Study on antimicrobial potency of alliin-rich black-garlic (*Allium sativum*) extract on soil micro biome

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By

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I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of her Master of Technology in Food Technology and Biochemical Engineering studies during academic session 2021-2023.

All information in this document has been obtained and presented in accordance with academic rules and ethical conduct.

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Certificate of Recommendation

The thesis entitled 'Study on antimicrobial potency of alliin-rich black-garlic (*Allium sativum*) extract on soil micro biome' prepared under the supervision of Dr. Paramita Bhattacharjee by Anwesha Mukherjee (Class Roll No. 002110902006, Registration No. 160270 of 2021-22), a student of M. Tech final year, has been evaluated by us and found satisfactory. It is therefore being accepted for the partial fulfillment of the requirement for awarding the degree of Master of Technology in Food Technology and Biochemical Engineering.

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This is to certify that Anwesha Mukherjee has carried out research work entitled ‘Study on antimicrobial potency of alliin-rich black-garlic (*Allium sativum*) extract on soil micro biome’ under my direct supervision in the Department of Food Technology and Biochemical Engineering, Jadavpur University, Kolkata. I am satisfied that she has carried out this work independently and with care and confidence. I hereby recommend that this dissertation be accepted in partial fulfillment of the requirements for the degree of Master of Technology in Food Technology and Biochemical Engineering.

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Anwesha Mukherjee
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Dedicated to

my Parents

and

my Teachers

CONTENTS

SYNOPSIS

The study aims to investigate the antimicrobial properties of alliin-rich black garlic extract (BGE) when applied to soil, with a focus on its impact on soil microorganisms. Black garlic (BG), a fermented form of regular garlic, is known for its elevated levels of bio active compounds, particularly alliin and allicin, which have demonstrated antimicrobial effects against various pathogens. This study seeks to explore the potential of using black garlic extract as a natural antimicrobial agent to manage soil-borne microorganisms, thereby promoting healthy soil ecosystems and sustainable agricultural practices. Microorganisms play a crucial role in soil health and nutrient cycling, and their balance is essential for maintaining a productive environment. The research might explore whether the extract has any inhibitory effects on harmful microbes or if it promotes the growth of beneficial ones. The synopsis hints at an exploration of the potential applications of the black garlic extract in agriculture. If the extract demonstrates strong antimicrobial properties, it could have implications for managing soil-borne diseases, improving plant health, and enhancing crop yields in a more sustainable and natural way. The study would likely involve laboratory experiments to test the extract's impact on various soil microorganisms, potentially including bacteria, fungi, and other microorganisms. The results could shed light on the extract's potential as an eco-friendly alternative to synthetic antimicrobial agents in agriculture. In summary, the synopsis describes a research project focused on assessing the antimicrobial potency of a BGE rich in alliin when applied to soil. The study aims to contribute to our understanding of natural alternatives for managing soil health and potentially improving agricultural practices. The study anticipates the identification of alliin-rich BGE as a promising natural antimicrobial agent for soil management. Evidence of reduced disease incidence in treated soil, highlighting practical applications in agriculture.

This research contributes to the growing body of knowledge on sustainable agriculture by exploring alternative methods for controlling soil-borne pathogens. The study put light on cost-effective and environmentally friendly approach to enhancing soil health and crop productivity.

Chapter 1 focuses on the presence of alliin in BGE (Soxhlet extraction using green solvent) using the HPTLC system and then quantifying it. **Section 1.1** elaborates **Introduction and Review of Literature** about the chemical composition of alliin and preservation of alliin in the black garlic cloves, making it available for further study. This section also deals on the presence of various bacteria in soil which is harmful for plant growth and to environment in general. The significance of current research has been emphasized in relation to the significance of alliin as a chemical. This section mainly emphasizes on action of alliin on total plate count of soil. The emphasis in **Section 1.2** is on the **Materials and Methods** employed in this section's research. The preparation of the BG and the method suitable for alliin extraction are discussed in this section. This section also includes the process parameters employed for study of soil sample with black garlic extract's antimicrobial potency. **Results and Discussion** regarding the yield of BGE and its effect in soil as well as prawn sample are presented in It further confirms the antimicrobial affect on soil and not that much on prawn samples which may be due to various reasons.

Chapter 2 presents the **Summary and Future Prospects** of this research. It provides an overall inference and the broad objectives of this project. The chapter explains the utility of the results obtained for the common people and industries. This chapter explains the benefit of black garlic extract used in soil sample to reduce microbial load and make it suitable for use in agricultural field.

CHAPTER 1

Study on antimicrobial potency of alliin-rich black-garlic (*Allium sativum*) extract on soil micro biome

1.1

Introduction and Review of Literature

1.1.1 Introduction

In recent years, the search for natural and sustainable alternatives to synthetic antimicrobial agents has gained significant attention, particularly in the realm of agriculture. The overuse of chemical pesticides and antibiotics has raised concerns about environmental pollution, development of resistance, and potential negative impacts on human health through the food chain. As a result, there is a growing interest in exploring the antimicrobial properties of bio active compounds derived from natural sources.

Garlic (*Allium sativum*) has been recognized for its various health-promoting properties, many of which are attributed to its organosulfur compounds. Among these compounds, alliin (S-ally-L-cysteine sulfoxide) has garnered attention for its potential antimicrobial effects (Mansingh *et al.*, 2018). Alliin is primarily present in intact garlic cloves and is enzymatically converted to allicin when garlic is crushed or damaged. Allicin, in turn, is known for its potent antimicrobial activity against a wide range of pathogens. A notable variation of garlic is black garlic, which is produced through a unique fermentation process involving high temperature and humidity over an extended period. This process leads to significant biochemical changes within the garlic cloves, resulting in altered nutritional composition and bio active compound profiles. Notably, black garlic has been found to have increased levels of certain bio active compounds, including S-allyl cysteine (SAC), a derivative of alliin (Martínez-Casas *et al.*, 2017).Despite the established antimicrobial properties of alliin and its derivative, S-allyl cysteine, there is limited research on the potential of alliin-rich black garlic extract as an antimicrobial agent in soil. Soil microorganisms play a pivotal role in nutrient cycling, organic matter decomposition, and plant growth promotion (Smith *et al.*, 2014; Rowan *et al.*, 2015). The balance of these microbial communities is crucial for maintaining soil health and agricultural productivity.

In recent years, the exploration of natural antimicrobial agents has gained considerable attention due to the growing concerns over the emergence of antibiotic-resistant pathogens and the desire for more sustainable agricultural practices. (Slusarenko *et al.*, 2012) Among the myriad of potential sources, garlic (*Allium sativum*) has stood out as a remarkable reservoir of bio active compounds with diverse health-promoting properties. Alliin, a sulfur-containing compound present in garlic, has been associated with potent antimicrobial effects against a wide range of microorganisms, including bacteria, fungi, and viruses (Mirelman *et al.*, 1999).The process of fermentation results in the production of black garlic, characterized by enhanced levels of bio active components, including alliin (Luo *et al.*, 2019).

Fermented garlic, known as BG are produced at high temperature (60 to 90°C) with a high humidity (70% to 90%) for a period of 8-9 days (Martínez-Casas *et al.*, 2017) with the following changes in sensory properties when compared to fresh garlic: color (grows darker), taste (induces sweetness and sourness), texture (creates a jelly-like mouth feel and increases chewiness), flavor (reduces pungent odor and off-flavor), and other physicochemical properties (Zhang *et al.*, 2016; Lu *et al.*, 2017; Borlinghaus *et al.*, 2014).

The present study delves into the unexplored realm of agricultural applications for BG-derived alliin as a natural antimicrobial agent in soil (Moon *et al.*, 2008). By investigating the antimicrobial potency of alliin-rich BGE, this research seeks to address the dual challenges of maintaining soil health and mitigating the risk of soil-borne diseases in a sustainable manner. The study aims to contribute to a deeper understanding of the potential benefits of utilizing alliin-rich BGE as an alternative to synthetic antimicrobial agents in soil management. The management of soil health and the control of soil-borne diseases are critical factors influencing agricultural productivity and sustainability. As conventional methods of soil disease control often involve the use of synthetic chemicals, there is a growing interest in exploring natural alternatives that are environmentally friendly and pose minimal risks to human health and ecosystems. Garlic (*Allium sativum*) and its bio active compound, alliin, have been recognized for their potential antimicrobial properties. (Wang *et al.*, 2019) This study investigates the antimicrobial potency of alliin-rich BGE when applied to soil, with potential implications for enhancing soil health and agricultural practices.

Garlic has been used for centuries for its medicinal and culinary attributes. One of its primary bio active compounds is alliin, a sulfur-containing compound that is enzymatically converted to allicin upon tissue damage. Allicin has been extensively studied for its broad-spectrum antimicrobial properties, which include inhibitory effects on bacteria, fungi, and even some viruses.(Mirelman e*t al.*, 1999) BG, a fermented form of garlic, is known to exhibit altered bio active profiles compared to fresh garlic(FG), potentially leading to enhanced biological activities (Lawson *et al.*, 2001).

Recently, the exploration of plant-derived substances as antimicrobial agents has gained attention due to concerns about the emergence of antibiotic-resistant microbes and the negative environmental impacts of chemical pesticides. Harnessing the antimicrobial potential of alliin-rich black garlic extract could provide a natural and sustainable solution for mitigating soil-borne diseases and promoting soil health.

1.1.2 Review of Literature

1.1.2.1 Composition of black garlic

The Latin name for garlic cloves, *Allium sativum L.*, being part of the Liliaceae family, from which the term "allicin" originated. Garlic does not naturally contain allicin; it can only be made when the cloves are broken or crushed. Alliin is broken down into allicin with the enzyme alliinase (also known as alliin-lyase), which is activated by the wounds (Omar and Al-Wabel, 2010). Only one of the four stereoisomers of the precursor alliin, a non-protein oxygenated sulfur amino acid, is present in garlic ((+)-S-allyl-L-cysteine-sulfoxide) (Ankri and Mirelman, 1999). When a garlic clove invades, the membranes get damaged, alliin and allinase react rapidly (the formation is finished in 6s), and allicin is created, deactivating the invader and ensuring the clove's defense (Lawson and Wang, 2005). BG is a fermented form of garlic that has gained significant attention in recent years due to its unique taste, aroma, and potential health benefits. Many studies have been conducted to investigate the antimicrobial properties of black garlic and its potential applications in food preservation and healthcare. Garlic has long been known for its antimicrobial properties, attributed to the presence of bio active compounds such as allicin and other sulfur compounds (Fitrotin and Hidayah, 2019). These compounds have been shown to have broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria. However, the antimicrobial properties of BG are even more potent compared to raw garlic. BG is produced when garlic freely breaks down in a regulated setting, such as one with monitored humidity and temperature (Zeng *et al.*, 2013; Kim *et al.*, 2016; Qiu *et al.*, 2018). BG is high in alliin but contains less allicin, therefore after fermentation; the distinctive garlic flavor is diminished. The component of it includes many compounds (Allison *et al.*, 2006), out of which, its organosulfur elements (Figure 1), such as ajoene, s-allyl-cysteine, alliinare the main bioactive components (Mansingh *et al.*, 2018). Enzymatic browning and Maillard processes occur in garlic as a result of heat treatment.

BG is distinct from FG due to the fact it has lower moisture content, a higher pH, and increased browning. It also contains larger amounts of protein, lipid, carbohydrate, ash, total sugar, and reducing sugar. According to (Ryu *et al.* 2017), BG tends to have greater quantities of total and reducing sugars, than FG, but less allicin. The fermentation process of BG produces four to eight times as much s-allyl-cysteine than FG does (Bae *et al.*, 2012; Bae *et al.*, 2014; Sasaki *et al.*, 2007). As the concentration of amino acids such as aspartic acid, threonine, and serine increases. Correspondingly raise the levels of free sugar and minerals in BG (Choi *et al.*, 2008; Kanf *et al.*, 2016).

1.1.2.2 Properties of black garlic extract

1.1.2.2.1 Antioxidant property

Black garlic extract (BGE) high in alliin is notable for having antioxidant properties. It is rich in phenolic, alkaloids, and flavonoids with -OH groups attached to the aromatic carbon ring that eases unpaired electrons (Lu *et al.*, 2017). When compared to FG, the antioxidant activity of BG is the most potent, according to a research using the DPPH procedure, FG had a 20.21% antioxidant activity and BG had a 79.30% (Krovánková *et al.*, 2018). 2,2′-azino-bis-3-ethylbenzothiazoline-6-ulfonic acid (ABTS) showed 99.95% activity of BG in a study of free radical scavenging compared to FG's 35.92% (Toledano-Medina *et al.*, 2016). . BG also has a positive impact on the body's ability to cope with stress, which is influenced by emotional and physiological circumstances. According to the research by (Hosseini and Hosseinzadeh, 2015), oral administration of BG to mice decreased the stress hormone corticosterone by 43% and increased serotonin and dopamine levels.

1.1.2.2.2 Anti-cancer activity

According to (Almatroodi *et al.*, 2019), BG has a strong therapeutic effect on a number of cancers including lung cancer, colon cancer, breast cancer, liver cancer, and leukemia. By raising the protein level of Bax while lowering the level of Bcl-2, BGE caused an increase in the protein expression of Bax/Bcl-2 (Ahmed *et al.*, 2018). By blocking the phosphatidylinositol 3-kinase protein kinase B signaling pathway, it also decreased the HT29 colon cancer cell. It contributed to the expression of serine/threonine kinase and phosphorylated-serine/threonine kinase being down-regulated and phosphates being up-regulated (Liu *et al.*, 2018).

1.1.2.2.3 Anti-microbial activity

High antibacterial properties of the BG as compared to the FG have been observed (Jeong *et al.*, 2016).The study on the antimicrobial activity of BG pomace extract was more effective against gram-positive bacteria (*Staphylococcus aureus* and *Listeria* sp.) as compared to gram-negative bacteria (*Escherichia coli* and *Salmonella* sp.) (Kang *et al.*, 2017). BG's antibacterial activity against *Streptococcusmutans* and *Enterococcus faecalis* significantly increased, according to a study of its antibacterial activity utilising the Kirby-Bauer diffusion method (Halimah *et al.*, 2021).

1.1.2.3. Plate count in soil

This measurement provides valuable insights into soil health, microbial diversity, and potential environmental impacts (Berg *et al.*, 2011). The TPC method involves diluting soil samples, plating them on appropriate growth media, and counting the visible microbial colonies after incubation (Park *et al.*, 2021). It refers to a microbiological method used to estimate the number of viable microorganisms present in soil samples (Hedjaroud *et al.*, 2019).This method provides insights into the microbial population and overall health of the soil. The procedure involves diluting soil samples, inoculating them onto agar plates, incubating the plates, and then counting the visible colonies that develop (Oliver *et al.*, 2010). This count reflects the number of colony-forming units (CFUs) per gram of soil. The total plate count (TPC) is commonly used in environmental and agricultural studies to assess soil quality and microbial diversity.

1.1.2.4. Effect of black garlic in soil

BG is garlic that has undergone a fermentation process, leading to changes in its chemical composition and potential bioactivity (Kim *et al.*, 2014). Research in this area aims to understand how BG could influence soil properties and contributes to sustainable agricultural practices. BG's compounds, including alliin-derived compounds, have shown antimicrobial properties that could affect soil microbial populations (Buchmann *et al.*, 2015). Some studies suggest that BG extracts can inhibit the growth of certain soil-borne pathogens, contributing to disease suppression (Edberg *et al.*, 2004). BG contains various bioactive compounds that may enhance soil health by promoting beneficial microbial communities and nutrient cycling. These compounds could positively influence soil structure, organic matter decomposition, and overall soil fertility. Some research indicates that the application of BG or its derivatives to soil can promote plant growth and development (Zhang *et al.*, 2020). This could be attributed to the bioactive compounds in BG that support nutrient availability, root development, and stress tolerance in plants. The potential for BG to act as a natural alternative to synthetic chemical inputs could have positive environmental implications by reducing the need for chemical fertilizers and pesticides.

1.1.2.5. Broad objective of the present investigation:

To determine the antimicrobial activity of alliin, a bioactive compound present in BG, against a range of pathogenic microorganisms commonly found in soil, to assess the potential synergistic or additive effects of alliin against soil-borne pathogens, aiming to enhance the overall antimicrobial activity, to assess the persistence and stability of alliin in soil under different environmental conditions, including variations in pH, temperature, salinity and to explore potential applications of alliin as a natural antimicrobial agent for sustainable agriculture, including its use as a biocontrol agent against soil-borne plant pathogens and as an alternative to synthetic chemical pesticides. Also, provide insights into the practical implications and feasibility of utilizing alliin from BG as a soil amendment or plant protection strategy, considering factors such as effectiveness, environmental safety, and cost-effectiveness.

1.1.2.6 The specific objectives of the present investigation:

1. To prepare BG from fresh garlic cloves
2. To perform Soxhlet extraction using green solvent for BG powder
3. To quantify the amount of alliin present in BG extract using HPTLC analysis
4. To check the antibacterial potency of alliin-rich BG extract against soil sample
5. To check the antibacterial potency of alliin-rich BG extract against prawn sample

1.2

Materials and Methods

1.2.1. Materials

Authenticated FG (*Allium sativum*) (Figure 2a) was procured from Jaydev Agro International ExportCompany, Maharashtra, India. Specialty Chemicals such as alliin (>98% HPLC grade) was procured from M/s Sigma-Aldrich, Munich, Germany. Silica gel 60 (F_{254}) coated aluminium plates were obtained from M/s E-Merck, Maharashtra, India. Low density polyethylene (LDPE) pouches (dimensions 0.25 m × 0.18 m) and aluminum (Al) foil was purchased from Prince Plastic Pvt. Ltd., West Bengal, India. All other chemicals used were of analytical grade.

1.2.2. Methods

1.2.2.1. Fermentation of raw garlic

After procurement, the garlic samples were cleaned of dirt and placed in a rice cooker (Rize Excel 1.2 L, Kutchina, West Bengal, India) under warm mode (70°C) for a period of 8-9 days for controlled fermentation according to the method described by Lee *et al*. (2009).

1.2.2.2. Preparation of black garlic powder

Fermentation rendered the white garlic cloves black which were then peeled and cleaned. The obtained BG cloves were ground into powder using mortar pestle. The obtained powder was wrapped in an aluminum foil, followed by packing in LDPE pouch which was kept in a desiccators for future analyses.

1.2.2.3. Soxhlet extraction of alliin from black garlic powder

Extraction of alliin from BG powder (10 g) was carried out using Soxhlet extractions in three different green solvent systems i.e water (BG1), ethanol-water in the ratio 1:1 (BG2) and ethanol (BG3). BG powder to the solvent systems were in the ratio 1:20 and the extraction was conducted for 2 h.

1.2.2.4. Concentration of the black garlic extracts (BGE)

BGE (BG1, BG2 and BG3) were concentrated in a rotary vacuum evaporator (M/s R1160 Superfit Continental, Maharashtra, India) at 45°C and 50 mbar Hg. The yield of the respective concentrated extracts was determined gravimetrically. Subsequently, the extracts were dissolved in distilled water (D.W) and stored in amber colored glass vials at -18°C, until further analyses.

1.2.2.5. Identification of the extracts

1.2.2.5.1. Quantification of alliin using high-performance thin layer chromatography (HPTLC) method

HPTLC analyses of BGE were conducted to estimate their alliin content in accordance to the method described by (Kanaki *et al.,* 2005). All extracts were applied in the form of bands, 8 mm wide with 13.6 mm spacing between consecutive bands, using a Camag Linomat V (M/s Camag, Switzerland) on Al TLC plates (200 mm × 100 mm), coated with silica gel 60 (F_{254}). The plates were sprayed with a saturated solution of Ninhydrin reagent and kept at 100°C for 5 min in a hot air oven for color development. HPTLC analysis was carried out using Camag HPTLC unit (TLC scanner IV) at 450 nm employing VisionCATS 3.0.20196.1 software (Muttenz, Switzerland). The R_f value of alliin was 0.82.

1.2.2.6. Characterization of the soil

Soil samples taken were tested for the following attributes: pH, moisture and salinity. Table 4 provides the values of these parameters before and after application of BGE to the soil samples.

1.2.2.6.1. Application of BGE on soil sample

In order to check the microbial load and the effect of BGE on microbes of soil, samples of soil were collected from one location of Jadavpur University Main Campus using a clean dry sterile polythene bag along with sterile spatula. 1g of the soil sample was dissolved in 10ml of D.W to make soil suspensions. Depending on the amount of alliin present in BGE, treatment of soil sample was conducted by varying parameters such as treatment time (1hr and 2hr) and amount of soil (10g and 20g). These treatment conditions have been tabulated in Table 1, Table 2 and Table 3 for control sample, 10 g and 20 g, respectively.

1.2.2.6.3. TPC of soil samples

Soil samples were collected and a series of dilutions of the sample was prepared to obtain a suitable colony count (Burns *et al.*, 2021). A common dilution series involving diluting the sample in a 10-fold manner (e.g., $10^{\wedge-1}$, $10^{\wedge-2}$, $10^{\wedge-3}$, etc.) was carried out by adding a 1 ml volume of the sample to 9 ml of D.W. Inoculation was conducted using sterile pipettes by transferring a known volume of each diluted sample onto separate agar plates (Bradshaw *et al.*, 2019) It was then spread evenly over the surface of the agar using a sterile inoculating loop or needle inside the laminar airflow chamber to maintain sterile condition. The inoculated plates were closed with lids and the plates were placed upside-down in an incubator set to the appropriate temperature of 37°C for a specified incubation period of 24 h. This allows viable microbial colonies to grow. After incubation, we counted the visible colonies on the plates. The TPC was obtained by multiplying the colony count on the plate by the reciprocal of the dilution factor. This gives the number of viable microorganisms per unit volume of the original sample. The results were expressed in terms of colony-forming units (CFUs) per gram (CFU/g) of the original sample. These values provide an estimate of the microbial load present in the soil sample.

1.2.2.7. Characterization of the prawns

De-shelled and beheaded prawns were purchased from local Jadavpur market at the time of purchase (Figure 2b). Attributes (Table 7) of the raw prawns were determined. The samples were first washed in running tap water, weighed and macerated using a mixer-grinder.

1.2.2.7.1. Application of BGE on prawn samples

In addition to the soil sample, the antimicrobial potency of alliin in BG extract was examined on a food sample, such as U16/U20 (i.e., 15 quantities per pound) Indian tiger prawns. In order to check microbial load and the effect of BGE on prawns, the samples were first washed in running tap water. The prawns in small batches (10-20 g) were aseptically transferred into petridishes containing varying amounts of BGE (4-6 g depending on the alliin content which varied from 15-30 µg/ml) into which the samples were dipped for varying time (30 min, 60 min) as have been tabulated in Tables 5 and 6. The total plate counts were determined using the same procedure described in sections 1.2.2.6.2 and 1.2.2.6.3.

1.3

RESULTS AND DISCUSSION

1.3.1. Black garlic powder obtained from fermented raw garlic

Garlic cloves after fermentation turned into a brownish black color (Figure 3a) with a decreased characteristic garlic odor. Fermented BG cloves yielded 95.67% of powder (Figure 3b).

1.3.2. Yield of concentrated Soxhlet extract (BGE)

The employed solvent systems produced 1.27-4.96 g of concentrated Soxhlet extracts. The lowest yield (1.27 g) was obtained while using ethanol as the extracting solvent and the highest (4.96 g), when ethanol: water (1:1) was used. Water as the extracting solvent yielded 4.54 g of extract (Figure 3c), thus establishing that water and ethanol: water (1:1) was the best solvent system.

1.3.3. Quantification of alliin in BG1 and BG2

Alliin contents of 1.8 mg/g and 1.322 mg/g were found to be present in BG1 and BG2, respectively, having an R_f value of 0.82 under chromatographic conditions mentioned in section 1.2.2.5.1. Alliin was not quantified in BG3 owing to its poor solubility in ethanol. BG1 had the highest content of alliin since the bioactive compound is highly soluble in water (Iberl *et al*., 1990) (Figure 4).

1.3.3.1. Microbial load of soil samples

The analysis demonstrated a significant reduction in the TPC of soil samples treated with BG extract containing alliin. The reduction amounted to 2 log cycles from 10^5 to 10^3, indicating a substantial decrease in the overall microbial population. (Table 4) This reduction suggests the potential antimicrobial effect of alliin present in BG extract. It is found that alliin extracted from BG exhibited significant antimicrobial properties, effectively suppressing soil-borne microbes (Figure 5, Figure 6 and Figure 7). Furthermore, it is anticipated that the presence of alliin in the soil lead to improved plant health and reduced disease incidence (Pizzeghello *et al.*, 2020). It is important to note that BGE if not used judiciously may negatively impact beneficial soil microorganisms.

1.3.3.2 Microbial load of prawn samples

The results demonstrated no significant reduction in the total plate count of the prawn samples treated with BG extract containing alliin. The reduction amounted to a slight decrease in CFU/g value indicating no substantial decrease in the overall microbial population (Figure 8, Figure 9 and Figure 10). This reduction show no effect of antimicrobial effect of alliin present in BG extract on prawn sample which could have been due to treatment time and treatment method (dip/coat), temperature and environmental conditions. Another factor that may have influenced the results is the quantity of BGE used in the treatment. If the concentration or volume of the extract was insufficient, it could have led to an inadequate exposure of the prawn samples to the antimicrobial compounds in the extract. Optimal concentrations and exposure time should be determined through further experimentation to evaluate the potential antimicrobial effects of alliin on prawn samples.

1.4

CONCLUSION AND FUTURE SCOPE OF WORK

This work discusses the effects of black garlic extract containing alliin on the total plate count in soil samples. The study demonstrates a significant reduction in the total plate count, indicating the potential antimicrobial properties of alliin present in black garlic extract. These findings highlight the potential of black garlic extract as a natural soil amendment to control microbial populations while preserving the beneficial microorganisms essential for soil health and agricultural productivity. Further research is needed to elucidate the specific mechanisms and optimize the application of black garlic extract in soil management practices. This study on the antimicrobial properties of alliin from black garlic and its effect on soil could open new possibilities for sustainable agriculture practices. By harnessing the natural power of alliin, farmers may reduce their dependence on synthetic chemicals while promoting soil health and plant productivity. The findings of this study may contribute to the development of environmentally friendly strategies to manage soil-borne diseases and enhance agricultural sustainability.

In conclusion, the results of this study suggest that the black garlic extract containing alliin did not demonstrate a significant reduction in the TPC or overall microbial population of the prawn samples. The limited decrease in CFU numbers indicates no substantial antimicrobial effect. Further investigations should be conducted to address potential limitations, such as sample contamination and the optimization of extract concentration, to obtain more definitive conclusions regarding the antimicrobial properties of alliin in black garlic extract on prawn samples.

The emergence of antibiotic-resistant bacteria has become a significant global health concern, necessitating the exploration of alternative antimicrobial agents. This work investigates the antimicrobial properties of alliin, a compound derived from black garlic, and its effect on soil. The antimicrobial properties of alliin extracted from black garlic against common soil-borne pathogens. The study aims to determine the potential of alliin as a natural antimicrobial agent and evaluate its impact on soil microorganisms. Soil samples were collected and treated with varying concentrations of alliin. Parameters such as soil pH, moisture, salinity, microbial activity were analyzed. The results showed that the addition of alliin to the soil significantly influenced soil pH, with higher concentrations causing a decrease in pH levels.

The study investigated the antimicrobial potency of an alliin-rich black garlic (*Allium sativum*) extract on soil. Alliin is a sulfur-containing compound found in garlic that is known for its potential antimicrobial properties. Black garlic is garlic that has undergone a fermentation process, resulting in changes to its chemical composition and potential bioactivity. The research involved extracting alliin-rich compounds from black garlic and applying them to soil samples. The antimicrobial effects were then assessed by measuring the inhibition or reduction of microbial growth in the treated soil. The study aimed to determine whether the black garlic extract could effectively inhibit the growth of various microorganisms present in the soil, potentially contributing to soil health and agricultural practices.

The study on the antimicrobial potency of alliin-rich black garlic extract on soil opens up several future prospects and research directions. Further research could involve conducting field trials to assess the practical application of the black garlic extract in real agricultural settings. This would provide insights into its effectiveness in promoting plant growth, enhancing crop yields, and reducing the incidence of soil-borne diseases. Mechanism of Action: Investigating the specific mechanisms through which the black garlic extract exerts its antimicrobial effects on soil microorganisms could provide a deeper understanding of its mode of action. This knowledge could potentially lead to the development of more targeted and effective applications. Environmental Impact: Assessing the potential environmental impact of using black garlic extract in agriculture is crucial. Researchers could explore its effects on non-target organisms, soil ecology, and overall ecosystem health.

Developing formulations of the black garlic extract that are easy to apply and stable under various environmental conditions would be valuable for practical agricultural use. Comparison Studies: Comparative studies with other natural antimicrobial agents or conventional chemical treatments could provide insights into the relative efficacy and advantages of using alliin-rich BGE in soil management. Commercial Applications: If proven effective and safe, the development of commercial products based on alliin-rich BGE for soil treatment could be explored.

The antimicrobial potency of alliin-rich BGE on soil presents a promising avenue for improving soil health, crop production, and sustainable agricultural practices. Further research and development are needed to fully understand its potential and realize its practical applications in the field. It is worth considering other potential limitations of the study, such as the experimental design, sample size, or variations in the prawn samples themselves. These factors can influence the reliability of the results. Replication of the study with larger sample sizes and improved experimental controls would be beneficial in providing more robust conclusions.

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Abbreviations

BG- Black Garlic

BG1- Soxhlet extraction of black garlic extracts using water

BG2- Soxhlet extraction of black garlic extracts using ethanol: water (1:1)

BGE- Black garlic extract

CFU- Colony Forming Unit

DMSO- Dimethyl Sulfoxide

FG- Fresh Garlic

HPTLC- High Performance Thin Layer Chromatography

PBS- Phosphate Buffer Solution

RH- Relative Humidity

Std- Standard

TLC- Thin Layer Chromatography

Table1: Control Sample (Amount of Alliin =0 µg/ml)

Amount of soil(in gm)	Amount of Alliin (µg/ml)	Time of incubation (in hr)	CFU/1gm
10	0	1	23000
10	0	2	48000
20	0	1	120000
20	0	2	145000

Table 2: Amount of Alliin =15 µg/ml

Amount of soil(in gm)	Amount of Alliin (µg/ml)	Time of incubation (in hr)	CFU/1gm
10	15	1	1800
10	15	2	1450
20	15	1	1620
20	15	2	1820

Table 3: Amount of Alliin = 30 µg/ml

Amount of soil (g)	Amount of Alliin (µg/ml)	Time of incubation (h)	CFU/g
10	30	1	160
10	30	2	490
20	30	1	210
20	30	2	680

Table 4: Characterization of soil

Soil parameter	Initial	After 1 h	After 2 h
pH	6.5	6.2	6.0
Salinity	3.072	3.068	3.042
Moisture	28	31	32

Table 5: Control Sample (Amount of Alliin =0 µg/ml) for prawn sample

Amount of small Indian tiger prawn (g)	Amount of Alliin (µg/ml)	Time of incubation (min)	CFU/g
10	0	30	220000
10	0	60	218000
20	0	30	234000
20	0	60	No growth

Table 6: Amount of Alliin =15 µg/ml for prawn sample

Amount of small Indian tiger prawn (g)	Amount of Alliin (µg/ml)	Duration of application (min)	CFU/g
10	15	30	180000
10	15	60	216000
20	15	30	Dense growth
20	15	60	Dense growth

Table 7: Characterization of Prawn Sample

Prawn Parameters	Initial	After 1 h	After 1 day
pH	6.83	6.42	6.28
Moisture	78%	76.2%	71%

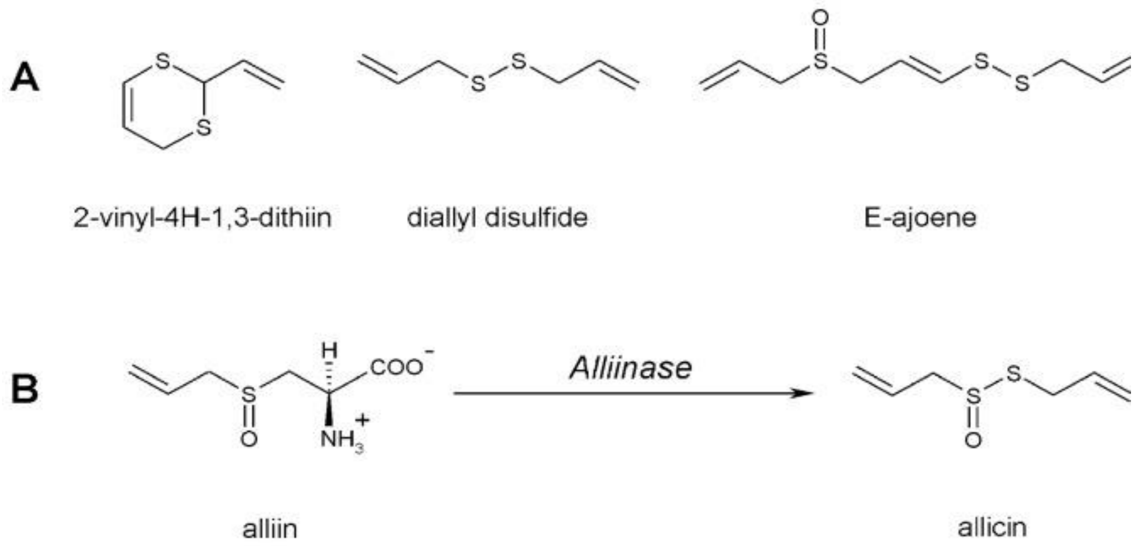


Figure 1: Formation of alliin and allicin in garlic

2 a)

2 b)

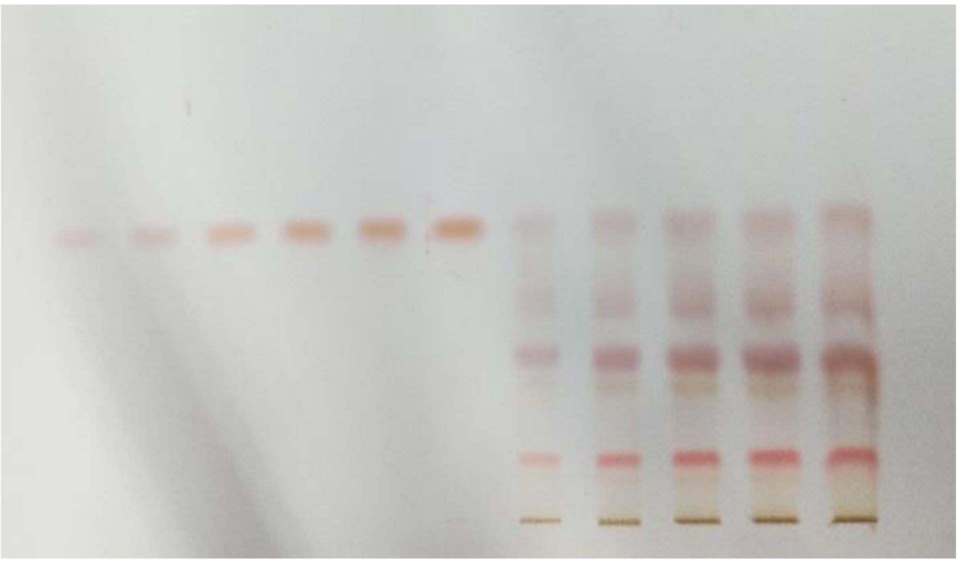
Figure 2 a) Soil sample used for study and b) Prawn sample used for study

3 a)

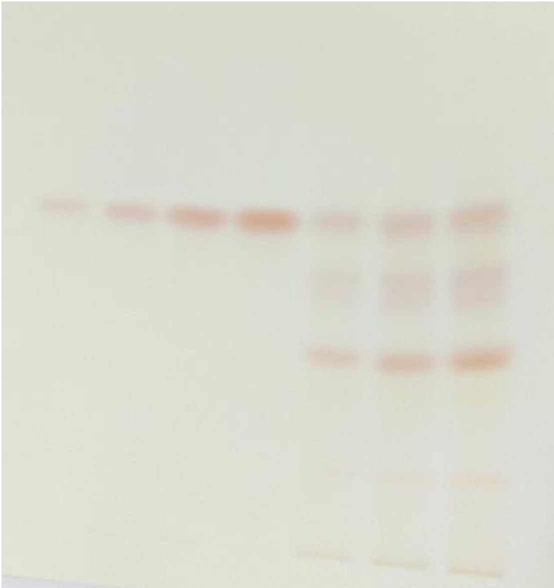
3 b)

3 c)

Figure 3 a) Black garlic cloves on fermentation for a period of 9-10 days, b) Black garlic powder obtained on grinding and c) Black garlic extract



4 a)



4 b)

Figure 4: Plate developed for a) BG1 and b) BG2 extracts along with Std. alliin for HPTLC run

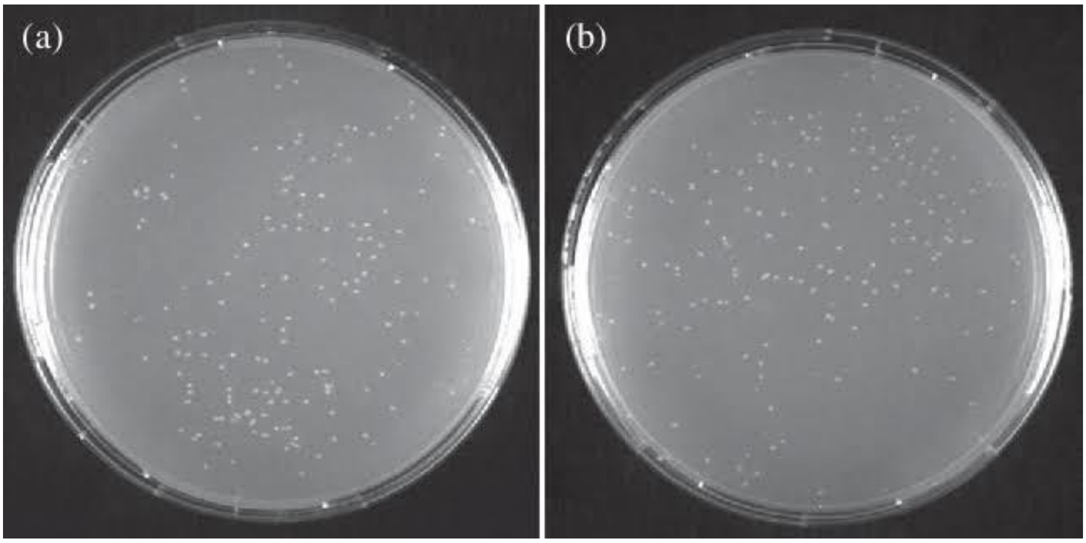


Figure 5: Control plates for soil sample

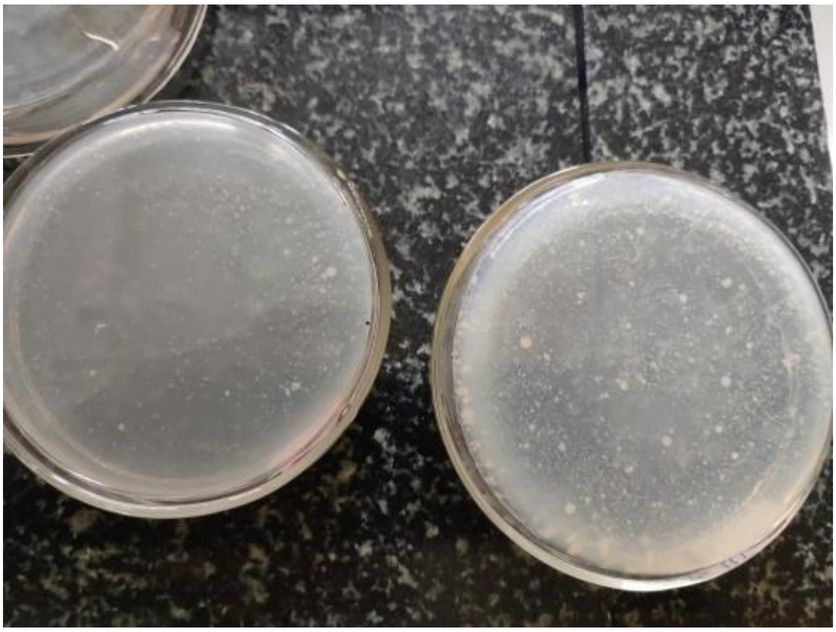


Figure 6: Plates for soil sample with 10g soil

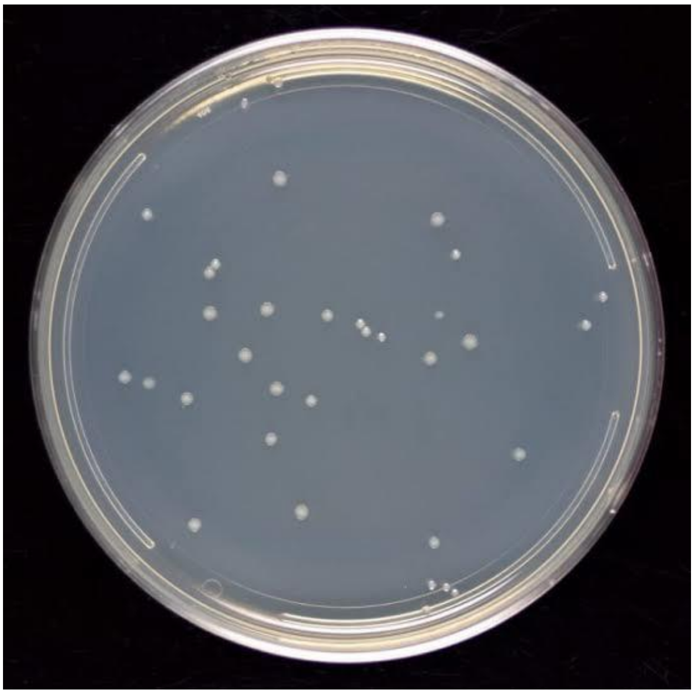


Figure 7: Plates for soil sample with 20g soil

Figure 8: Control plates for prawn sample

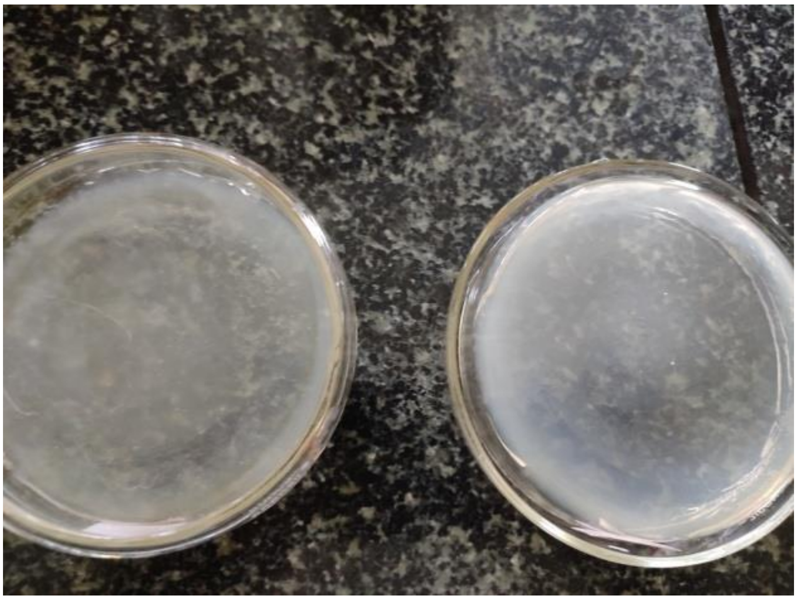


Figure 9: Plates for 10g prawn sample

Figure 10: Plates for20g prawn sample