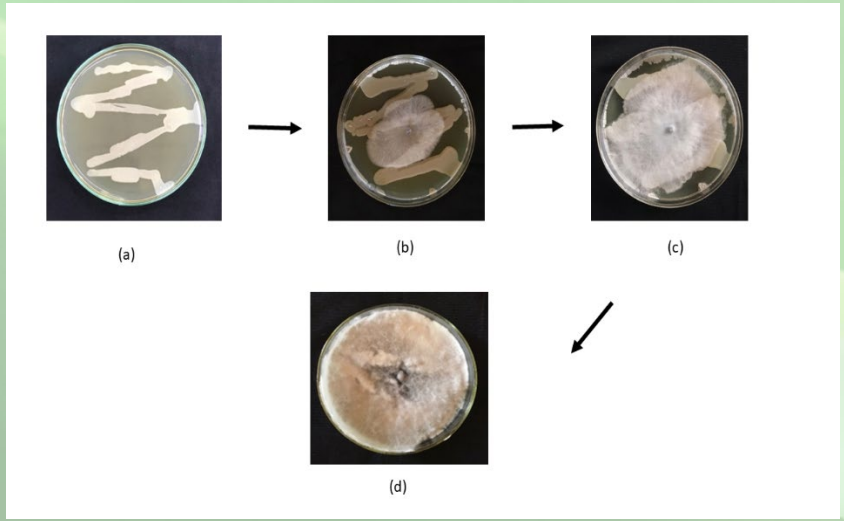
Plate 9: Gradual growth of *Trichoderma* in Dual culture with Sooty mould (a) 1 DAC (b) 2 DAC (c) 3 DAC (d) 4 DAC (e) 5 DAC

The colony of *Trichoderma* and Sooty mould grow normally without any interruption until the second day after inoculation. The third day Sooty mould mycelial growth is slightly inhibited by the *Trichoderma.* Because normally the growth of Sooty mould will reach 9cm after 3 days of incubation. but the mycelium was only reached 6cm and other spaces of the Petri dish was encroached by *Trichoderma*
Gradually *Trichoderma* was spread to the petri dish fully and suppress the sooty mould. Growth of Sooty mould in control is 9cm and the growth of Sooty mould in dual culture in 0. So the results revealed that the inhibition percentage of the *Trichoderma* to Sooty mould fungus is calculated as 100%. So the *Trichoderma* fully control the sooty mould. Sooty mould have the antifungal activity against many fungus through their mechanisms such as mycoparasitism, competition and production of antifungal compounds (Mukhopadhyay and Kumar 2020).

Evaluation of the antifungal activity of *Pseudomonas* species against sooty mould fungus

Table *6*: Day-by-day growth of Sooty mould in control and in *Pseudomonas* inoculated culture plate

	Colony diameter cm		
	1st DAC	**2nd DAC**	**3rd DAC**
Dual culture	2.7	5.2	9
Pure culture	2.9	5.8	9



Sooty mould culture was grown normally like the control one without any interruption in the dual culture . Within three days the fungus was grown fully to 9cm in diameter and covered the petri dish fully.There is no suppression effect of *Pseudomonas* for sooty mould fungus. So radial growth of the pathogen in control and the radial growth of the pathogen in dual culture were equal of 9 cm (0% inhibition).0. *Pseudomonas* inhibit many fungus species (Goswami et al. 2015). Due to the superficial growth and environmental conditions may be unsuitable for controlling of the sooty mould with *Pseudomonas* bacteria.

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References

Abdulaziz, Abdullah A., Mark Lloyd G. Dapar, Agnes T. Aranas, Roland Anthony R. Mindo, Clint Kenny Cabrido, Muhmin Michael E. Manting, Mark Anthony Torres, and Cesar G. Demayo. 2019. "Qualitative Evaluation of the Antimicrobial, Antioxidant, and Medicinally Important Phytochemical Constituents of the Ethanolic Extracts of the Leaves of Gliricidia Sepium (Jacq.) Walp." *Pharmacophore* 10(4):72–83.

Abdullah, Syahriel, Januarius Gobilik, and Khimphin Chong. 2012. "Preliminary Phytochemical Study and Antimicrobial Activity from Various Extract of Cynodondactylon (L.) Pers. (Bermuda) against Selectedpathogens." *International Journal of Pharmacy and Pharmaceutical Sciences* 4(SUPPL. 5):227–30.

Abioye, J. O. K., E. Manaja, and A. L. Olokun. 2022. "Screening Asclepias Syriaca for Antimicrobial Activity." *Journal of Advances in Microbiology* (March):1–7. doi: 10.9734/jamb/2022/v22i330441.

Aguirre-hudson, Begoña, Qing Tian, Derek Peršoh, Manpreet K. Dhami, and Aisyah S. Alias. 2014. "The Sooty Moulds." (July). doi: 10.1007/s13225-014-0278-5.

Akpuaka, A., M. M. Ekwenchi, D. A. Dashak, and A. Dildar. 2013. "Biological Activities of Characterized Isolates of N-Hexane Extract of Azadirachta Indica A.Juss (Neem) Leaves." *New York Science Journal* 6(16):119–24.

Anon. 1991. "Review Antibiotics Produced." 23(11):1991.

Apisariyakul, Amphawan, Nongnuch Vanittanakom, and Duang Buddhasukh. 1995. "Antifungal Activity of Turmeric Oil Extracted from Curcuma Longa (Zingiberaceae)." *Journal of Ethnopharmacology* 49(3):163–69. doi: 10.1016/0378-8741(95)01320-2.

Arunkumar, S., and M. Muthuselvam. 2009. "Analysis of Phytochemical Constituents and Antimicrobial Activities of Aloe Vera L. against Clinical Pathogens." *World Journal of Agricultural Sciences* 5(5):572–76.

Balakumar, S., S. Rajan, T. Thirunalasundari, and S. Jeeva. 2011. "Antifungal Activity of Ocimum Sanctum Linn. (Lamiaceae) on Clinically Isolated Dermatophytic Fungi." *Asian Pacific Journal of Tropical Medicine* 4(8):654–57. doi: 10.1016/S1995-7645(11)60166-1.

Banerjee, Arnab, Sant Gahira, Guru Vishwavidyalaya, Jayanta Kumar Datta, and Srimanta Gupta. 2009. "Antifungal Activities and Chemical Characterization of Neem Leaf Extracts on the Growth of Some Selected Fungal Species in Vitro Culture Medium Geochemical and Geospatial Studies of Birbhum District View Project Environmental Monitoring Project View Proje." (July 2014).

Batiha, Gaber El Saber, Amany Magdy Beshbishy, Lamiaa G. Wasef, Yaser H. A. Elewa, Ahmed A. Al-Sagan, Mohamed E. Ab. El-Hack, Ayman E. Taha, Yasmina M. Abd-Elhakim, and Hari Prasad Devkota. 2020. "Chemical Constituents and Pharmacological Activities of Garlic (Allium Sativum L.): A Review." *Nutrients* 12(3):1–21. doi: 10.3390/nu12030872.

Bhuyar, Prakash, Mohd Hasbi Ab Rahim, Gaanty Pragas Maniam, and Natanamurugaraj Govindan. 2021. "ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS IN MEDICINAL PLANT Centella Asiatica AND STUDY THE EFFECTS ON FUNGAL ACTIVITIES." *Journal of Microbiology, Biotechnology and Food Sciences* 10(4):631–35. doi: 10.15414/jmbfs.2021.10.4.631-635.

Chakraborty, Pritha, Papiya Deb, Sudeshna Chakraborty, Bohnisikha Chatterjee, and Jayanthi Abraham. 2015. "Cytotoxicity and Antimicrobial Activity of Colocasia Esculenta." *Available Online Www.Jocpr.Com Journal of Chemical and Pharmaceutical Research* 7(12):627–35.

Chandra, Deepak, and Kundan Prasad. 2017. "Phytochemicals of Acorus Calamus (Sweet Flag)." (January).

Chen, Cun, Chun Hong Liu, Jing Cai, Wei Zhang, Wei Liang Qi, Zheng Wang, Zhi Bin Liu, and Yi Yang. 2018. "Broad-Spectrum Antimicrobial Activity, Chemical Composition and Mechanism of Action of Garlic (Allium Sativum) Extracts." *Food Control* 86:117–25. doi: 10.1016/j.foodcont.2017.11.015.

Chukanhom, Kanit, Prasatporn Borisuthpeth, and Kishio Hatai. 2005. "Antifungal Activities of Aroma Components from Alpinia Galanga against Water Molds." *Biocontrol Science* 10(3):105–9. doi:

10.4265/bio.10.105.
Dellavalle, Paola Díaz, Andrea Cabrera, Diego Alem, Patricia Larrañaga, Fernando Ferreira, and Marco Dalla Rizza. 2011. "Antifungal Activity of Medicinal Plant Extracts against Phytopathogenic Fungus Alternaria Spp." *Chilean Journal of Agricultural Research* 71(2):231–39. doi: 10.4067/s0718-58392011000200008.
Dhami, Manpreet K., Bevan S. Weir, Michael W. Taylor, and Jacqueline R. Beggs. 2013. "Diverse Honeydew-Consuming Fungal Communities Associated with Scale Insects." *PLoS ONE* 8(7):1–12. doi: 10.1371/journal.pone.0070316.
Díaz Dellavalle, Paola, Andrea Cabrera, Diego Alem, Patricia Larrañaga, Fernando Ferreira, and Marco Dalla Rizza. 2011. "Antifungal Activity of Medicinal Plant Extracts against Phytopathogenic Fungus Alternaria Spp." *Chilean Journal of Agricultural Research* 71:231–39.
Elkousy, Rawah H., Zeinab N. A. Said, Mohamed A. Abd El-Baseer, and Salwa A. Abu El wafa. 2021. "Antiviral Activity of Castor Oil Plant (Ricinus Communis) Leaf Extracts." *Journal of Ethnopharmacology* 271(January):113878. doi: 10.1016/j.jep.2021.113878.
Galawat, Fadil, and Mitsuyasu Yabe. 2012. "Profit Efficiency in Rice Production in Brunei Darussalam: A Stochastic Frontier Approach." *Journal of the International Society for Southeast Asian Agricultural Sciences* 18(1):100–112.
Goswami, Debahuti, Siddhartha Narayan Borah, Jiumoni Lahkar, Pratap Jyoti Handique, and Suresh Deka. 2015. "Antifungal Properties of Rhamnolipid Produced by Pseudomonas Aeruginosa DS9 against Colletotrichum Falcatum." *Journal of Basic Microbiology* 55(11):1265–74. doi: 10.1002/jobm.201500220.
Grover, Madhuri, Tapan Behl, Tarun Virmani, Saurabh Bhatia, Ahmed Al-Harrasi, and Lotfi Aleya. 2021. "Chrysopogon Zizanioides—a Review on Its Pharmacognosy, Chemical Composition and Pharmacological Activities." *Environmental Science and Pollution Research* 28(33):44667–92. doi: 10.1007/s11356-021-15145-1.
Hughes, Stanley J. 2018. "Sooty Moulds." 5514(1976):693–820. doi: 10.1080/00275514.1976.12019958.
Ipm, U. C. 2006. "Sooty Mold." *Encyclopedia of Entomology* (September):2055–2055. doi: 10.1007/0-306-48380-7_3983.
J. S. Smith, Jr., and W. L. Tedders. 1980. "Light Measurements for Studying Sooty Mold Growth on Simulated Pecan Foliage." *Transactions of the ASAE* 23(2):0481–84. doi: 10.13031/2013.34607.
Jang, M. H., X. L. Piao, J. M. Kim, S. W. Kwon, and J. H. Park. 2008. "Inhibition of Cholinesterase and Amyloid-&bgr; Aggregation by Resveratrol Oligomers from Vitis Amurensis." *Phytotherapy Research* 22(4):544–549. doi: 10.1002/ptr.
Kahramanoğlu, Ibrahim, Chuying Chen, Jinyin Chen, and Chunpeng Wan. 2019. "Chemical Constituents, Antimicrobial Activity and Food Preservative Characteristics of Aloe Vera Gel." *Agronomy* 9(12). doi: 10.3390/agronomy9120831.
Karaca, Ayten. 2011. "Biology of Earthworms (Soil Biology, 24)." (November 2010). doi: 10.1007/978-3-642-14636-7.
Kaushik, A., S. Basu, V. S. Batra, and M. Balakrishnan. 2018. "Industrial Crops & Products Fractionation of Sugarcane Molasses Distillery Wastewater and Evaluation of Antioxidant and Antimicrobial Characteristics." *Industrial Crops & Products* 118(February):73–80. doi: 10.1016/j.indcrop.2018.03.040.
Khanchandani, Nisha, Prachi Shah, Twinkle Kalwani, Anujkumar Ardeshna, and Darshan Dharajiya. 2019. "Antibacterial and Antifungal Activity of Ashwagandha (Withania Somnifera L.): A Review." *Journal of Drug Delivery and Therapeutics* 9(5-s):154–61. doi: 10.22270/jddt.v9i5-s.3573.
Kucuk, Cigdem, and Merith Kicanic. 2003. "Isolation of Trichoderma Spp . and Determination of Their Antifungal , Biochemical and Physiological Features." *Turkish Journal of Biology* 27(January 2003):247–53.
Kumar, R. Sunish, N. Ayyadurai, P. Pandiaraja, A. V. Reddy, Y. Venkateswarlu, O. Prakash, and N. Sakthivel. 2005. "Characterization of Antifungal Metabolite Produced by a New Strain Pseudomonas Aeruginosa PUPa3 That Exhibits Broad-Spectrum Antifungal Activity Biofertilizing Traits." *Journal of Applied Microbiology* 98(1):145–54. doi: 10.1111/j.1365-2672.2004.02435.x.
Kumar, Vinod. 2014. "Antimicrobial Activity of Rhizome Extract of Acorus Calamus Against Different Micro-Organisms." 2(June):3663.
Length, Full. 2008. "Antimicrobial Activity of Leaf Extracts of Senna Obtusifolia (L)." *Journal of Pharmacy and Pharmacology* 2(1):007–013.
Li, Wen Ru, Qing Shan Shi, Qing Liang, Xiao Mo Huang, and Yi Ben Chen. 2014. "Antifungal Effect and Mechanism of Garlic Oil on Penicillium Funiculosum." *Applied Microbiology and Biotechnology* 98(19):8337–46. doi: 10.1007/s00253-014-5919-9.
Maheshwari, M., R. Ramkumar, V. P. Arun, B. Anandharaj, and M. Sathya. 2021. "Evaluation of Phytochemical Analysis and Antibacterial Activity of Andrographis Echioides (L) Nees (Green Chiretta)." *International Journal of Botany Studies* 6(4):378–83.
Mandavgane, Sachin A., and Bhaskar D. Kulkarni. 2020. "Valorization of Cow Urine and Dung: A Model

Biorefinery." *Waste and Biomass Valorization* 11(3):1191–1204. doi: 10.1007/s12649-018-0406-7.

Mansoori, Aadil, Nitesh Singh, Sharad Kumar Dubey, Tarun K. Thakur, Noam Alkan, Subha Narayan Das, and Anirudh Kumar. 2020. "Phytochemical Characterization and Assessment of Crude Extracts From Lantana Camara L. for Antioxidant and Antimicrobial Activity." *Frontiers in Agronomy* 2(November). doi: 10.3389/fagro.2020.582268.

Mendy, T. K., A. Misran, T. M. M. Mahmud, and S. I. Ismail. 2019. "Antifungal Properties of Aloe Vera through in Vitro and in Vivo Screening against Postharvest Pathogens of Papaya Fruit." *Scientia Horticulturae* 257(April):108767. doi: 10.1016/j.scienta.2019.108767.

Mesquita, Naasoom Luiz Santos, Armínio Santos, Rafael José Vilela de Oliveira, Poliana Prates de Souza Soares, Quelmo Silva de Novaes, and José Luiz Bezerra. 2022. "Sooty Mould Caused by Capnodium Alfenasii on Azadirachta Indica." *Journal of Phytopathology* 170(7–8):574–78. doi: 10.1111/jph.13120.

Milenković, Aleksandra, and Ljiljana Stanojević. 2021. "Black Pepper: Chemical Composition and Biological Activities." *Advanced Technologies* 10(2):40–50. doi: 10.5937/savteh2102040m.

Mohamed, Amal A., Sami I. Ali, and Farouk K. El-Baz. 2013. "Antioxidant and Antibacterial Activities of Crude Extracts and Essential Oils of Syzygium Cumini Leaves." *PLoS ONE* 8(4). doi: 10.1371/journal.pone.0060269.

Mohana, Sarayu, Bhavik K. Acharya, and Datta Madamwar. 2009. "Distillery Spent Wash: Treatment Technologies and Potential Applications." *Journal of Hazardous Materials* 163(1):12–25. doi: 10.1016/j.jhazmat.2008.06.079.

Mu, Jingjuan, Xianping Li, Jiaguo Jiao, Guanning Ji, Jun Wu, Feng Hu, and Huixin Li. 2017. "Biocontrol Potential of Vermicompost through Antifungal Volatiles Produced by Indigenous Bacteria." *Biological Control* 112:49–54. doi: 10.1016/j.biocontrol.2017.05.013.

Muhammad, Aminu, Suleiman Yusuf Mudi, A Muhammad, and S Y Mudi. 2011. "Phytochemical Screening and Antimicrobial Activities of Terminalia Catappa , Leaf Extracts Phytochemical Screening and Antimicrobial Activities of Terminalia Catappa, Leaf Extracts." *Nigerian Society for Experimental Biology* 23(1):35–39.

Mukhopadhyay, Ria, and Deepak Kumar. 2020. "Trichoderma: A Beneficial Antifungal Agent and Insights into Its Mechanism of Biocontrol Potential." *Egyptian Journal of Biological Pest Control* 30(1). doi: 10.1186/s41938-020-00333-x.

Narendhiran, S., S. Mohanasundaram, J. Arun, R. V Rannjith, L. Saravanan, L. Catherine, and M. Subathra. 2014. "Phytochemical Screening and Antimicrobial Activity of Thespesiapopulnealinn Phytochemical Screening and Antimicrobial Activity of Thespesiapopulnea Linn." (January).

Nazli, Rahila, Tehmina Sohail, Bushra Nawab, and Zahra Yaqeen. 2011. "Antimicrobial Property of Gliricidia Sepium Plant." 24(1):51–55.

Noumi, Emira, Mejdi Snoussi, Hafedh Hajlaoui, Najla Trabelsi, Riadh Ksouri, Eulogio Valentin, and Amina Bakhrouf. 2011. "Chemical Composition, Antioxidant and Antifungal Potential of Melaleuca Alternifolia (Tea Tree) and Eucalyptus Globulus Essential Oils against Oral Candida Species." *Journal of Medicinal Plants Research* 5(17):4147–56.

Nusra, M. S. F., and M. H. Haroon. 2016. "Antifungal Activity of Asparagus Racemosus (Willd)." *5th Annual Science Research Sessions-2016* 210–16.

Obafemi, C. A., D. A. Akinpelu, O. O. Taiwo, and A. Adeloye. 2007. "Antimicrobial Activity of Solvent Extracts of *Terminalia Catappa* Linn Leaves." *Ife Journal of Science* 8(1).

OWOYALE, J. A., G. A. OLATUNJI, and S. O. OGUNTOYE. 2005. "Antifungal and Antibacterial Activities of Alocoholic Extract of Seena Alata Leaves." *Journal of Applied Sciences and Enviornment Managment* 9(3):105107.

Passos, Juliana Lanna, Luiz Claudio Almeida Barbosa, Antonio Jacinto Demuner, Elson Santiago Alvarenga, Cleiton Moreira Da Silva, and Robert Weingart Barreto. 2012. "Chemical Characterization of Volatile Compounds of Lantana Camara l. and l. Radula Sw. and Their Antifungal Activity." *Molecules* 17(10):11447–55. doi: 10.3390/molecules171011447.

Patel, Pinal, Nivedita Patel, Dhara Patel, Sharav Desai, and Dhananjay Meshram. 2014. "Phytochemical Analysis and Antifungal Activity of Moringa Oleifera." *International Journal of Pharmacy and Pharmaceutical Sciences* 6(5):144–47.

Pogaku, Ravindra, Awang Bono, and Christopher Chu. 2013. "Developments in Sustainable Chemical and Bioprocess Technology." *Developments in Sustainable Chemical and Bioprocess Technology* 1–424. doi: 10.1007/978-1-4614-6208-8.

Pratap, Chandran R., S. Manju, M. V. Vysakhi, P. K. Shaji, and Nair G. Achuthan. 2014. "Antibacterial and Antifungal Activities of Thespesia Populnea Leaf Extracts against Human Pathogens." *International Journal of PharmTech Research* 6(1):290–97.

Rashed, Khaled, and Monica Butnariu. 2014. "Antimicrobial and Antioxidant Activities of Bauhinia Racemosa Lam. and Chemical Content." *Iranian Journal of Pharmaceutical Research* 13(3):1073–80.

Rongai, Domenico, Patrizio Pulcini, Giovanni Di Lernia, Paolo Nota, Pjerin Preka, and Filomena Milano. 2019. "Punicalagin Content and Antifungal Activity of Different Pomegranate (Punica Ganatum L.) Genotypes." *Horticulturae* 5(3):1–9. doi: 10.3390/horticulturae5030052.

S., Zorofchian Moghadamtousi, Abdul Kadir H., Hassandarvish P., Tajik H., Abubakar S., and Zandi K. 2014. "A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin." *BioMed Research International* 2014.

Sah, Dr. Mithilesh Kumar, Binod Singh, and Prasant Singh. 2021. "Literary Review on Ageing Process and It'S Natural Ayurveda Approach." *Journal of Ayurveda Campus* 2(1):110–19. doi: 10.51648/jac.41.

Saharkhiz, Mohammad Jamal, Amir Alam Kamyab, Narges Khatoon Kazerani, Kamiar Zomorodian, Keyvan Pakshir, and Mohammad Javad Rahimi. 2015. "Chemical Compositions and Antimicrobial Activities of Ocimum Sanctum L. Essential Oils at Different Harvest Stages." *Jundishapur Journal of Microbiology* 8(1):1–7. doi: 10.5812/jjm.13720.

Sasidharan, Indu, and a Nirmala Menon. 2010. "Comparative Chemical Composition and Antimicrobial Activity Fresh & Dry Ginger Oils (Zingiber Officinale Roscoe)." *International Journal of Current Pharmaceutical Research* 2(4):4–7.

Schmidt, Alexander R., Christina Beimforde, Leyla J. Seyfullah, Sarah Elena Wege, Heinrich Dörfelt, Vincent Girard, Heinrich Grabenhorst, Matthias Gube, Jochen Heinrichs, André Nel, Patricia Nel, Vincent Perrichot, Joachim Reitner, and Jouko Rikkinen. 2014. "Amber Fossils of Sooty Moulds." *Review of Palaeobotany and Palynology* 200:53–64. doi: 10.1016/j.revpalbo.2013.07.002.

shmi, Ra, D. V. Pathak, and R. Kumar. 2019. "Effect of Ricinus Communis L on Microorganisms: Advantages and Disadvantages." *International Journal of Current Microbiology and Applied Sciences* 8(04):878–84. doi: 10.20546/ijcmas.2019.804.099.

Shubin, Li, Huang Juan, Zhou Renchao, Xu Shiru, Jin Yuanxiao, and Ned B. Klopfenstein. 2014. "Fungal Endophytes of Alpinia Officinarum Rhizomes: Insights on Diversity and Variation across Growth Years, Growth Sites, and the Inner Active Chemical Concentration." *PLoS ONE* 9(12):1–21. doi: 10.1371/journal.pone.0115289.

Singh, Nameirakpam Bunindro, Moirangthem Lakshmipriyari Devi, Thokchom Biona, Nanaocha Sharma, Sudripta Das, Jharna Chakravorty, Pulok Kumar Mukherjee, and Yallappa Rajashekar. 2023a. "Phytochemical Composition and Antimicrobial Activity of Essential Oil from the Leaves of Artemisia Vulgaris L." *Molecules* 28(5). doi: 10.3390/molecules28052279.

Singh, Nameirakpam Bunindro, Moirangthem Lakshmipriyari Devi, Thokchom Biona, Nanaocha Sharma, Sudripta Das, Jharna Chakravorty, Pulok Kumar Mukherjee, and Yallappa Rajashekar. 2023b. "Phytochemical Composition and Antimicrobial Activity of Essential Oil from the Leaves of Artemisia Vulgaris L." *Molecules* 28(5). doi: 10.3390/molecules28052279.

Singh Rawat, Nawal, Surender Singh Lathwal, Girish J. Panchbhai, Ravi Kumar Pal, Amit Kumar Jha, and Mohabat Singh Jamra. 2020. "Physical, Microbial and Biochemical Composition of Bio-Pesticides Based on Cow Urine and Dung with Medicinal Plants." *~ 754 ~ Journal of Pharmacognosy and Phytochemistry* 9(5):754–58.

Sumathy, Dr. V. Judia Harriet. 2017. "A Comparative Study on the Phytochemicals, Antioxidant, Antidiabetic and Antimicrobial Activity of the Leaves of Solanum Nigrum and Solanum Trilobatum." *World Journal of Pharmaceutical Research* 4(1):272–85. doi: 10.20959/wjpr201710-9340.

Umar, Nura Muhammad, Thaigarajan Parumas ivam, Nafiu Aminu, and Seok Ming Toh. 2020. "Phytochemical and Pharmacological Properties of Curcuma Aromatica Salisb (Wild Turmeric)." *Journal of Applied Pharmaceutical Science* 10(10):180--194. doi: 10.7324/JAPS.2020.1010018.

Wuthi-Udomlert, Mansuang, Pavena Kupittayanant, and Wandee Gritsanapan. 2010. "In Vitro Evaluation of Antifungal Activity of Anthraquinone Derivatives of Senna Alata." *J Health Res* 24(3):117–22.