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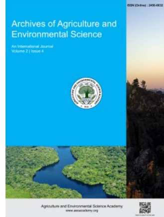

ORIGINAL RESEARCH ARTICLE

# In-vitro evaluation of antifungal effects of botanical extracts against *Colletotrichum lindemuthianum* causing anthracnose of beans

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**ABSTRACT**

Anthracnose of beans caused by *Colletotrichum lindemuthianum* is regarded as one of the most damaging diseases of legumes, producing major losses in productivity and quality every year. Several fungicides are available for control but are dangerous to the health of the environment and organisms, so developing organic solutions is imperative. The in-vitro study includes five botanicals namely neem, mugwort, garlic, ginger, and wild sage, to evaluate their capacity to inhibit the mycelial growth of *C. lindemuthianum*. Analysis was done with the poisoned food technique with four replications for each botanical used in a completely randomized design (CRD). The maximum growth inhibition (100%) was observed in garlic at 10% concentration, followed by ginger (81.15%), while the minimum growth inhibition (36.58%) was observed in wild sage at 10% concentration. Garlic, neem, and mugwort exhibited greater efficiency at lower concentrations, whereas ginger and wild sage demonstrated increased efficiency with higher concentrations. The in-vitro study needs to be tested in field conditions to determine the practical efficiency of the botanicals, and a focus should be made on using garlic and ginger to find organic solutions against the anthracnose of beans.

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## INTRODUCTION

*Colletotrichum lindemuthianum* is considered a devastating pathogen causing anthracnose in beans under cool and humid conditions with yield losses of up to 100% (Dhiman *et al.*, 2020). Lindemuth discovered the fungus in Germany in 1875 (Kadege *et al.*, 2022; Nabi *et al.*, 2022). The pathogen is very genetically variable and has co-evolved with the host, making breeding for anthracnose resistance difficult. Co-evolution is rapid and continuing. The discrepancies in virulence of two races of anthracnose against 139 varieties of beans were discovered (Barrus, 1911). More than 25 different *C. lindemuthianum* races have been recognized in Brazil (Nunes *et al.*, 2021). In Tanzania, output losses remain very significant (40–80%), with an estimated annual cost of $304 million (Mohammed, 2013). Field losses in these Sudanese locations could reach 90% due to seedling, leaf, stem, and pod infections under disease-friendly circumstances (Nalupya, 2021). Bean anthracnose is a widespread disease, and infected seeds are the most essential mechanism for spreading this fungus (Degu *et al.*, 2020). At first, symptoms manifest as dark-red to black lesions along the veins on the lower leaf surfaces; however, lesions may occur at any plant location. Cotyledons may exhibit rust-colored flecks, while petioles, leaves, and leaf veins have lesions that vary from brick-red to purple or black. Symptoms on pods appear as brown, deep cankers surrounded by black rings. Lesions on stems are depressed and frequently elongate. Seed lesions are dark with a white or reddish core. Lesions on severely diseased plants consolidate, causing the plant to die entirely or in part. When the disease infects the seedlings, yield losses are usually severe (Muimba-Kankolongo, 2018).

Bean anthracnose is best controlled by prevention (Manjunatha

*et al.*, 2022). According to epidemiological data, the first opportunity for controlling seed-borne infections is to eliminate or restrict pathogen inoculum in the seed-producing field. Among the management measures used to minimize seed-borne disease in the seed production field are resistant hosts and cultural, chemical, and biological infection control tactics (Yousef, 2021). Botanicals are also being developed as safer alternatives to traditional fungicides for plant disease control (Gwinn, 2018). Natural product-based fungicides break down quickly, lowering their environmental danger (Warra & Prasad, 2020). Plants' antifungal activity and usefulness as potential sources of natural fungicides are well known since they contain many aromatic secondary metabolites such as phenols, phenolic acids, quinines, flavones, flavonoids, flavonols, tannins, and coumarins. Compounds like carvacrol, eugenol, and thymol, possessing phenolic structures, exhibit noteworthy activity against infections (Šernaitė, 2018). Research has consistently demonstrated the fungicidal activity of botanicals against *C. lindemuthianum* over time (Bosquez-Molina *et al.*, 2010; Peraza-Sánchez *et al.*, 2005; Sharma *et al.*, 2022; Wilson *et al.*, 1997). Different fungicides are traditionally used to control the disease. The indiscriminate application of chemical fungicides not only increases input costs but also affects the environment and human health (Ashraf, 2016). The antifungal and antioxidant properties of several botanical plants help reduce disease development and environmental hazards (Kavita, 2013). The study aimed to assess the effectiveness of locally available indigenous botanicals in Nepal against the *C. lindemuthianum* pathogen responsible for bean anthracnose. Further, the goal of our research is to investigate these medicinally essential plants to find solutions to various diseases using biological approaches rather than synthetic chemical methods.

## MATERIALS AND METHODS

### Isolation of the pathogen

Winter bean leaves with distinctive symptoms of anthracnose were obtained from the field of the Institute of Agriculture and Animal Science (IAAS), Rupandehi, Nepal. For about a week, the sample plant was incubated at 24±2°C to allow fungal development. The contaminated leaf was then cut into small 4-5 mm pieces and surface sterilized with 1% sodium hypochlorite (NaOCl). They were eventually rinsed three times with distilled water. Under aseptic conditions, the chopped leaves were dried on sterile blotter paper and placed on Petri dishes with sterilized potato dextrose agar (PDA) media. The Petri plates were kept in an incubator (24±2°C) for ten days and inspected daily for any infection. The conidia were examined under a microscope to validate morphology in comparison to reference cultures of anthracnose.

**Table 1.** List of botanicals that had been tested against *C. lindemuthianum*.

| S. No. | Scientific Name | Common Name | Parts used |
|---|---|---|---|
| 1 | *Azadirachta indica* | Neem | Leaf |
| 2 | *Artemisia vulgaris* | Mugwort | Leaf |
| 3 | *Allium sativum* | Garlic | Bulb |
| 4 | *Zingiber officinale* | Ginger | Rhizome |
| 5 | *Lantana camara* | Wild sage | Leaf |

### Plant extract preparation

Five medicinal plants were obtained from the local area namely neem, mugwort, garlic, ginger, and wild sage (details presented in Table 1). The freshly harvested botanicals were washed with tap water to remove debris from the surface, followed by surface drying. 100 grams of each botanical were taken and macerated into a fine paste using a sterile mortar and pestle while adding 100 ml of distilled water. To maintain constant concentration, the weight-to-volume ratio of botanicals to water was kept constant throughout the procedure. The resulting solution was then filtered using a cotton cloth and centrifuged at 5000 rpm for 10 minutes. To prevent contamination from microorganisms, the extracts were maintained in separate flasks and autoclaved at 121°C at 15 psi for 20 minutes.

### Media preparation

The medium used in the experiment was potato dextrose agar (PDA), which was made by combining ready-made PDA powder with distilled water. The analysis was carried out using the "poisoned food technique" as described by (Adhikari *et al.*, 2018), in which two concentrations (10% and 20%) of each plant extract were infused with PDA, resulting in a total of ten unique growth media. To obtain 10% and 20% concentrations of plant extract, 10 and 20 ml of each plant extract were mixed with 90 and 80 ml of PDA medium, respectively. They were then put onto a sterilized Petri plate and allowed to solidify. Pure PDA media was kept separately as a control, and four replications for each unique media and control were made by pouring molten PDA (20 ml each) into Petri dishes.

### Experimental design and antifungal activity assay

The entire experiment was conducted in a completely randomized design (CRD), with five botanicals (10% and 20% concentrations each) along with a control and four replications of each concentration of botanical extracts, yielding 44 petri plates. The fungal development in each treatment was continuously observed, and the diameter of the mycelium in each plate was measured every 24 hours. The percentage of mycelium growth inhibition (MGI) was estimated for the antifungal activity assay using the formula described by (Shao *et al.*, 2021; Wei *et al.*, 2021).

$$\text{Percentage inhibition} = \frac{(C - T)}{C} \times 100$$

Where,

C = control colony diameter (mm) and T = test plate colony diameter (mm).

### Statistical analysis

Data were statistically analyzed using R (version 4.3.1), and mean comparisons were made using the least significant difference (LSD) at a 5% level of significance.

## RESULTS AND DISCUSSION

### Effect of botanical extracts on the linear diameter colony growth of *C. lindemuthianum*

Different botanical extracts from diverse origins had a significant ($p \leq 0.01$) effect on the linear diameter colony growth of the *C. lindemuthianum* pathogen 24, 48, 72, 96, 120, and 144 hours after inoculation in growth media. Garlic at 10% concentration was shown to be the most efficient of all the botanicals examined, with no colony formation of *C. lindemuthianum* observed after 144 hours of inoculation. After 144 hours, the control petri plate was completely colonized by fungus, and the reading was halted. In the final reading, there was no pathogen colony formation due to the antagonistic impact of garlic (0 mm), followed by ginger (43.5 mm). The control had the highest colony growth (89.25 mm), followed by the wild sage (67 mm), as shown in Table 2. Similarly, a significant difference ($p \leq 0.01$) was observed between several botanical extracts of 20% concentration in mean linear diameter colony growth at 24, 48, 72, 96, 120, and 144 hours after inoculation. Initially, no colony growth was seen in garlic and ginger extracts, whereas the control had the maximum colony growth (16.5 mm), followed by mugwort (8.75 mm). After 144 hours, the control petri plate was nearly fully colonized with fungus, and the reading was halted where mugwort (62.5 mm) had the highest colony growth, followed by wild sage (62 mm). Garlic had the slowest colony growth (17mm), followed by ginger (29.25mm) (Table 3). From tables 2 and 3, the lowest mycelium growth of 0 mm and 17 mm was recorded after 144 hours in garlic at concentrations of 10% and 20%, followed by ginger, in which mycelium growth of 43.5 mm and 29.25 mm was recorded at respective 10% and 20% concentrations after 144 hours of inoculation against control.

### Percent growth inhibition of *C. lindemuthianum* in different botanical extracts

In-vitro, all plant extracts significantly ($p \leq 0.01$) suppressed the colony growth of the *C. lindemuthianum* infection. The highest reduction in colony growth was reported at 10% concentrations of garlic, neem, and mugwort, while ginger and wild sage inhibited colony growth at 20% concentrations. Among the botanicals tested, garlic had the highest colony growth inhibition percentage (100% and 92.86% in 10 and 20% concentrations, respectively), followed by ginger (68.94% and 81.15% in 10 and 20% concentrations, respectively), and wild sage (36.58%), followed by mugwort (47.82%) at 10% concentration, and mugwort (37.54%), followed by wild sage (39.33%) at 20% concentration (Tables 4 and 5). Among the many botanicals and concentrations tested, garlic produced the best results.

**Table 2.** In-vitro efficacy of different botanical extracts (10% concentration) on growth of *C. lindemuthianum*.

| S. No. | Treatments | Mean colony diameter (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 24 hour | 48 hour | 72 hour | 96 hour | 120 hour | 144 hour |
| 1 | Neem (*Azadirachta indica*) | $8.25^b$ | $14.75^b$ | $23^c$ | $34^c$ | $42.75^c$ | $48.75^c$ |
| 2 | Mugwort (*Artemisia vulgaris*) | $2^c$ | $13.75^b$ | $26.25^c$ | $43^b$ | $52^b$ | $59.5^b$ |
| 3 | Garlic (*Allium sativum*) | $0^c$ | $0^d$ | $0^e$ | $0^e$ | $0^d$ | $0^d$ |
| 4 | Ginger (*Zingiber officinale*) | $0^c$ | $6^c$ | $15^d$ | $26^d$ | $34.5^c$ | $43.5^c$ |
| 5 | Wild sage (*Lantana camara*) | $8.5^b$ | $16.5^b$ | $30^b$ | $43.25^b$ | $54^b$ | $67^b$ |
| 6 | Control | $16.5^a$ | $31.5^a$ | $47.75^a$ | $63.75^a$ | $77.25^a$ | $89.25^a$ |
| | LSD (p=0.05) | 2.95*** | 4.8*** | 3.63*** | 6.39*** | 8.61*** | 10.06*** |
| | CV% | 33.75 | 23.48 | 10.32 | 12.3 | 13.35 | 13.2 |

*Average of four replication, LSD = Least significant difference, CV = Coefficient of variation. LSD value with triple asterisk indicates highly significant difference between treatments.

**Table 3.** In-vitro efficacy of different botanical extracts (20% concentration) on growth of *C. lindemuthianum*.

| S. No. | Treatments | Mean colony diameter (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 24 hour | 48 hour | 72 hour | 96 hour | 120 hour | 144 hour |
| 1 | Neem (*Azadirachta indica*) | $8.5^b$ | $16.5^c$ | $22.75^b$ | $34.25^b$ | $40.75^b$ | $50^b$ |
| 2 | Mugwort (*Artemisia vulgaris*) | $8.75^b$ | $18.5^b$ | $28^b$ | $42^b$ | $52^b$ | $62.5^b$ |
| 3 | Garlic (*Allium sativum*) | $0^c$ | $0^d$ | $0^d$ | $5.25^d$ | $12.5^c$ | $17^c$ |
| 4 | Ginger (*Zingiber officinale*) | $0^c$ | $0^d$ | $10^c$ | $17.75^c$ | $24^c$ | $29.25^c$ |
| 5 | Wild sage (*Lantana camara*) | $8.5^b$ | $17.5^{bc}$ | $26.5^b$ | $41.25^b$ | $50.25^b$ | $62^b$ |
| 6 | Control | $16.5^a$ | $31.5^a$ | $47.75^a$ | $63.75^a$ | $77.25^a$ | $89.25^a$ |
| | LSD (p=0.05) | 4.02*** | 1.71*** | 5.65*** | 11.16*** | 14.37*** | 16.51*** |
| | CV% | 38.42 | 8.24 | 16.91 | 22.07 | 22.61 | 21.51 |

*Average of four replication, LSD = Least significant difference, CV = Coefficient of variation. LSD value with triple asterisk indicates highly significant difference between treatments.

**Table 4.** Percent growth inhibition of *C. lindemuthianum* in different botanical extracts of 10% concentration.

| S. No. | Treatments | Percent growth inhibition (%) | | | | | | Mean Growth Inhibition (%) |
|---|---|---|---|---|---|---|---|---|
| | | 24 hour | 48 hour | 72 hour | 96 hour | 120 hour | 144 hour | |
| 1 | Neem (*Azadirachta indica*) | 49.61[b] | 53.05[c] | 51.68[c] | 46.65[c] | 44.62[bc] | 45.37[bc] | 48.5 |
| 2 | Mugwort (*Artemisia vulgaris*) | 87.5[a] | 56.38[c] | 44.95[cd] | 32.41[d] | 32.47[cd] | 33.22[cd] | 47.82 |
| 3 | Garlic (*Allium sativum*) | 100[a] | 100[a] | 100[a] | 100[a] | 100[a] | 100[a] | 100 |
| 4 | Ginger (*Zingiber officinale*) | 100[a] | 79.98[b] | 68.17[b] | 59.1[b] | 55.17[b] | 51.21[b] | 68.94 |
| 5 | Wild sage (*Lantana camara*) | 47.71[b] | 47.55[c] | 36.97[d] | 32.17[d] | 30.11[d] | 24.95[d] | 36.58 |
| 6 | Control | - | - | - | - | - | - | - |
| | LSD (p=0.05) | 19.31*** | 17.05*** | 9.29*** | 11.79*** | 13.09*** | 12.8*** | - |
| | CV% | 16.65 | 16.78 | 10.21 | 14.47 | 16.55 | 16.67 | - |

*Average of four replication, LSD = Least significant difference, CV = Coefficient of variation. LSD value with triple asterisk indicates highly significant difference between treatments.

**Table 5.** Percent growth inhibition of *C. lindemuthianum* in different botanical extracts of 20% concentration.

| S. No. | Treatments | Percent growth inhibition (%) | | | | | | Mean Growth Inhibition (%) |
|---|---|---|---|---|---|---|---|---|
| | | 24 hour | 48 hour | 72 hour | 96 hour | 120 hour | 144 hour | |
| 1 | Neem (*Azadirachta indica*) | 45.75[b] | 47.29[b] | 52.22[c] | 46.22[c] | 47.15[b] | 43.98[b] | 47.10 |
| 2 | Mugwort (*Artemisia vulgaris*) | 45.72[b] | 41.12[b] | 41.54[c] | 34.18[c] | 32.71[b] | 29.99[b] | 37.54 |
| 3 | Garlic (*Allium sativum*) | 100[a] | 100[a] | 100[a] | 91.92[a] | 84.10[a] | 81.11[a] | 92.86 |
| 4 | Ginger (*Zingiber officinale*) | 100[a] | 100[a] | 78.94[b] | 72.12[b] | 68.71[a] | 67.15[a] | 81.15 |
| 5 | Wild sage (*Lantana camara*) | 47.04[b] | 44.21[b] | 44.09[c] | 35.25[c] | 34.87[b] | 30.54[b] | 39.33 |
| 6 | Control | - | - | - | - | - | - | - |
| | LSD (p=0.05) | 29.14*** | 7.23*** | 12.89*** | 19.34*** | 20.79*** | 20.37*** | - |
| | CV% | 28.55 | 7.21 | 13.51 | 22.94 | 25.78 | 26.74 | - |

*Average of four replication, LSD = Least significant difference, CV = Coefficient of variation. LSD value with triple asterisk indicates highly significant difference between treatments.

**Table 6.** List of botanicals along with chemically active constituents present in them.

| Name of plant | Family | Major active chemical constituent | Reference |
|---|---|---|---|
| Neem (*Azadirachta indica*) | Meliaceae | Azadirachtin<br>Nimonol<br>Epoxyazadiradione<br>Quercetin | (Latif *et al.*, 2020)<br>(Bello *et al.*, 2018)<br>(Gyanwali *et al.*, 2023) |
| Mugwort (*Artemisia vulgaris*) | Asteraceae | Luteolin<br>quercetin 3-glucoside<br>Eriodictyol<br>Camphor<br>Thujone | (Soon *et al.*, 2019)<br>(Abiri *et al.*, 2018)<br>(Ekiert *et al.*, 2020) |
| Garlic (*Allium sativum*) | Amaryllidaceae | Allicin<br>Alliin<br>Ajoene | (Rolim *et al.*, 2020)<br>(Bazaraliyeva *et al.*, 2022) |
| Ginger (*Zingiber officinale*) | Zingiberaceae | Gingerols<br>Shogaols<br>Paradols | (Mao *et al.*, 2019)<br>(Bhattarai *et al.*, 2018) |
| Wild sage (*Lantana camara*) | Verbenaceae | beta-caryophyllene<br>zingiberene<br>gamma-curcumene<br>alpha-humulene | (Aliyu *et al.*, 2020)<br>(Delgado-altamirano *et al.*, 2019) |

## Constituents present in botanical extracts

The botanicals chosen for analysis have antifungal characteristics, as demonstrated by various research over the years: *Azardirachta indica* (Khan *et al.*, 2021; Nn *et al.*, 2018; Gyanwali *et al.*, 2023); *Artemisia vulgaris* (Ekiert *et al.*, 2020); *Allium sativum* (Carreón-Delgado *et al.*, 2023); *Zingiber officinale* (Li *et al.*, 2021); *Lantana camara* (Ozturk & Hakeem, 2019). The unique chemicals found in certain plant species make them effective against a specific type of pest. Thus, documentation of these valuable plants is essential to conserving knowledge through multiple generations (Lengai *et al.*, 2020). The botanicals having antifungal properties with unique compounds present in them are listed in Table 6. Over the years, several scientists have reported the antifungal efficacy of many botanical extracts against various plant diseases. In-vitro evaluation was conducted against *C. lindemuthianum* using botanical extracts of *Zingiber officinale, Pongamia pinnata, Azadirachta indica, Eucalyptus globules, and Ocimum sanctum* (Bagade *et al.*, 2020). They showed the highest mycelium growth inhibition with ginger, which is in close conformity with our experiment. Vasuki *et al.* (2020) reported that the mycelial growth inhibition of garlic at 5 and 10% concentrations was superior to that of 11 different

botanical extracts used in an experiment against *C. lindemuthianum*. According to Choudhary *et al*. (2017), botanicals were used to control the growth of *C. lindemuthianum* mycelia on *Vigna radiata*. At 10% concentration, they observed antagonistic effects of botanicals on mycelia proliferation. *A. sativum* (80.56%), *A. indica* (78.83%), *Z. officinale* (74.38%), and *Datura* (70.91%) were found to be effective in inhibiting mycelia growth. Botanicals *A. sativum*, *Z. officinale*, and *A. indica* were found to be beneficial in suppressing the mycelia growth of *C. gloeosporioides* in-vitro (Handiso & Alemu, 2017; Chourasia, 2021).

The present study is in close conformity with the above works. Garlic extracts reduced the growth of the pathogens tested in this study. This finding is consistent with previous research on garlic's antifungal effects. Garlic contains a chemical called di-allyl thiosulfinate (allicin), which inhibits bacterial and fungal growth (Cavallito *et al*., 1944). Garlic extract has been used in agriculture as a natural fungicide to reduce fungal diseases in crops. Garlic extract has been demonstrated in studies to efficiently prevent the growth of various plant pathogenic fungi, including *Fusarium oxysporum*, *Alternaria alternata*, *Colletotrichum* species, and *Botrytis cinerea* (Soteyome & Theeramongkol, 2023).

### Conclusion

From this experiment, it became clear that of the five botanical extracts tested in-vitro against *C. lindemuthianum*, garlic at 10% inhibited 100% mycelial development, whereas ginger at 20% inhibited 81.15% mycelial growth. In-vitro study indicate that garlic and ginger have intriguing potential, whereas neem, mugwort, and wild sage all demonstrated inhibitory effects, albeit at a lower level. As a result, using botanicals rather than drugs to control the disease can be considered a safer option. Thus, these plant extracts could be used as a novel fungicide alternative to toxic chemical fungicides. However, these in-vitro study findings should be validated in field circumstances before being used in the field.

## DECLARATIONS

### Author contribution statement

Conceptualization, methodology, Investigation: N.P.P., P.G., B.T., S.P., R.K., and R.K.; Software, validation, Data curation, Writing, original draft preparation: N.P.P. review and editing: N.P.P. and P.G.; Supervision: R.P. All authors have read and agreed to the published version of the manuscript.

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## REFERENCES

Abiri, R., Silva, A. L. M., de Mesquita, L. S. S., de Mesquita, J. W. C., Atabaki, N., de Almeida, E. B., Shaharuddin, N. A., & Malik, S. (2018). Towards a better understanding of Artemisia vulgaris: Botany, phytochemistry, pharmacological and biotechnological potential. *Food Research International*, *109*(October 2017), 403–415. https://doi.org/10.1016/j.foodres.2018.03.072

Adhikari, M., Kharel, N., Gaire, L., Poudel, R., Shrestha, S. M., Gaire, S. P., & Acharya, B. (2018). In vitro evaluation of different chemicals against Rhizoctonia solani by poisoned food technique. In *Field Crop*, 1(3), 6–9).

Aliyu, R. H., Dasikwo, S. Y., Alanana, J. A., & Ombugadu, N. A. (2020). Phytochemical screening and the ethnobotanical importance of the leaves of wild *SAGE ( Lantana camara L .)*. *6*(1).

Ashraf, Y. (2016). *In Vitro and In Vivo Management of Alternaria Leaf Spot of Brassica Plant Pathology & Microbiology*, *7*(7). https://doi.org/10.4172/2157-7471.100036

Bagade, A. R., Giri, G. K., Shingne, A. W., & Usendi, P. N. (2020). In vitro Evaluation of Fungicides, Botanicals and Bio-agents against *Colletotrichum lindemuthianum* causing Anthracnose of Bean. *International Journal of Current Microbiology and Applied Sciences*, *9*(8), 3103–3110. https://doi.org/10.20546/ijcmas.2020.908.352

Barrus, M. F. (1911). Variation of varieties of beans in their susceptibility to anthracnose. *Phytopathology*, 1(6), 190-195

Bazaraliyeva, A., Moldashov, D., Turgumbayeva, A., Kartbayeva, E., Kalykova, A., Sarsenova, L., & Issayeva, R. (2022). Chemical and biological properties of bioactive compounds from garlic (Allium sativum). *Pharmacia*, *69*(4), 955–964. https://doi.org/10.3897/pharmacia.93.e93604

Bello, O. A., Ayanda, O. I., Aworunse, O. S., & Olukanmi, B. I. (2018). *Pharmacognosy Reviews*. *1*(2), 8–15. https://doi.org/10.4103/phrev.phrev

Bhattarai, K., Pokharel, B., Maharjan, S., & Adhikari, S. (2018). *Chemical Constituents and Biological Activities of Ginger Rhizomes from Three Different Regions of Nepal*. *1*(1), 1–12.

Bosquez-Molina, E., Jesús, E. R. de, Bautista-Baños, S., Verde-Calvo, J. R., & Morales-López, J. (2010). Inhibitory effect of essential oils against *Colletotrichum gloeosporioides* and *Rhizopus stolonifer* in stored papaya fruit and their possible application in coatings. *Postharvest Biology and Technology*, *57*(2), 132–137. https://doi.org/10.1016/j.postharvbio.2010.03.008

Carreón-Delgado, D. F., Hernández-Montesinos, I. Y., Rivera-Hernández, K. N., del Sugeyrol Villa-Ramírez, M., Ochoa-Velasco, C. E., & Ramírez-López, C. (2023). Evaluation of pretreatments and extraction conditions on the antifungal and antioxidant effects of garlic (Allium sativum) peel extracts. *Plants*, 12(1), 217. https://doi.org/10.3390/plants12010217

Cavallito, C. J., Buck, J. S., & Suter, C. M. (1944). Allicin, the Antibacterial Principle of Allium sativum. II. Determination of the Chemical Structure. *Journal of the American Chemical Society*, *66*(11), 1952–1954. https://doi.org/10.1021/ja01239a049

Choudhary, R. S., Simon, S., & Bana, S. R. (2017). Efficacy of plant extracts against anthracnose (*Colletotrichum lindemuthianum*) of green gram (*Vigna radiata*). *International Journal of Chemical Studies*, *5*(4), 769-772.

Degu, T., Yaregal, W., & Gudisa, T. (2020). Status of Common Bean (Phaseolus vulgaris L.) Diseases in Metekel Zone, North West Ethiopia. *Journal of Plant Pathology & Microbiology Research Article 1 J Plant Pathol Microbiol*, *11*(5), 494. https://doi.org/10.35248/2157-7471.20.11.494

Delgado-altamirano, R., L, R. I., Monzote, L., Becker, I., & Rivero-cruz, J. F. (2019). *Chemical Constituents with Leishmanicidal Activity from a Pink-Yellow Cultivar of Lantana camara var. aculeata (L.) Collected in Central Mexico*. https://doi.org/10.3390/ijms20040872

Dhiman, S., Badiyal, A., Katoch, S., Pathania, A., Sharma, S. K., & Sharma, P. . N. (2020). Introgression of Anthracnose Resistance Gene from Common Bean Land Race KRC-8 into Elite Cultivar Jawala. *Himachal Journal of Agricultural Research*, *46*(1), 56–61.

Ekiert, H., Pajor, J., Klin, P., Rzepiela, A., Slesak, H., & Szopa, A. (2020). Significance of *Artemisia vulgaris* L. (Common Mugwort) in the history of medicine and its possible contemporary applications substantiated by phytochemical and pharmacological studies. *Molecules*, *25*(19). https://doi.org/10.3390/molecules25194415

Gwinn, K. D. (2018). Bioactive Natural Products in Plant Disease Control. In *Studies in Natural Products Chemistry* (1st ed., Vol. 56). Elsevier B.V. https://doi.org/10.1016/B978-0-444-64058-1.00007-8

Gyanwali, P., Khanal, R., Pokharel, N. P., Tharu, B., Koirala, R., Paudel, S., & Paudel, R. (2023). Invitro analysis of antifungal effects of botanicals on sclerotinia sclerotiorum causing white mold disease. *Agriculture and Food Sciences Research*, *10*(2), 8–13. https://doi.org/10.20448/aesr.v10i2.5186

Handiso, S., & Alemu, T. (2017). A Comparative Study on Aqueous Extracts of Some Indigenous Medicinal Plants and Fungicides against Anthracnose (*Colletotrichum* spp ) of Chili ( *Capsicum* spp ). 7(1), 9–15.

Kadege, E. L., Venkataramana, P., Assefa, T., Ndunguru, J. C., Mukankusi, C. M., Rubyogo, J. C., & Mbega, E. R. (2022). Pathogenicity and approaches for management of anthracnose in common bean (*Phaseolus vulgaris*) in Africa. *International Journal of Agriculture & Biology*. https://doi.org/10.17957/IJAB/15.1978

Kavita, D. (2013). Efficacy of different botanicals against Alternaria brassicae in in vitro conditions. *International Journal of Science and Research*, 4, 791-793.

Khan, M. R., Chonhenchob, V., & Huang, C. (2021). Antifungal Activity of Propyl Disulfide from Neem (*Azadirachta indica*) in Vapor and Agar Diffusion Assays against Anthracnose Pathogens (*Colletotrichum gloeosporioides* and *Colletotrichum acutatum*) in Mango Fruit.

Latif, M. J., Hassan, S. M., Sharif Mughal, S., Aslam, A., Munir, M., Shabbir, N., Mushtaq, M., & Perveiz, S. (2020). Therapeutic Potential of *Azadirachta indica* (Neem) and Their Active Phytoconstituents against Diseases Prevention. *Online) Journal of Chemistry and Chemical Sciences*, *10*(3), 98–110.

Lengai, G. M. W., Muthomi, J. W., & Mbega, E. R. (2020). Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. *Scientific African*, *7*. https://doi.org/10.1016/j.sciaf.2019.e00239

Li, X., Ao, M., Zhang, C., Fan, S., Chen, Z., & Yu, L. (2021). Zingiberis Rhizoma Recens: A Review of Its Traditional Uses, Phytochemistry, Pharmacology, and Toxicology. *Evidence-Based Complementary and Alternative Medicine*, *2021*. https://doi.org/10.1155/2021/6668990

Manjunatha, V., Bhattacharjee, D., & Flores, C. (2022). Disease Management of Faba Beans. *Faba Bean: Chemistry, Properties and Functionality*, 357–394. https://doi.org/10.1007/978-3-031-14587-2_14/COVER

Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Beta, T., & Li, H. Bin. (2019). Bioactive compounds and bioactivities of ginger (*zingiber officinale* roscoe). *Foods*, *8*(6), 1–21. https://doi.org/10.3390/foods8060185

Mohammed, A. (2013). An Overview of Distribution, Biology and the Management of Common Bean Anthracnose. *Journal of Plant Pathology & Microbiology*, *04* (08). https://doi.org/10.4172/2157-7471.1000193

Muimba-Kankolongo, A. (2018). Leguminous Crops. *Food Crop Production by Smallholder Farmers in Southern Africa*, 173–203. https://doi.org/10.1016/B978-0-12-814383-4.00010-4

Nabi, A., Lateef, I., Nisa, Q., Banoo, A., Rasool, R. S., Shah, M. D., Ahmad, M., & Padder, B. A. (2022). Phaseolus vulgaris-Colletotrichum lindemuthianum pathosystem in the post-genomic era: An update. *Current Microbiology*, *79*(2), 36.

Nalupya, K. (2021). *Evaluation of the ciat phaseoulus core collection for resistance to anthracnose* (Doctoral Dissertation, The University of Zambia).

Nn, Z., Kf, C., Magga, B., & Tm, A. (2018). *Antifungal effect of neem (Azadirachta indica) leaf extracts on mango fruit post-harvest rot agents in Yola , Adamawa state*. *7*(1), 23–26.

Nunes, M. P. B. A., Gonçalves-Vidigal, M. C., Martins, V. S. R., Xavier, L. F. S., Valentini, G., Vaz Bisneta, M., & Vidigal Filho, P. S. (2021). Relationship of *Colletotrichum lindemuthianum* races and resistance loci in the Phaseolus vulgaris L. genome. *Crop Science*, *61*(6), 3877–3893. https://doi.org/10.1002/csc2.20601

Ozturk, M., & Hakeem, K. R. (2019). Plant and human health: Pharmacology and therapeutic uses. In *Plant and Human Health: Pharmacology and Therapeutic Uses* (Vol. 3, Issue January). https://doi.org/10.1007/978-3-030-04408-4

Peraza-Sánchez, S. R., Chan-Che, E. O., & Ruiz-Sánchez, E. (2005). Screening of Yucatecan plant extracts to control Colletotrichum gloeosporioides and isolation of a new pimarene from Acacia pennatula. *Journal of Agricultural and Food Chemistry*, *53*(7), 2429–2432. https://doi.org/10.1021/jf040422i

Rolim, L. A., Santos, F. C. M. dos, Chaves, L. L., Gonçalves, M. L. C. M., Freitas-Neto, J. L., Nascimento, A. L. da S. do, Soares-Sobrinho, J. L., Albuquerque, M. M. de, Lima, M. do C. A. de, & Rolim-Neto, P. J. (2020). Enhanced Reader.pdf. In *Nature* (Vol. 388, pp. 539–547).

Šernaitė, L. (2018). Plant extracts: antimicrobial and antifungal activity and appliance in plant protection (Review) Plant extracts: antimicrobial and antifungal activity and appliance in plant protection ( Review ). *01*.

Shao, J., Pei, Z., Jing, H., Wang, L., Jiang, C., Du, X., Jiang, C., Lou, Z., & Wang, H. (2021). Antifungal activity of myriocin against Fusarium graminearum and its inhibitory effect on deoxynivalenol production in wheat grains. *Physiological and Molecular Plant Pathology*, *114*, 101635. https://doi.org/10.1016/J.PMPP.2021.101635

Sharma, N., Thakur, R., Rana, D., Sharma, P. ., & Basandrai, A. K. (2022). Combating bean anthracnose through integrated disease management. *Plant Disease Research*, *37*(1), 15–22. https://doi.org/10.5958/2249-8788.2022.00003.8

Soon, L., Ng, P. Q., Chellian, J., Madheswaran, T., Panneerselvam, J., Gupta, G., Nammi, S., Hansbro, N., Hsu, A., Dureja, H., Mehta, M., Satija, S., Hansbro, P. M., Dua, K., Collet, T., & Chellappan, D. K. (2019). Therapeutic potential of artemisia vulgaris: An insight into underlying immunological mechanisms. *Journal of Environmental Pathology, Toxicology and Oncology*, *38*(3), 205–216. https://doi.org/10.1615/JEnvironPatholToxicolOncol.2019029397

Soteyome, T., & Theeramongkol, P. (2023). *Preparation and processing of garlic extract and its further application on anti-fungal activity*. *10*, 2021–2035.

Vasuki, E., Rajinimala, N., Kannan, R., & Sabarinathan, K. G. (2020). *Bioefficiency of Botanicals against Colletotrichum lindemuthianum causing Anthracnose in Blackgram*. *9*(8), 460–466.

Warra, A. A., & Prasad, M. N. V. (2020). African perspective of chemical usage in agriculture and horticulture—their impact on human health and environment. In *Agrochemicals Detection, Treatment and Remediation: Pesticides and Chemical Fertilizers*. LTD. https://doi.org/10.1016/B978-0-08-103017-2.00016-7

Wei, L., Chen, C., Chen, J., Lin, L., & Wan, C. (2021). Possible fungicidal effect of citral on kiwifruit pathogens and their mechanisms of actions. *Physiological and Molecular Plant Pathology*, *114*(February), 101631. https://doi.org/10.1016/j.pmpp.2021.101631

Wilson, C. L., Solar, J. M., El Ghaouth, A., & Wisniewski, M. E. (1997). Rapid evaluation of plant extracts and essential oils for antifungal activity against Botrytis cinerea. *Plant Disease*, *81*(2), 204–210. https://doi.org/10.1094/PDIS.1997.81.2.204

Yousef S. (2021). *Bean Anthracnose Control Using Different Beneficial Bacteria*. January, 1–20.