International Journal of Sustainability and Advanced Integrated Research
Volume 2, Issue 2 (April-June 2026) | ISSN: 3107-7854
DOI: https://doi.org/10.65339/ijsair.V2.I2.634

Allium sativum (Garlic), and *Citrus sinensis* (Orange Peel) Extract as Alternative Ingredient for Eco-friendly Mold Remover Spray

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Publication history: Received: May 22, 2026; Revised: June 12, 2026; Accepted: June 17, 2026
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Abstract

This study evaluated the antifungal effectiveness of *Allium sativum* (garlic) and *Citrus sinensis* (orange peel) extracts as ingredients in an eco-friendly mold remover spray against Aspergillus niger. Guided by the Input–Process–Output (IPO) framework, the study employed an experimental research design to determine the effectiveness of different extract concentrations and combinations. Garlic and orange peel extracts were prepared through ethanol extraction and tested using the paper disk diffusion method at the Department of Science and Technology (DOST) Region II. Antifungal activity was measured through the zone of inhibition, and data were analyzed using mean, One-Way Analysis of Variance (ANOVA), and Tukey's Honestly Significant Difference (HSD) test. The results showed that all extract formulations exhibited antifungal activity against Aspergillus niger. The combination containing 70% orange peel extract and 30% garlic extract produced the highest mean zone of inhibition among the plant-based treatments and was classified as active. Garlic extract demonstrated strong antifungal activity, confirming its role as the primary contributor to mold inhibition, while orange peel extract provided additional inhibitory effects when combined with garlic. Statistical analysis revealed a significant difference among the treatment concentrations, indicating that extract ratios influenced antifungal effectiveness. Although all plant-based treatments were less effective than the positive control (Fluconazole), they demonstrated measurable antifungal properties and potential for household mold management. The findings suggest that garlic and orange peel extracts can serve as natural and eco-friendly alternatives for mold control, with garlic exhibiting greater antifungal effectiveness. Future studies are recommended to investigate additional concentrations, extraction methods, solvents, and fungal species to further optimize the formulation. This study aligns with SDG 3 (Good Health and Well-Being) by promoting safer alternatives for mold control and SDG 12 (Responsible Consumption and Production) through the use of biodegradable plant-based materials. The study contributes to environmental and public health sustainability by encouraging the development of natural, affordable, and environmentally responsible household cleaning products.

Keywords: Allium Sativum, Aspergillus Niger, Citrus Sinensis, Eco-Friendly Mold Remover Spray, Ethanolic Extracts, Mold Inhibition, Zone of Inhibition

1. Introduction

Mold infestation remains a persistent problem in indoor environments, particularly in humid and poorly ventilated areas where fungal growth threatens both human health and structural integrity. Conventional mold removers are effective but often contain chemicals that release volatile organic compounds (VOCs), posing health and environmental concerns. Consequently, there is growing interest in plant-based antifungal alternatives such as *Allium sativum* (garlic) and *Citrus sinensis* (orange peel), which possess bioactive compounds with demonstrated antifungal properties. This study investigates the effectiveness of combined garlic and orange peel extracts as eco-friendly ingredients for a mold remover spray against Aspergillus niger.

1.1 Background of the Study on Garlic and Orange Peel-Based Eco-Friendly Mold Remover Spray

Indoor mold growth is a widespread concern due to its association with respiratory illnesses, allergic reactions, and asthma exacerbation, particularly among children and immunocompromised individuals (World Health Organization [WHO], 2023). Mold commonly develops in humid and poorly ventilated environments, making its control a significant public health concern. Conventional cleaning agents such as chlorine bleach and ammonia-based sprays are frequently used to eliminate mold; however, these products release volatile organic compounds (VOCs) that may negatively affect indoor air quality and contribute to environmental pollution (United States Environmental Protection Agency [EPA], 2024). Natural alternatives have gained attention because of their safety and environmental benefits. *Allium sativum* contains sulfur-based compounds such as allicin that disrupt fungal cell membranes and interfere with fungal metabolism (Kumawat et al., 2023). Likewise, *Citrus sinensis* peel contains limonene and flavonoids with antimicrobial and antifungal properties capable of inhibiting fungal growth, including Aspergillus niger (Anwar et al., 2023). In the Philippine setting, where tropical conditions promote indoor mold growth, there is a need for affordable, safe, and environmentally sustainable mold control solutions (Department of Health Philippines, 2022).

1.2 Themes and Insights from Related Literature on Antifungal Properties of Garlic and Orange Peel Extracts

Literature consistently demonstrates the antifungal potential of *Allium sativum* and *Citrus sinensis*. Garlic owes its effectiveness to sulfur-containing compounds such as allicin and diallyl trisulfide (DAT), which disrupt fungal cell membranes and metabolic processes, resulting in fungal inhibition and cell death (Kumar et al., 2021). Ethanolic garlic extracts exhibit stronger antifungal activity against Aspergillus niger than aqueous extracts because ethanol extracts a wider range of bioactive compounds (Mehta & Rao, 2022). Enhanced formulations, including DAT nanoemulsions, further improve antifungal efficiency and stability (Zhang et al., 2023). Similarly, *Citrus sinensis* contains limonene and citric acid, compounds known to disrupt fungal membranes and create conditions unfavorable for fungal growth (Anwar et al., 2023; Sharma and Tripathi, 2021). Studies have reported inhibition of fungal growth through orange peel essential oils and concentrated extracts (Budhathoki and Poudel, 2023; Viuda-Martos et al., 2021; Liu et al., 2021). In addition to antifungal activity, orange peel extracts contribute to environmental sustainability through the utilization of agricultural waste and support circular economy practices (Olaitan, 2024; Handayani et al., 2019). Research also indicates that combining garlic and orange peel extracts may provide synergistic antifungal effects. Garlic demonstrates strong inhibitory activity against Aspergillus niger (Rafsanjani and Trimulyono, 2023), while orange peel essential oil similarly exhibits antifungal properties (Budhathoki and Poudel, 2023). These findings suggest the potential effectiveness of combining both extracts into a household mold remover spray.

1.3 Local, National, and Global Context of Mold Infestation and Control

Globally, mold contamination remains a public health and environmental concern due to its effects on indoor air quality and human health. Aspergillus niger is among the most common indoor molds, thriving in damp environments and colonizing surfaces such as wood, fabrics, wallpapers, and ventilation systems (Smith, 2021; Lopez et al., 2022). Exposure to its spores may cause allergic reactions, respiratory illnesses, chronic sinusitis, and severe infections among immunocompromised individuals (Ali et al., 2020; Chen et al., 2021; Torres, 2024). Beyond health impacts, mold contributes to structural deterioration and economic losses through the degradation of organic materials and household furnishings (Nguyen & Tran, 2020; Patel, 2023). In the Philippines, warm temperatures and high humidity levels create favorable conditions for mold growth, increasing the need for practical and sustainable mold management strategies (Department of Health Philippines, 2022). Although chemical fungicides remain widely used, concerns regarding toxicity, environmental contamination, and indoor air pollution have encouraged the search for natural alternatives (Kim & Park, 2021; Chung, 2022; Kulkarni, 2024).

1.4 Theoretical or Conceptual Basis of Eco-Friendly Mold Control

This study is anchored on the principle that plant-derived bioactive compounds can serve as effective and environmentally sustainable antifungal agents. Garlic-derived compounds such as allicin and DAT inhibit fungal growth by disrupting cell membranes and metabolic processes (Kumar et al., 2021; Zhang et al., 2023). Orange peel compounds, particularly limonene and citric acid, interfere with fungal membrane integrity and growth mechanisms (Anwar et al., 2023; Sharma and Tripathi, 2021). The study adopts the Input–Process–Output (IPO) framework. The inputs consist of *Allium sativum* extract, *Citrus sinensis* extract, and solvent materials. The process involves extraction, preparation, mixing, filtration, and preservation procedures designed to obtain active antifungal compounds. The output is an eco-friendly mold remover spray formulated from combined garlic and orange peel extracts for testing against Aspergillus niger.

1.5 Research Gaps and Rationale for Developing a Combined Garlic–Orange Peel Mold Remover Spray

Previous studies have extensively examined the antifungal properties of garlic and orange peel extracts individually. Garlic extracts have demonstrated significant inhibition against Aspergillus species and Aspergillus niger (Lima et al., 2021; Yasmeen et al., 2020), while orange peel extracts have shown similar antifungal activity through limonene-rich essential oils (Budhathoki and Poudel, 2023; Viuda-Martos et al., 2021). Despite these findings, limited studies have explored the combined effectiveness of *Allium sativum* and *Citrus sinensis* extracts as an eco-friendly household mold remover spray. Existing research largely focuses on laboratory-based antimicrobial testing rather than practical indoor applications. Therefore, further investigation is necessary to determine whether combining these natural extracts enhances antifungal effectiveness and provides a viable alternative to conventional chemical-based mold removers.

1.6 Alignment with Relevant Sustainable Development Goals (SDGs)

This study aligns with SDG 3: Good Health and Well-Being by promoting safer mold control methods that may reduce exposure to mold-related respiratory illnesses and harmful chemicals commonly found in conventional cleaners. It also supports SDG 12: Responsible Consumption and Production through the use of biodegradable plant-based materials and the productive utilization of orange peel by-products. Furthermore, the study contributes to SDG 13: Climate Action by encouraging environmentally responsible alternatives that reduce dependence on chemical cleaning agents and their associated environmental impacts.

1.7 Importance of the Study to Multidisciplinary Sustainability

The study contributes to public health sustainability by exploring safer mold management strategies that minimize exposure to harmful chemicals and indoor pollutants. It supports environmental sustainability through the use of biodegradable and non-toxic plant-based extracts while promoting waste reduction through the utilization of citrus peel by-products. The research also contributes to socio-economic sustainability by providing a potentially affordable and accessible mold control solution for households. Additionally, it supports innovation and community sustainability by encouraging the development of environmentally responsible cleaning products derived from locally available natural resources.

2. Material and methods

2.1 Research Design

The study utilized an experimental research design to determine the antifungal activity of *Allium sativum* and *Citrus sinensis* extracts in controlling household mold. Experimental research involves the deliberate manipulation of independent variables to determine their effects on dependent variables and is appropriate for establishing cause-and-effect relationships through quantitative analysis (Skreeumar, 2024). This design enabled the researchers to formulate and evaluate different extract concentrations and combinations to assess their effectiveness as safer and more environmentally friendly alternatives to conventional mold removers.

2.2 Locale of the Study

The study was conducted in several locations to facilitate material preparation, testing, and analysis. Raw materials were obtained from the Baguio City Public Market, while preliminary preparation activities were carried out at Saint Louis School of Pacdal, Inc., located in Baguio City. Antifungal testing and data collection were conducted at the Department of Science and Technology (DOST) Regional Office Region II in Tuguegarao City, where laboratory analyses were performed under controlled conditions.

2.3 Respondents of the Study

No human respondents participated in this study. The experimental subject was Aspergillus niger, a common mold species used to evaluate the antifungal activity of the formulated extracts under laboratory conditions.

2.4 Sample and Sampling Procedure

Fresh garlic bulbs (Allium sativum) and mature sweet oranges (*Citrus sinensis*) were purchased from local vendors in Baguio City. Garlic samples were selected based on firmness and the absence of visible contamination, while oranges were chosen based on ripeness and freedom from fungal infection. The materials were transported in clean containers and processed immediately to preserve their bioactive compounds, as proper handling helps maintain phytochemical content (Azwanida, 2021). Prior to extraction, garlic cloves were separated, washed thoroughly, air-dried, peeled, and blended into a fine paste. Proper washing removes contaminants that may affect extraction efficiency (Azwanida, 2022), while blending increases the surface area and improves the release of phytochemical compounds (Sasidharan et al., 2021). Similarly, sweet oranges were washed thoroughly, and the peels were carefully removed while avoiding the white pith. The peels were chopped into smaller pieces, dried under sunlight for two days, and ground into coarse particles. Drying minimizes moisture content and preserves phytochemicals by preventing microbial growth (Azwanida, 2021), while grinding enhances extraction efficiency by increasing surface area (Sasidharan et al., 2021).

2.5 Research Instrument

The study utilized common laboratory and household materials, including mason jars, zesters or knives, blenders, sterile containers, Whatman filter paper No. 1, vodka as an ethanol-based solvent, garlic, and orange peels. Vodka was selected as the extraction solvent because ethanol effectively extracts bioactive compounds from plant materials (Tiwari et al., 2023). Garlic extraction was performed by blending garlic with ethanol at a 1:1 ratio and allowing the mixture to stand for 24 hours to facilitate the extraction of sulfur-containing compounds such as allicin (Rahman et al., 2021; Sharma & Singh, 2022). The extract was then filtered using Whatman filter paper No. 1 (Kim et al., 2023). For orange peel extraction, dried peel particles were soaked in ethanol for 48–72 hours with occasional agitation to improve extraction efficiency. Ethanol maceration is commonly used to extract flavonoids and essential oils from citrus peels (Santos et al., 2021). The mixture was subsequently filtered using Whatman filter paper No. 1 (Nguyen & Tran, 2022). The resulting extracts were combined into five treatment formulations with a total volume of 50 mL per treatment. Treatment 1 consisted of 100% orange peel extract, while Treatment 2 consisted of 100% garlic extract. Treatment 3 contained 70% garlic extract and 30% orange peel extract, Treatment 4 contained 30% garlic extract and 70% orange peel extract, and Treatment 5 contained equal proportions of garlic and orange peel extracts (50:50). These formulations served as the experimental treatments for antifungal testing.

2.6 Data Gathering Procedure

Prior to experimentation, the researchers secured permission from the school administration and formally requested microbiological testing services from the Department of Science and Technology (DOST) Region II. The antifungal activity of the extracts was evaluated using the paper disk diffusion method described in A Guidebook to Plant Screening: Phytochemical and Biological by Guevarra (2005). Ethanolic extracts of garlic and orange peel at concentrations of 30%, 50%, 70%, and 100%, including the combination ratios of 70:30, 50:50, 30:70, 1:0, and 0:1, were dispensed into assay wells at 50 µL per treatment. Fluconazole served as the positive control, while distilled water served as the negative control. Each treatment was tested in triplicate across three experimental trials to ensure reliability and accuracy. Following incubation, the zones of inhibition were measured in millimeters and recorded systematically. Antifungal effectiveness was determined based on the diameter of the inhibition zones, with larger zones indicating stronger antifungal activity. The most effective formulation was identified based on the greatest inhibition against Aspergillus niger.

2.7 Data Analysis Techniques

The effectiveness of the mold remover formulations was evaluated through the measurement of zones of inhibition using standard agar diffusion techniques (Shafique et al., 2022). The zone of inhibition, measured in millimeters, represented the diameter of the clear area where fungal growth was prevented, with larger diameters indicating stronger antifungal activity (Ali et al., 2021). The inhibition zones were interpreted using the following qualitative classifications: zones measuring less than 10 mm were considered inactive, those measuring 10–13 mm were classified as partially active, zones measuring 14–19 mm were considered active, and zones greater than 19 mm were interpreted as very active. These classifications are commonly used in evaluating antimicrobial activity through disk diffusion assays (Balouiri, Sadiki, & Ibnsouda, 2016; Lozada et al., 2021). The mean zone of inhibition for each treatment concentration was computed using the statistical results provided by DOST. The mean values served as representative measures of antifungal performance for each treatment group. To determine whether significant differences existed among treatment means, a one-way Analysis of Variance (ANOVA) was employed (Montgomery, 2019). ANOVA was used to establish whether observed differences among treatments resulted from the extract formulations rather than random variation (Field, 2018). The null hypothesis stated that the eco-friendly mold remover spray formulated from *Allium sativum* and *Citrus sinensis* extracts produced no significant antifungal effect, whereas the alternative hypothesis stated that the formulation produced significant inhibition against mold growth. The null hypothesis was rejected when the computed F-value exceeded the critical value or when the p-value was less than 0.05 (Montgomery, 2019). When significant differences were detected, Tukey's Honestly Significant Difference (HSD) test was applied to identify which treatment groups differed significantly from one another (Field, 2018).

2.8 Ethical Considerations

The study was conducted following appropriate ethical and laboratory safety standards. Antifungal testing of *Allium sativum* and *Citrus sinensis* extracts against Aspergillus niger was performed under controlled laboratory conditions to ensure safety and accuracy. No human participants or animals were involved; therefore, informed consent and animal welfare considerations were not applicable. Plant materials were legally acquired from commercial sources, and all biological and chemical wastes were disposed of according to laboratory safety guidelines. Furthermore, all data provided by the Department of Science and Technology (DOST) were reported accurately and honestly, while all referenced sources were properly cited to uphold academic integrity and avoid plagiarism.

3. Results and Discussion

3.1 Antifungal Effectiveness of Garlic and Orange Peel Extract Formulations

3.1.1 Antifungal Activity of Different Extract Concentrations Against Aspergillus niger

The antifungal assay revealed varying levels of inhibition among the treatments. The combination containing 70% orange peel extract and 30% garlic extract produced the highest mean zone of inhibition of 14 mm and was classified as active. The 100% garlic extract yielded a mean inhibition zone of 13 mm, while the 100% orange peel extract produced a mean of 12.33 mm. The combination containing 70% garlic extract and 30% orange peel extract, as well as the equal-ratio formulation, both produced a mean inhibition zone of 12.67 mm. These treatments were classified as partially active. The positive control, Fluconazole, generated the largest inhibition zone with a mean of 31.33 mm and was classified as very active, whereas the negative control, distilled water, produced a mean inhibition zone of 6 mm and was classified as inactive. The findings indicate that both garlic and orange peel extracts possess antifungal properties capable of inhibiting the growth of Aspergillus niger. The highest-performing treatment was the formulation containing 70% orange peel extract and 30% garlic extract, suggesting a possible synergistic interaction between the bioactive compounds present in both plants. This observation supports the findings of Anazodo et al. (2024), who reported that garlic contains sulfur-containing phytochemicals that interfere with fungal growth. Similarly, Agustantina & Soekartono (2021) identified garlic as an effective antifungal agent against Aspergillus species. The antifungal activity of orange peel extract is likewise supported by Anwar et al. (2023), who demonstrated that *Citrus sinensis* peel oil significantly inhibits Aspergillus niger. The superior performance of Fluconazole confirms the effectiveness of established antifungal drugs, while the absence of inhibition in the negative control validates that the observed antifungal activity resulted from the plant extracts.

3.1.2 Comparative Performance of Natural Extract Formulations

The results showed only small differences among the plant-based treatments. While the formulation containing 70% orange peel extract and 30% garlic extract achieved the highest inhibition zone, the remaining extract combinations produced relatively similar inhibition values ranging from 12.33 mm to 13 mm. All natural formulations demonstrated measurable antifungal activity against Aspergillus niger. The relatively close inhibition values suggest that both *Allium sativum* and *Citrus sinensis* contribute substantially to antifungal effectiveness. The results indicate that combining the extracts can enhance inhibitory activity while maintaining the natural and eco-friendly characteristics of the formulation. These findings support the potential use of garlic and orange peel extracts as alternative mold control agents and suggest that optimized extract ratios may improve antifungal performance while reducing reliance on synthetic fungicides.

3.2 Significant Differences Among Treatment Concentrations

The One-Way Analysis of Variance (ANOVA) yielded a computed F-value of 2.8477 and a p-value of 1.36×10^{-10}. Since the p-value was lower than the 0.05 level of significance, the null hypothesis was rejected. This result indicates that significant differences existed among the antifungal effects of the different concentrations and combinations of garlic and orange peel extracts against Aspergillus niger. The rejection of the null hypothesis demonstrates that concentration ratios significantly influence mold inhibition. The differences observed among treatments were not caused by random variation but by the effectiveness of the extract formulations themselves. This finding highlights the importance of concentration optimization in developing plant-based antifungal products and confirms that variations in garlic and orange peel extract ratios can affect the level of fungal growth suppression.

3.3 Post-Hoc Comparison of Treatment Effectiveness

The post-hoc analysis revealed that most plant-based formulations exhibited minimal mean differences when compared with one another, indicating generally comparable antifungal performance among the natural extract treatments. However, notable differences were observed when the extract formulations were compared with the positive and negative controls. The positive control consistently produced substantially larger inhibition zones, whereas the negative control showed minimal or no inhibitory effect. These findings indicate that although the natural extract formulations performed similarly among themselves, they were considerably more effective than the negative control. The results further validate the antifungal properties of both garlic and orange peel extracts and support their potential use as eco-friendly alternatives for mold inhibition. While their effectiveness remains lower than that of Fluconazole, the observed inhibitory activity demonstrates their potential value in the development of natural antifungal products for household applications.

4. Conclusion and Recommendations

This study found that both *Allium sativum* (garlic) and *Citrus sinensis* (orange peel) extracts possess antifungal properties capable of inhibiting mold growth. Among the treatments, pure garlic extract demonstrated the highest antifungal effectiveness, while formulations with higher garlic concentrations also produced stronger inhibition. Although orange peel extract exhibited antifungal activity, its effectiveness was lower than that of garlic. The findings indicate that garlic serves as the primary contributor to antifungal activity, while orange peel extract may enhance the effectiveness of combined formulations, supporting their potential use as natural and eco-friendly mold control agents.

Future studies should investigate additional concentrations and combinations of garlic and orange peel extracts to identify the most effective formulation against mold. Researchers are also encouraged to evaluate the extracts against other fungal species, employ alternative extraction methods and solvents, increase the number of trials and samples, extend garlic fermentation time, incorporate rotary evaporation before filtration, utilize the agar disk diffusion method for antifungal testing, explore the use of orange peel essential oil obtained through Soxhlet extraction, and use commercial ethanol instead of vodka to improve extract quality, consistency, and testing reliability.

This study was limited to the evaluation of garlic and orange peel extracts against Aspergillus niger using specific extract concentrations, a paper disk diffusion method, and extracts prepared through ethanol extraction using vodka as the solvent. The study also relied on a limited number of treatment formulations and laboratory trials, which may restrict the generalizability of the findings to other fungal species, extraction procedures, and testing conditions.

Compliance with ethical standards

The authors declare no conflict of interest. This study received no external funding and was conducted using legally acquired plant materials and non-invasive laboratory procedures to evaluate the antifungal activity of *Allium sativum* and *Citrus sinensis* extracts against Aspergillus niger at the Department of Science and Technology (DOST). No human participants or animals were involved; therefore, informed consent and participant recruitment were not applicable. All data were recorded and reported honestly, and no identifying information was collected or disclosed, ensuring compliance with academic integrity and confidentiality standards.

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