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ISOLATION, CHARACTERIZATION AND MANAGEMENT OF ALTERNARIA LEAF BLIGHT OF TURNIP THROUGH BOTANICALS

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A B S T R A C T

Turnip is an important winter vegetable. Worldwide there are several fungal pathogens that cause leaf blight diseases on wide range of host plants including turnip. The isolated fungus from infected leaves were identified microscopically for morphological characterization and genetically by the nucleotide sequencing of amplified ITS1/ITS4 and BT2a/BT2b region of rDNA and identified fungus was found to be *Alternaria brassicicola*. Due to their reduced side effects, superior biodegradability, and lower toxicity when compared to other synthetic fungicides, natural compounds have recently received significant interest as an alternative to synthetic fungicides. Aqueous solutions of weed Flora like, *Solanum nigrum, Nicotiana plumbaginifolia, Trianthema portulacastrum, Malvestrum coromendelianum, Chenopodium album*, and *Parthenium hysterophorus* were tested against target pathogen. *Chenopodium album* concentrations in control exhibit maximum growth up to 75.67mm while in concentrations of 250, 500 and 1000ppm lessen growth up to 13.00, 12.33, and 00.00 mm. While *Solanum nigrum* against *A. brassicicola* at 250, 500 and 1000 ppm concentrations show reduction in fungal growth up to 29.67, 15.33, and 00.00 mm. While using *Nicotiana plumbaginifolia* against target pathogen in concentration of 250, 500 and 1000 ppm minimize growth up to 24.00, 18.33, and 00.00 mm. *Chenopodium album, Solanum nigrum and Nicotiana plumbaginifolia* give better results against *A. brassicicola*. Therefore, biological control is an excellent and safe approach to control fungal pathogens and to provide less chemical usage.

Keywords: Amplification, Antifungal assay, Bathu, Brassicaceae, Leaves, Nucleotide, Percentage inhibition.

INTRODUCTION

The turnip (*Brassica rapa* L.), which is grown in many nations across the world, is the most significant root crop in the family Cruciferae (Vogl, and Reiner, 2007; Wahocho, 2016). While larger types are specifically intended for animal consumption, the small, sensitive varieties are grown as a source of nourishment for humans. Various elements, including iron, calcium, carbohydrates, protein, and vitamins, are abundant in turnips (A, B, and C). Turnip farming has several possibilities in Pakistan due to the country's environment (Wahocho, 2016). In Punjab, total turnip production in the year of 2018-19 covers the area of about 8.9 thousand hectares with total production of 170.7 thousand tonnes (BOSP, 2021).

Foliar diseases are one of the limiting variables that have an impact on the majority of Brassicaceae farming. *Alternaria*, a fungus that belongs to the Brassicaceae family, is one of the most significant fungi that cause leaf spot disease (Reis and Boiteux, 2010). These pathogens frequently do not affect the size or weight of the harvested plant, but they do result in significant losses because of the affected plant's poor quality and appearance. On rare occasions, leafy brassicas like bok choy and Chinese cabbage may sustain severe damage and lose their commercial viability (Koike., 2007).

Dark leaf spot in brassica crops, such as turnips, is caused by *Alternaria brassicicola* (Schwein.). The disease has a significant impact on the entire world and can affect brassica crops like rapeseed or canola by up to 20 to 50%. All plant

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parts (seed, seedlings, pods, and leaves) are equally harmed by *A. brassicicola*, the cause of dark leaf blotches in both wild and cultivated varieties. The fungus may spread through seeds. It is possible to find mycelium both internally and externally. Mycelium that is able to survive on crop waste can potentially act as a disease inoculum (Köhl and Wolf, 2005).

To obtain healthy seeds, disease prevention in seed crops is crucial. Across the world, studies on plant extracts' antifungal properties have been conducted. Synthetic chemicals are harmful to the environment and human health. Due to detrimental consequences on the environment, plant scientists are worried about using more environmentally friendly and cost-effective resources to prevent plant diseases (Sasode *et al.,* 2012).

The majority of medicinal plants, including Eucalyptus, Neem, Pudina, and Datura, have been utilized to combat *Alternaria brassicae* both raw and boiled. Studies have also been done on eucalyptus and neem oils. In the studied forms (Crude, Boil, and Oil), all the understudies strongly prevent fungus growth. On Neem crude extract, the pathogen grew the least out of all the media. Also, the boiled Neem extract performed the best. On *A. brassicae*, Neem crude extract has been shown to exhibit antifungal properties (Sasode., 2012).

Therefore, the current study is focused on using plant extracts to prevent Alternaria leaf blight in turnips.

The goal of the current study was to isolate, identify, and manage Alternaria leaf blight of turnip. It was carried out at the Fungal Biotechnology Laboratory, Department of Plant Pathology, University of the Punjab, Lahore.

Surveying and collecting samples: In November-December 2019, to investigate the diseases of vegetable plants, a survey of different Hadyara vegetable fields was carried out in Lahore. Along with many other vegetable plants, the leaves of turnip (*Brassica rapa* L.) were discovered to have spots or lesions. After data on the appearance of spots and lesions on leaves, including their size, color, and shape, was collected, these plants were chosen so that the pathogens could be studied. Diseased plants and leaves were photographed for the purpose of reference and record. From three different plants, five damaged leaves were randomly chosen and sent to the lab in sterile plastic bags for pathogen detection. Samples were kept in freezer at 4 °C until.

Isolation and purification of Fungal Pathogens: Zuha's (2018) methodology was used to prepare the Potato Dextrose Agar (PDA) media. For isolation of fungal pathogens, each of the selected diseased leaves had at least 3–4 spots cut in to small pieces that were about 3 mm^2 (with some healthy leaf tissue). Then, using NaOCl and the Ranaware method. (2014) protocol. To purify the mycelia of the fungi, they were moved from the inoculated leaf pieces to the freshly prepared PDA plates and left to grow at 25 + 2 °C. Pure fungal cultures were kept at 4 °C.

Identifying the Pathogen: The morphogenic properties of the fungal strains being isolated were first used for identification, and afterwards, nucleotide sequence analysis of the internal transcribed spacer (ITS) sequence of rDNA and partial beta-tubulin (β-Tubulin) gene were used for confirmation.

Morphological identification: Morphological observations were done on pure cultures grown on PDA for 7 days at 25 + 2 °C. (Sohail.,2018). Photographs were taken to describe the macro- and micromorphological features. Each isolate's complete description was written based on its morphological characteristics. Pure fungal pathogen culture that was deposited at the University of the Punjab's Institute of Agricultural Sciences' First Fungal Culture Bank of Pakistan.

Molecular identification: Fungal genomic DNA extraction: Using Nucleon reagent B, the Amir (2015) technique for DNA isolation was carried out. Extracted DNA was incubated for 15 minutes at 65 °C before being kept at -20 °C until further usage. Following Amir's instructions, agarose gel electrophoresis was performed to examine the quality and integrity of the isolated DNA (2015).

PCR Amplification for DNA Sequence Analysis: The ITS region of rDNA and a partial beta-tubulin gene were amplified using the fungal genomic DNA as a template and the universal primer pairs ITS1/ITS4 and Bt2a/Bt2b (Amir., 2015). Detail of these primers is given in Table.

Table 1. Detail of the primers used for amplification of ITS or β-tubulin gene.

Sr. no	Gene	Primer name	Primer sequence
1.	ITS	ITS 1	5′-TCCGTAGGTGAACCTGCGG-3′
2.		ITS 4	5′-TCCTCCGCTTATTGATATGC -3′
3	β-tubulin	Bt_2a	5′-GGTAACCAAATCGGTGCTGCTTTC-3′
4		Bt_2b	5′-ACCCTCAGTGTAGTGACCCTTGGC-3′

Nucleotide BLAST Analysis: PCR products that had been amplified were sent for nucleotide sequencing. Nucleotide Basic Local Alignment Tool (BLAST) was used to analyze the resulting sequences. Sequence homology was noted and used to distinguish and identify various fungus strains.

Utilizing aqueous plant extracts for management:

Plant material collection: Fresh plant samples include above-ground sections of all weeds were obtained from the University of the Punjab's field and wasteland areas in Lahore, Pakistan.

Table 2**.** Detail of tested weeds against *Alternaria brassicicola*

Local names	Scientific names
Bathu	*Chenopodium album*
Congress booti	*Parthenium hysterophorus*
Itsit	*Trianthema portulacastrum* L.
Malvestrum	*Malvestrum coromendelianum*
Mako	*Solanum nigrum*
Giddar tumbako	*Nicotiana plumbaginifolia*

The top six weeds were washed 2 to 3 times with running water to get rid of dirt and other impurities. Plant material's extra water was drained through a strainer. After washing with distilled water and disinfecting them with 2% NaOCl, they were shade dried on blotter paper. The dried plants were finally powdered at medium revolution using an electrical home grinder.

Extraction of plant material: Distilled water was used to soak 20g of powdered plant material for 24 hours at room temperature. The soaking plant material was sterilized twice and filtered using Muslin Cloth and Whatman Filter Paper No. 1. The filtrate, which contained 20% of the original substance, was considered as stock extraction. The following formula was used to prepare the lower concentration of 1000 ppm:

For the control of *Alternaria barassicola*, a total of 8 concentrations were used 15.63, 31.25, 62.5, 125, 250, 500, and 100 ppm.

Antifungal assay: The inhibitory activity of phytoextracts of weed species against *Alternaria barassicola* was assessed in vitro using the food poisoning technique. After being autoclaved, weed plant extract concentrations (15.63 ppm–1000 ppm) were combined with potato dextrose agar (PDA) media and added to Petri plates at a temperature of about 40 °C. As a control, PDA material without any plant extract was used. With the help of a cork-borer, a pure culture of fungi that had been growing for a few days was inoculated into plates by placing its 5mm mycelial disc in the center of the plate. Incubation temperatures for the test treatments were 25±2°C.

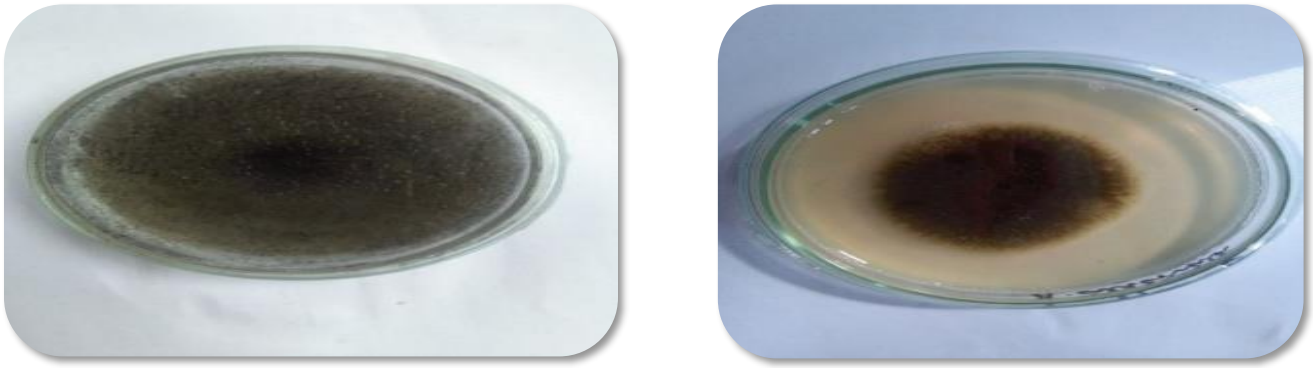
DATA COLLECTION

The in vitro tests were carried out with three replicates using a completely randomized design. After 10 days, the formula below was used to calculate the percentage of growth inhibition (Bajwa *et al.,* 2006).

RESULTS

Turnip disease samples were collected from Hadyara village for the survey, and through morphological and molecular analysis, the test fungus was identified.

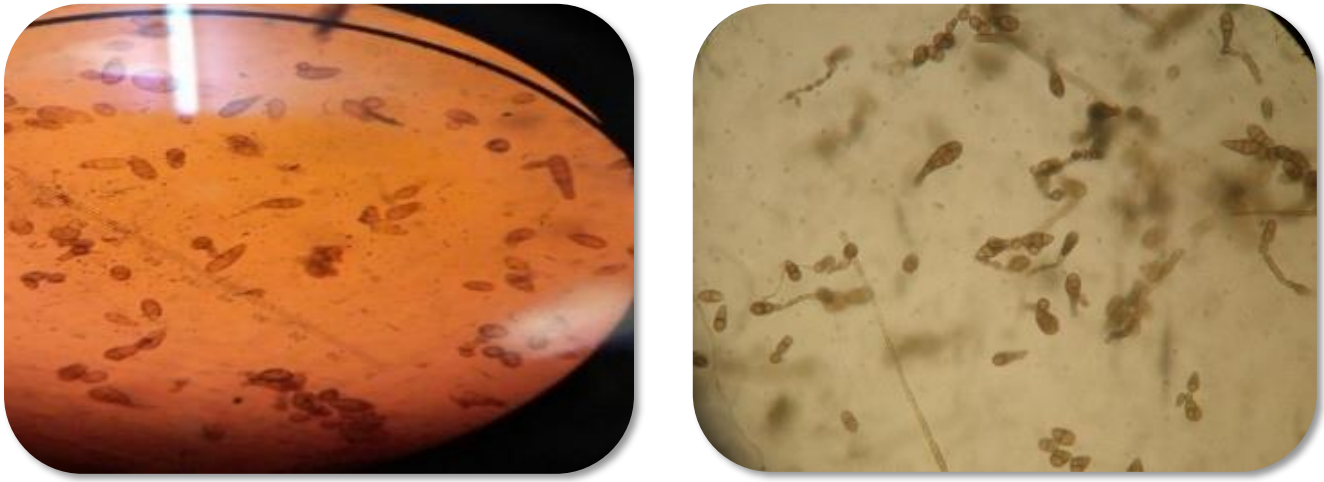
Morphological identification: Colony Characters: Many of the macro characters of the fungus under observation were studied. On PDA medium, it appeared as an evenly dispersed blackish grey to olive green colony that covered the entire medium plate. On the opposite side, its edges were smooth and blackish, while on MEA medium, they were brownish-black.



A B

Figure 1. Growth of isolated fungal culture (A) on the front side (B) reverse side

Micro Characters: Its conidia were maniform and ranged in color from light brown to hyaline. Conidia had a length range of 30.99 m to 42.47 m and a breadth range of 11.90 m to 17.37 m. It has septate hyphae. Each conidium has an average of 5 septations. Microspores have two septations, while macrospores have four to five. The fungus culture was identified as *Alternaria barassicicola* based on its shape.



A B

Figure 2. A and B showed the spores of *Alternaria brassicicola* under 10X and 40X microscope respectively

Molecular identification: Beta tubulin (Bt-a) and the internal transcribed spacer region were chosen as the primers for the polymerase chain reaction (PCR), which was used to amplify the isolate's genomic DNA (ITS). A Gel Documentation System was used to electrophorese, photograph, and evaluate the acquired PCR results. Images of the ITS and Bt-a PCR product amplification bands, are shown in figure 3.

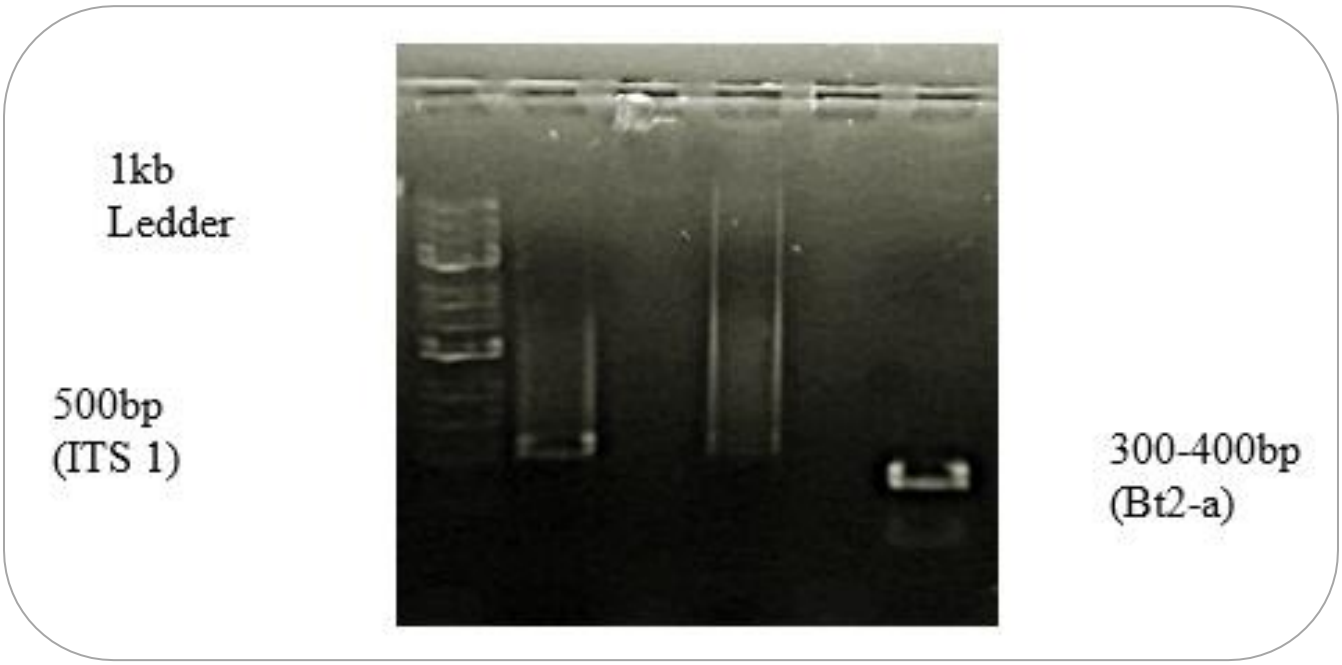


Figure 3. polymerase chain reaction (PCR) results of amplified ITS and Beta tubulin region

The PCR products were purified and forwarded for sequencing. Using the Basic Local Alignment Search Tool (BLAST), the sequences were compared to sequences from NCBI's GenBank. The results of a BLAST comparison revealed that the fungal isolates' similarity to known sequences in the NCBI database was 100%. The fungus was given the name *Alternaria brassicicola* as a result of the closest BLAST search. The submitted ITS primer product sequence of *A. brassicicola* was received from GenBank with the accession number MW415928.

Figure 4. ITS sequence alignment of MW415928 with the *Alternaria alternata* (MN615420.1) strain YZU 191238.

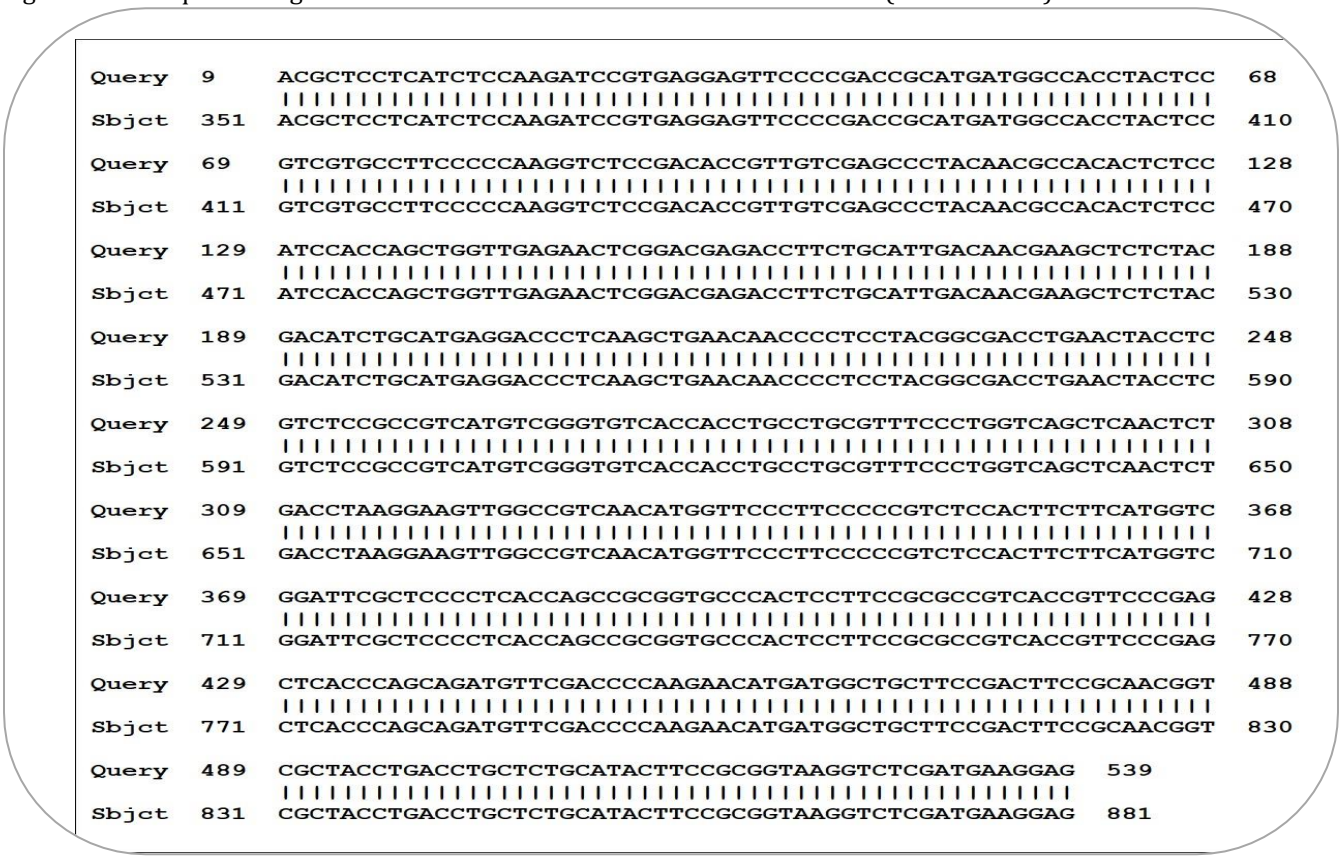


Figure 5. Beta-tubulin sequence alignment of (awaited Accession number sequence) with the *Alternaria brassicicola* (Y17084.1) isolate ICMP 1120-77.

Use of plant extracts for management: The effects of six weed flora's aqueous solutions on *Alternaria brassicicola* growth were assessed in the current study.

Plant extracts' effect on *Alternaria brassicicola* growth *:* The effects of the weeds' aqueous solutions were evaluated in the sample preparation on the growth of *A. brassicicola*. The effects of total eight concentrations on the development of *A. brassicicola* included (15.63, 31.25, 62.5, 125, 250, 500ppm) and 0ppm as a control. The effectiveness of Chenopodium album concentrations in preventing *A. brassicicola* growth was examined. Control displayed *A. brassicicola*'s maximum growth of 75.67 mm in each of these concentrations. *A. brassicicola'*s growth gradually slowed down as extract concentrations increased. However, the fungal growth was decreased to 13.0, 12.33, and 0.00 mm at concentrations of 62.5, 500, and 1000 ppm., respectively.

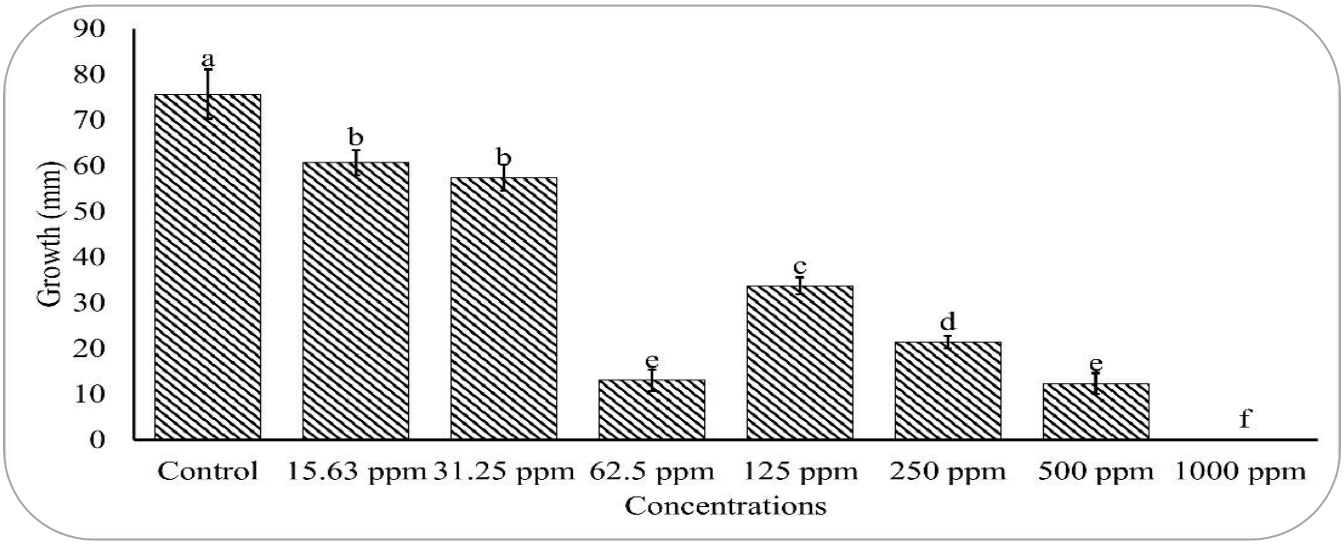


Figure 6. Effect of various *Parthenium hysterophorus* concentrations on *Alternaria brassicicola* growth. Vertical bars show standard errors of means of three replicates.

Significant results were obtained with the *Parthenium hysterophorus* concentrations tested against the growth of *A. brassicicola*. *A. brassicicola* grew to its maximum extent in the control at 75.67 mm, whereas concentrations of 62.5 ppm, 500 ppm, and 1000 ppm significantly inhibited fungal growth to 28.67 mm, 24.67 mm, and 13.67 mm. *A. brassicicola* growth gradually slowed down as extract concentrations raised.

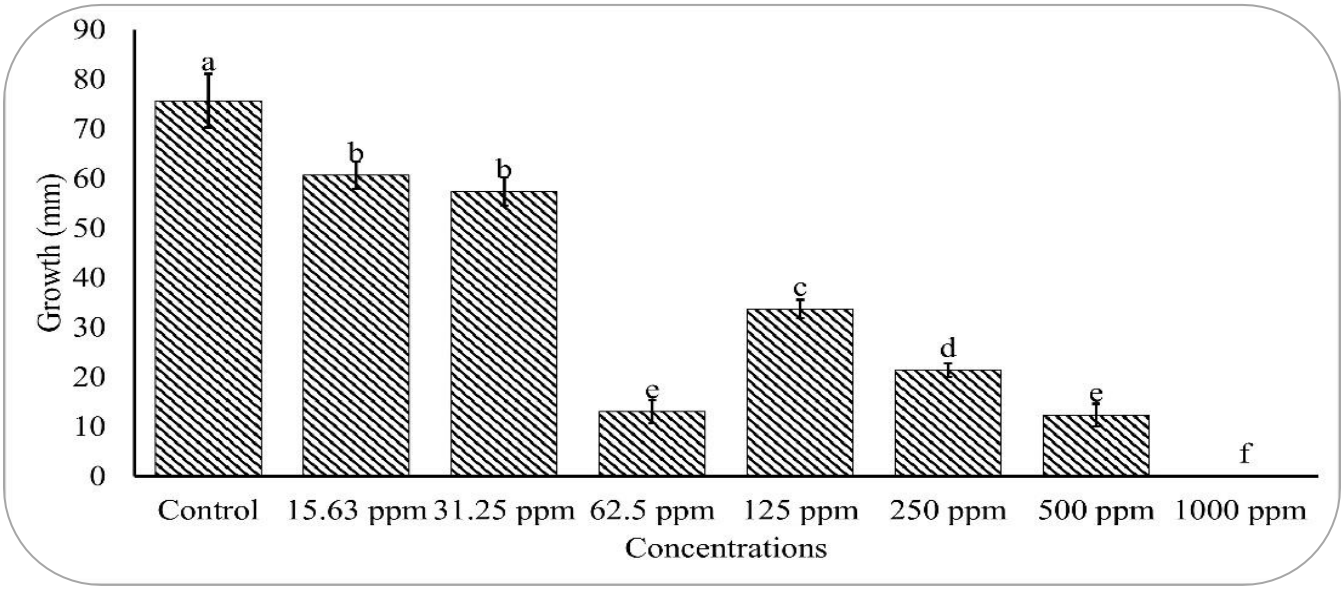


Figure 7. *Alternaria brassicicola* growth in response to various *Parthenium hysterophorus* concentrations*.* The vertical bar illustrates the standard deviation of means.

Trianthema portulacastrum concentrations were evaluated against *A. brassicicola*, and statistical analysis revealed substantial results regarding *A. brassicicola* growth. 75.67 mm was the highest growth in the control, while 39.33 mm, 37.00 mm, and 23.33 mm were the drastically reduced growth rates in the 62.5 ppm, 500 ppm, and 1000 ppm concentrations, respectively.

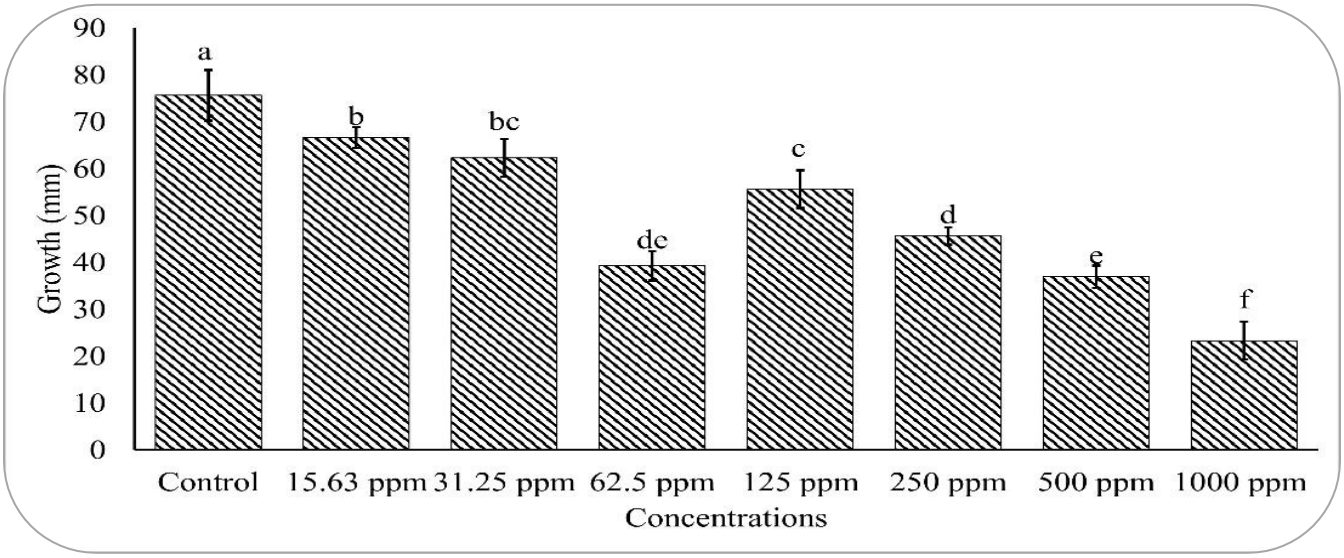


Figure 8. Effect of various *Trianthema portulacastrum* concentrations on *Alternaria brassicicola* growth. The vertical bar illustrates the standard deviation of means.

Results from the assessment of *Malvestrum coromandelianum* concentrations on the development of *A. brassicicola* were highly significant. *A. brassicicola* had a maximum growth rate of 75.67 mm in the control, whereas it considerably decreased at 500 ppm and 1000 ppm concentrations to 35.33 mm and 26.33 mm, respectively. *A. brassicicola* growth gradually slowed down as extract concentrations increased.

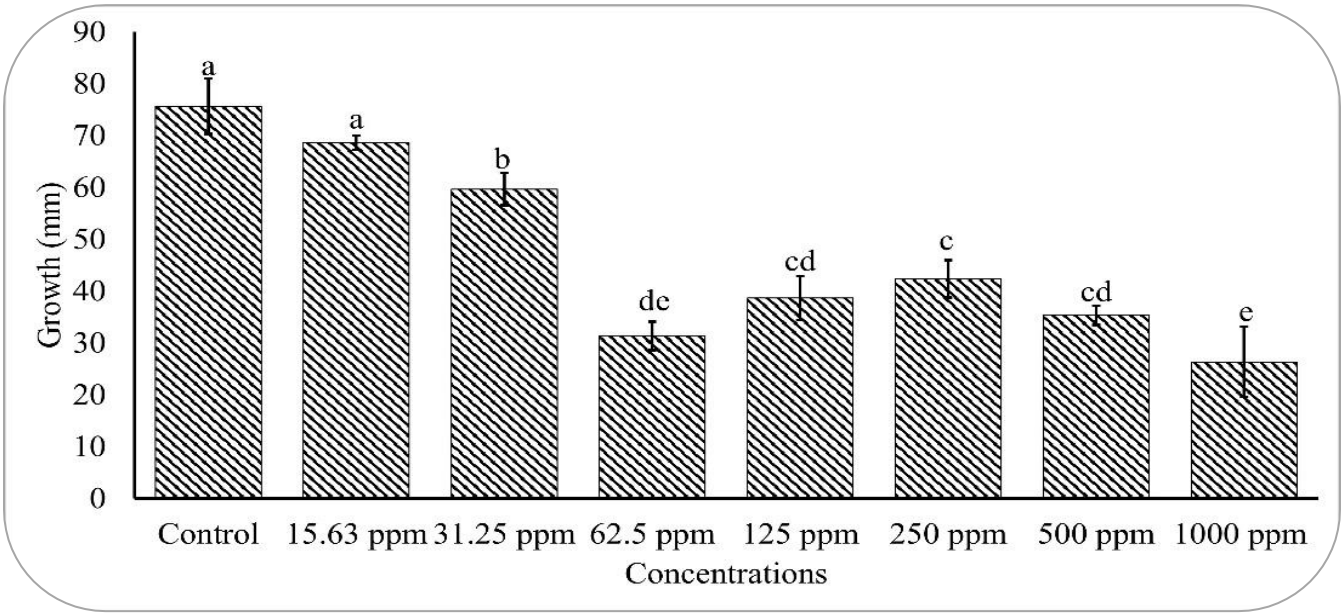


Figure 9. *Malvestrum coromandelianum's* effect on *Alternaria brassicicola* growth at various concentrations. The vertical bar illustrates the standard deviation of means.

The results showed statistical significance when *Solanum nigrum* concentrations were tested against the growth of *A. brassicicola*. *A. brassicicola* had a maximum growth rate of 75.67 mm in the control, however it significantly decreased by 29.67 mm, 15.33 mm, and 0.00 mm at concentrations of 250 ppm, 500 ppm, and 1000 ppm. *A. brassicicola* growth gradually slowed down as extract concentrations increased.

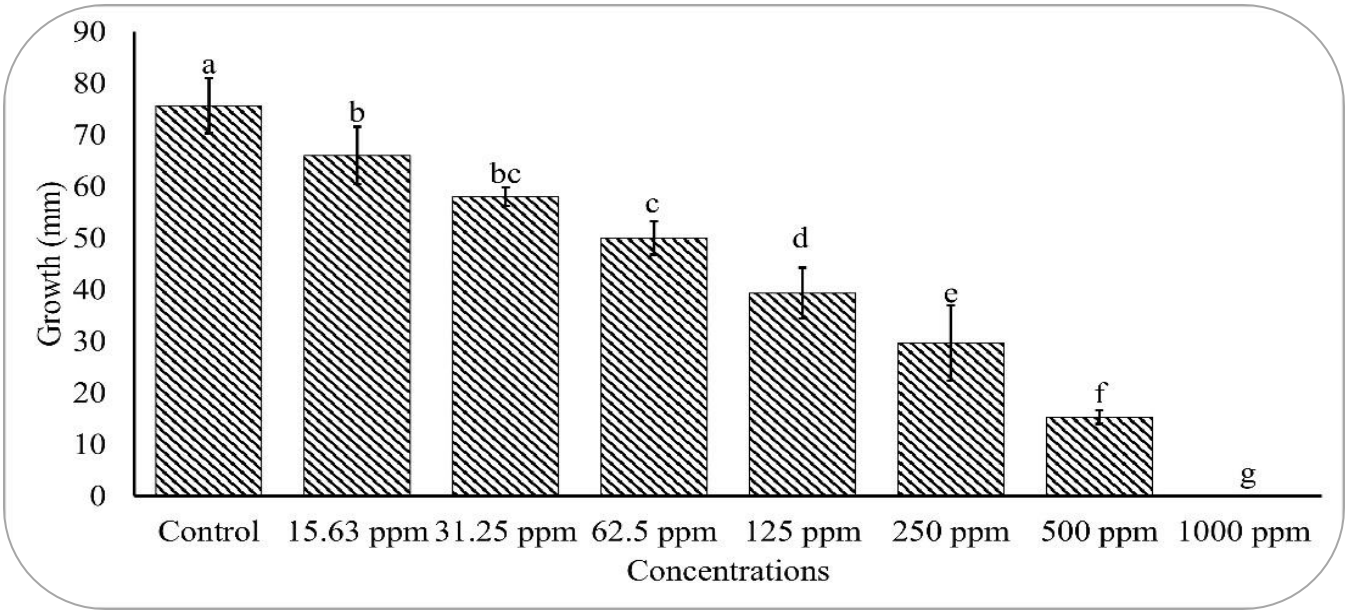


Figure 10. Effect of different concentrations of *Solanum nigrum* on the growth of *Alternaria brassicicola.* The vertical bar illustrates the standard deviation of means.

Significant results were obtained when the concentrations of *Nicotiana plumbaginifolia* were tested against the growth of *A. brassicicola*. *A. brassicicola* had a maximum growth of 75.67 mm in the control, however it considerably shrank at concentrations of 250, 500, and 1000 ppm by 24.00, 18.33, and 00.00 mm, respectively. *A. brassicicola* growth gradually slowed down as extract concentrations increased.

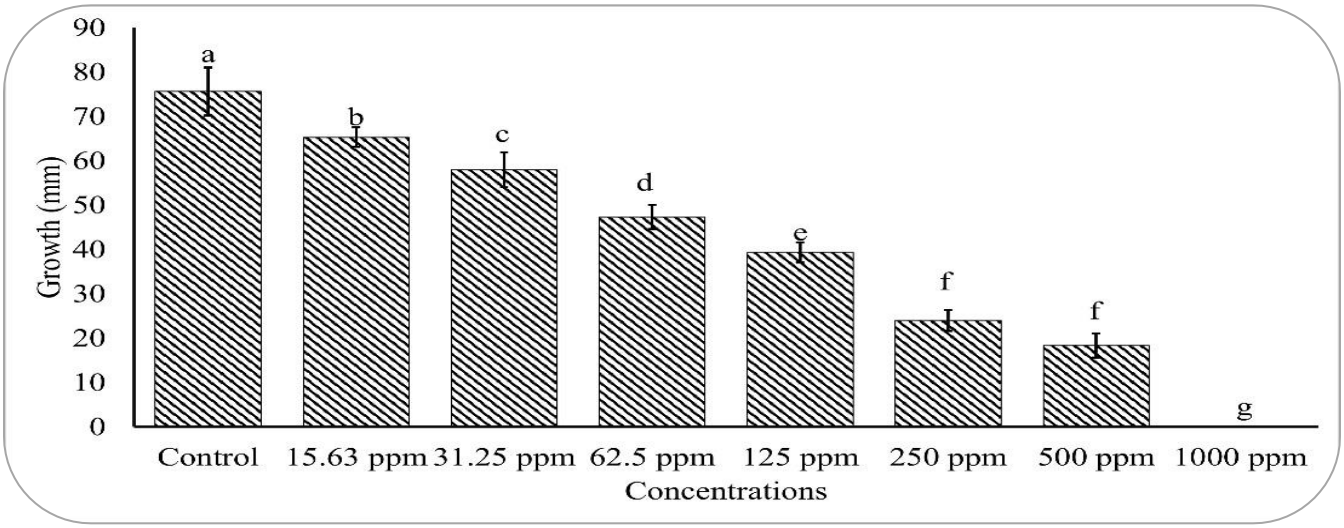


Figure 11. Effect of different concentrations of *Nicotiana plumbaginifolia* on the growth of *Alternaria brassicicola.* The vertical bar illustrates the standard deviation of means.

Effect of plant extracts on *Alternaria brassicicola* percentage inhibition*:* In this work, the percentage inhibition of *A. brassicicola* in the aqueous solutions of several weeds, including *Chenopodium album, Parthenium hysterophorus, Trianthema portulacastrum L., Malvestrum coromandelianum, Solanum nigrum*, and *Nicotiana plumbaginifolia* were assessed. Different concentrations of each weed extract were applied separately.

Chenopodium album concentrations were employed to combat the percentage inhibition (PI) of *A. brassicicola*. The findings of the statistical study on the PI of *A. brassicicola* concentrations of C. album were highly significant. The concentration of 62.5 ppm after 1000 ppm and 500 ppm produced positive findings on PI, i.e., 100, 83.46, and 82.79%, respectively. The results from the other concentrations were not as favorable as those from these concentrations, where PI increases as extract dose is increased.

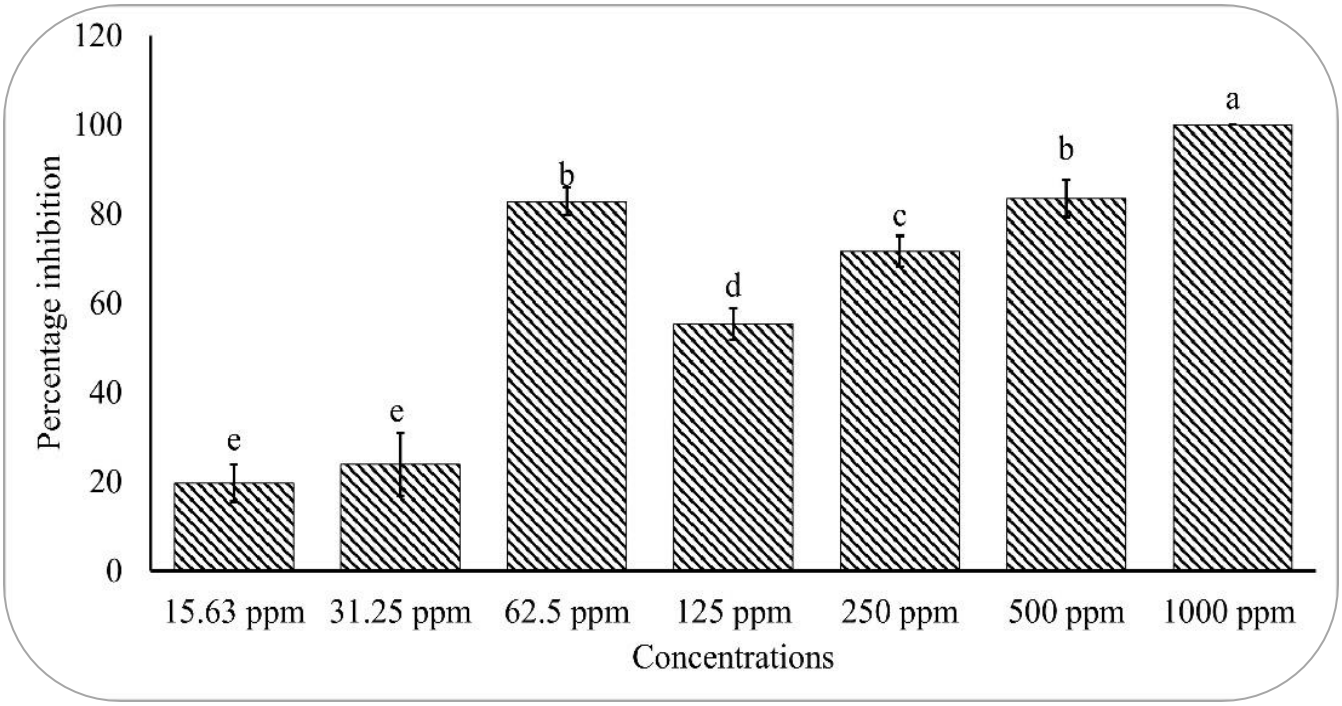


Figure 12. Effect of different concentrations of *Chenopodium album* on the percentage inhibition (PI) of *Alternaria brassicicola.* The vertical bar illustrates the standard deviation of means.

Chenopodium album concentrations were utilized to fight the percentage inhibition (PI) of *A. brassicicola*. The findings of the statistical study on the PI of *A. brassicicola* concentrations of *C. album* were highly significant. The concentration of 62.5 ppm after 1000 ppm and 500 ppm produced positive findings on PI, i.e., 100, 83.46, and 82.79%, respectively. The results from the other concentrations were not as favorable as those from these concentrations, where PI rises as extract dose is increased.

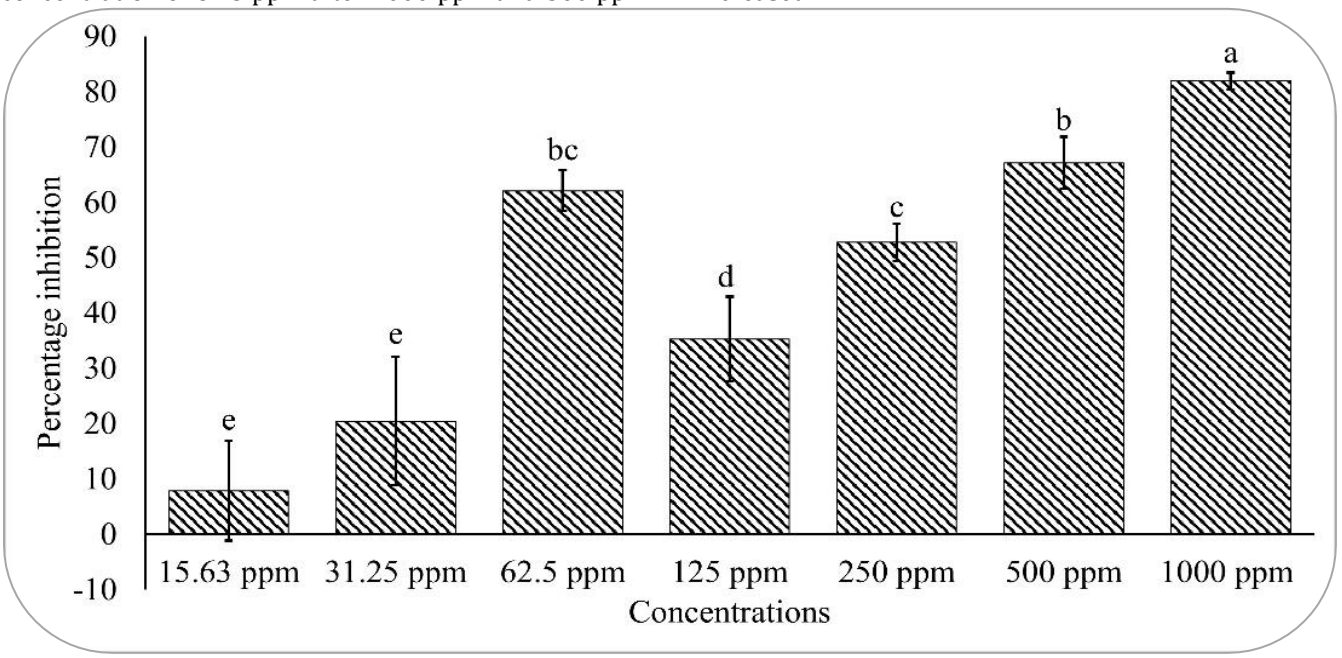


Figure 13. Effect of different concentrations of *Parthenium hysterophorus* on the percentage inhibition (PI) of *Alternaria brassicicola.* The vertical bar illustrates the standard deviation of means.

The percentage inhibition (PI) of *A. brassicicola* was examined using different concentrations of *Trianthema portulacastrum*. Significant results on PI for concentrations of 62.5 ppm, 500 ppm, and 1000 ppm were obtained at 47.68, 51.16, and 68.87%, respectively. *A. brassicicola's* PI increases as PI extract concentrations were raised.

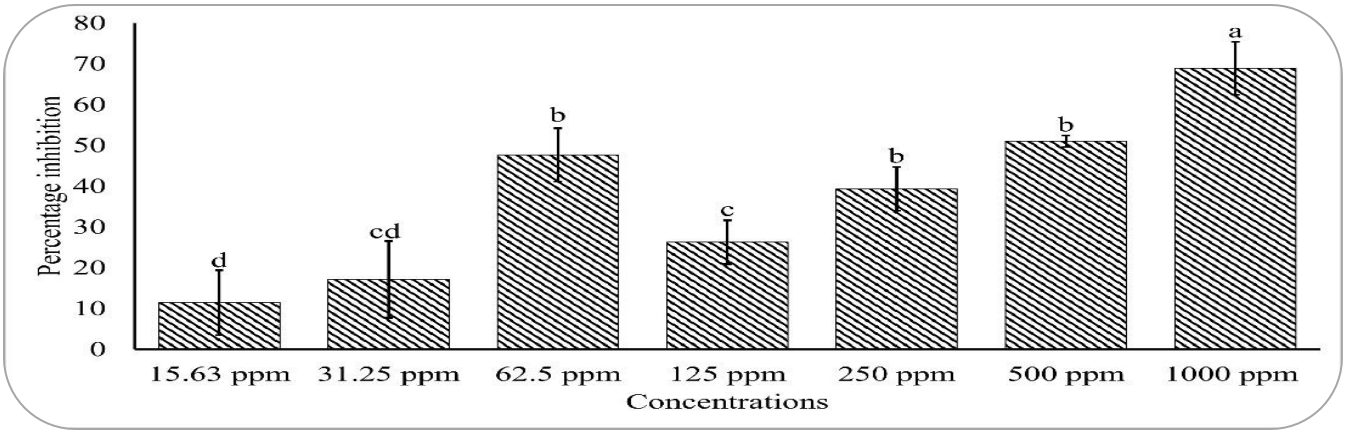


Figure 14. Effect of different concentrations of *Trianthema portulacastrum* on the percentage inhibition (PI) of *Alternaria brassicicola.* The vertical bar illustrates the standard deviation of means.

To calculate the percentage of inhibition, *Malvestrum coromendelianum* concentrations were tested against *A. brassicicola* (PI). At concentrations of 62.5 ppm, 500 ppm, and 1000 ppm, or 58.55, 53.21, and 65.55%, respectively, the highest PI was found. As plant extract concentrations were increased, the PI of *A. brassicicola* increased.

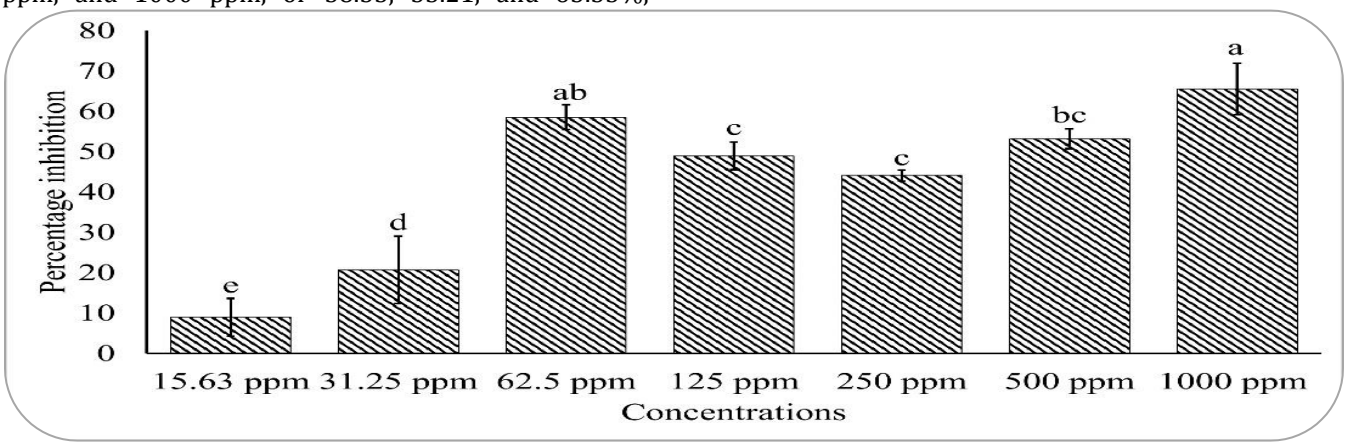


Figure 15. Effect of different concentrations of *Malvestrum coromendelianum* on the percentage inhibition (PI) of *Alternaria brassicicola.* The vertical bar illustrates the standard deviation of means.

The percentage inhibition (PI) of *A. brassicicola* was investigated using different amounts of *Solanum nigrum*. Results of the statistical analysis on PI were quite significant. The PI of *A. brassicicola* increased as *S. nigrum* concentration increased. At concentrations of 500 ppm and 1000 ppm, or 79.69 and 100% of *A. brassicicola*, respectively, the greatest PI was obtained.

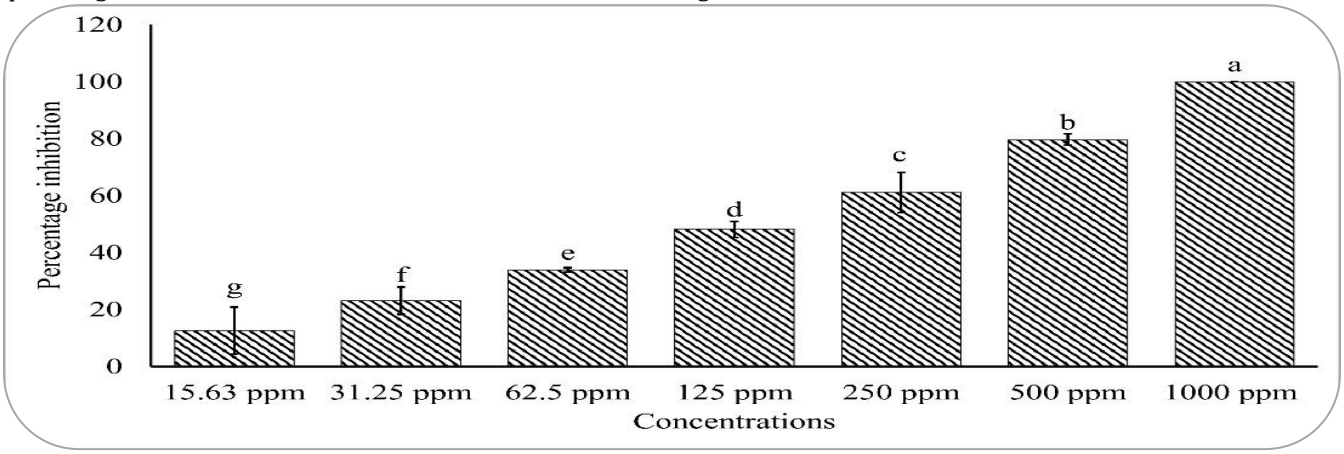


Figure 16. Effect of different concentrations of *Solanum nigrum* on the percentage inhibition (PI) of *Alternaria brassicicola.* The vertical bar illustrates the standard deviation of means.

The growth of *A. brassicicola* was evaluated using concentrations of *Nicotiana plumbaginifolia*, and the results were substantial. At concentrations of 500 and 1000 ppm, or 75.87 and 100% of *A. brassicicola*, respectively, the greatest PI was attained.

In the present study, we tested the effectiveness of six different weed extracts against the turnip blight pathogen *Alternaria brassicicola*. When test fungi are exposed to quantities of Chenopodium album, *A. brassicicola* is inhibited while growth of the test fungi is reciprocally stimulated. Reported that the C. album leaf extract had the strongest antifungal effects against. Also mentioned is how powerful C. album is in combating *Aschochyta rabiei*. Demonstrates that different fungi were unable to grow when C. album leaf extracts were used. such as *Rhizoctonia solani, Pythium aphanidermatum, Sclerotinia sclerotium,* and *Fusarium solani.*

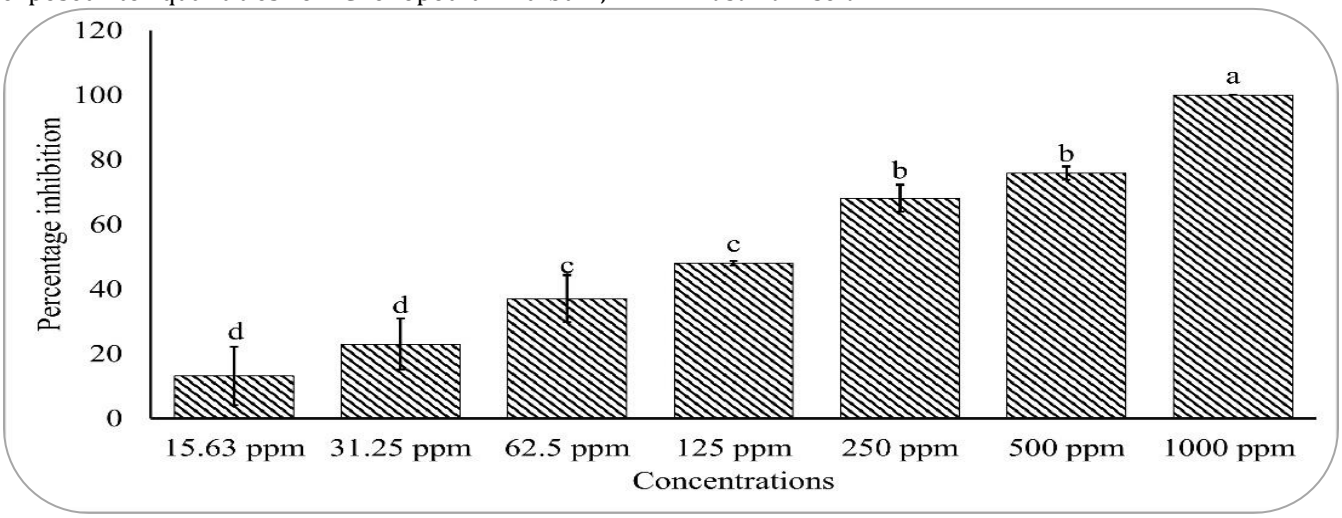


Figure 17. Effect of different concentrations of *Nicotiana plumbaginifolia* on the percentage inhibition (PI) of *Alternaria brassicicola.* The vertical bar illustrates the standard deviation of means.

In this study, the *Parthenium hysterophorus* has a considerable impact on *A. brassicicola's* growth and percentage inhibition. The percentage inhibition against *Alternaria brassicae* by *P. hysterophorus* leaf extract was significantly reduced in 2016, according to Aqeel and Yaseen. Pal *et al.* (2013) and Tapwal *et al.* (2011) it has been reported that *P. hysterophorus* leaf extracts can effectively prevent the Alternaria Spp.

In the current study, *Trianthema portulacastrum* shows excellent results against the percentage inhibition. Abd El-Gawad *et al.* (2016) described the ability of *T. portulacastrum* to combat a variety of fungal diseases, including *Fusarium moniliforme*, *F. oxysporum*, and *F. solani.*

When exposed to various concentrations of these weeds in our studies, the percentage inhibition was significantly impacted by the leaf extracts of *Nicotiana plumbaginifolia*, *Solanum nigrum*, and *Malvestrum coromendelianum*. Mushtaq *et al.* (2012) report that the antibacterial capabilities of the extracts of *Malvastrum coromandelianum*, *Amaranthus viridis*, and *Lantana camara* are good against the seed-borne fungus i.e., *Drechslera biseptata*, *Alternaria alternata*, *Aspergillus niger*, and *Fusarium solani*. These extracts were particularly successful at inhibiting the radial growth of seed-borne fungus. the soilborne pathogens *Rhizoctonia solani*, *Rhizoctonia oryzae*, *Fusarium fujikuroi*, *Fusarium oxysporum*, *Pythium ultimum*, and *Pyricularia oryzae* were tested against four weed extracts, *Melilotus indicus*, *Melilotus alba*, *Medicago parviflora*, and *Solanum nigrum*. Khan (2018) evaluated the effectiveness of these weed extracts as antifungals. They evaluated the minimal fungicidal and inhibitory concentrations of various weed extracts. According to Pushpavathi *et al.* (2017), *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Curvularia* sp.*, Alternaria* sp.*,* and *Fusarium* sp. are just a few of the soilborne fungal pathogens that the methanolic extract of *Nicotiana plumbaginifolia* demonstrated remarkable antifungal activity against.

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Muhammad B. Chattha	:	Design and supervised the experiment
Muhammad B. Razzaq	:	Conduct and perform the experiment
Shazia Shafique	:	Reviewed the manuscript
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Hafiza H. E. Peerzada	:	Prepared figures and help in doing experiments