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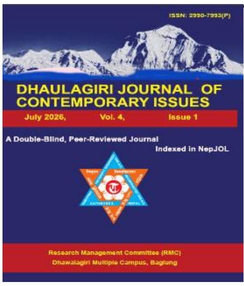

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Isolation of Seed-Borne Fungi of Wheat and Their Control Using Aqueous Plant Extract of *Ageratum houstonianum* Mill

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Abstract

Seed-borne fungi are important constraints in wheat production and storage because they reduce seed quality, germination, and crop productivity. Plant-based extracts are increasingly considered eco-friendly alternatives to synthetic fungicides for managing seed-borne fungal pathogens. Seeds of *Triticum aestivum* L. were collected from three households in Kathekhola Rural Municipality-8, Gandaki Province, Nepal, and were tested for seed-borne fungi using the standard blotter technique. A total of 25 wheat seeds were examined in this laboratory-based study**.** On testing, five different fungi, namely *Aspergillus niger*, *Fusarium oxysporum*, *Aspergillus flavus*, *Alternaria* sp., and *Alternaria alternata*, were isolated, among which *Aspergillus niger* showed the highest incidence. However, *Alternaria alternata* and *Fusarium oxysporum* were selected for in vitro inhibition tests. The aqueous leaf extract of *Ageratum houstonianum* Mill. was prepared for mycelial inhibition against *Alternaria alternata* and *Fusarium oxysporum* at concentrations of 25%, 50%, and 100%. *Fusarium oxysporum* was completely inhibited by *A. houstonianum* at 100% crude extract, while *Alternaria alternata* was inhibited by 88.09% at the same concentration. Inhibition gradually increased from lower to higher concentrations. The extract of *A. houstonianum* was more effective against *Fusarium oxysporum* than against *Alternaria alternata*. The findings of this study suggest that *A. houstonianum* leaf extract may serve as a potential eco-friendly alternative to synthetic fungicides for managing selected seed-borne fungi in wheat. However, further studies with larger seed samples, molecular identification of fungi, and field trials are needed to validate its practical application**.**

Keywords: Ageratum, Alternaria, Extract, Inhibition, Innoculation, Mycelium, PdA.

Introduction

Worldwide, wheat is one of the most important cereal crops, which serves as a staple food for billions of people. Providing about 20% of global dietary calories and protein, wheat has been considered central to food security strategies (Gupta et al., 2007). Production-wise, after rice and maize, wheat is the third major cereal crop grown in 707,505 hectares and produces 2.18 million metric tons annually (Subedi, 2019). Due to poor input use, pest and disease pressure, and inadequate irrigation, the production of wheat remains low (Bist et al., 2021). Despite of its importance, seed- borne fungi represent a major challenge in wheat cultivation and storage. Common seed-borne fungi include *Alternaria*, *Fusarium*, and *Penicillium,* which contaminate seeds during storage under humid and warm conditions. Seed- borne fungi are a global concern affecting cereals such as wheat, maize and rice. More than 150 species of fungi have been reported on cereal grains, with *Aspergillus, Fusarium, Alternaria,* and *Penicillium*. These fungi reduce germination rate, nutritional value and yield also produces harmful mycotoxins that threaten human and animal health (Christensen & Kaufmann, 1965).

According to Adhikari et al., 2015, fungi like *Alternaria alternata* and *Bipolaris sorokiniana* contaminate the wheat seeds during storage.

Synthetic fungicides are widely used to control these pathogens but they pose environmental and health risk due to its toxicity, persistence and pollution (Baka, 2014; Burt, 2004). As a result, researchers have increasingly explored eco-friendly alternatives such as plant extracts. Invasive species like *Ageratum houstonianum* contain bioactive compounds (alkaloids, flavonoids, tannins, saponins) with antifungal properties (Swami & Alane, 2013). Using these plants for biocontrol could reduce the dependence on synthetic chemicals and also divert the negative effects of invasive plants towards the concept of biocontrol.

This study investigates the antifungal efficacy of *A. houstonianum* leaf extracts against seed-borne fungi in wheat stored in Baglung, Nepal. By isolating fungi and testing plant extracts, the research aims to identify sustainable strategies for managing storage-related seed contamination. Plant-derived bioactive compounds offer a promising avenue for sustainable disease management, as Ageratum houstonianum is an invasive plant species that is easily and locally available. As its potential as an aqueous extract for controlling wheat seed-borne fungi remains underexplored, addressing this gap could provide an eco- friendly solution that not only manages fungal pathogens but also utilizes an invasive plant for beneficial agricultural purposes. Devkota and Sahu (2020) found that aqueous and methanol extracts of *A. houstonianum* reduced mycelial growth of *Fusarium* and *Alternaria*. Similarly, Chhetri and Jha (2016) demonstrated that A. houstonianum extracts controlled fungi in lentil seeds.

Invasive plants like *A. houstonianum* pose ecological challenges but may also offer opportunities for biocontrol. By utilizing their bioactive compounds, researchers can turn ecological problems into agricultural solutions. This dual role highlights the importance of integrating invasive species management with sustainable agriculture.

The result of this work is a completely laboratory based and lacks the real application in the field if this test can be done on the field against seed- borne fungal disease which has been significantly threatening wheat productivity by reducing the seed germination and crop yield, it could play a vital role in food security and rural livelihood.

Materials and Methods

Study area

The study was conducted in Baglung district, Gandaki Province, Nepal. Baglung is known as “Mini-Nepal” due to its diverse geography and climate.

Collection of seeds

Seeds were collected and mixed from the storage conditions of three houses from the village of Kathekhola-8, Baglung, Gandaki province, Nepal. The mixing was done to increase the chance of fungal isolation as all the houses' storage conditions were not the same.

Plant collection

Disease-free and mature leaves of Ageratum houstonianum were collected from Odharchaur of Baglung district, Gandaki province, Nepal, in March and April 2023. The leaves were washed with tap water, followed by sterile water, and shade-dried until they were dry for grinding to prepare powder.

Fungi isolation

Seed- borne fungi were isolated using the standard blotter technique described by De Tempe (1953). Altogether, 25 wheat seeds were examined for fungal isolation. The seeds were at first surface sterilized using tap water three times, followed by surface sterilization with 70% ethanol for 20-30 seconds. Then the seeds were again subjected to sterile or distilled water to remove the traces of the sterilizing agent. The sterilized seeds were then inoculated on moist sterile blotter papers, and the Petri dishes were incubated at 20°C for seven days at a constant temperature, typically at 22 ± 2°C. The number of seeds kept on blotter paper inside Petri dishes was five. After 7 days, fungal colonies developed from the seeds were observed based on colony color, growth pattern, sporulation, and microscopic characters. The isolated fungi were transferred to Potato Dextrose Agar (PDA) for further microscopic observation. The fungi were then preserved in slant for future use and study.

Fungi identification

Fungi were identified morphologically using monographs and literature. The isolated fungi were maintained in PDA culture slants and were identified with the help of books, monographs and using Ocular micrometer. Leaves of *A. houstonianum* were shade-dried, ground, and extracted using distilled water (Chhetri & Jha, 2016). Extract concentrations of 25%, 50%, and 100% were prepared by serial dilution.

Two fungi *Fusarium oxysporum* and *Alternaria alternata* were selected on the basis of their dominancy during isolation and for the control using poison food technique (Grover & Moore, 1962). After incubation using the poison food technique for around seven days, the average diameter of fungal colonies and percentage inhibition of mycelial growth were calculated using the formula given by (Rao & Shrivastava, 1994).

Table 1

Aqueous Extract Preparation of Leaf of Ageratum houstonianum

Concentration%	PDA (ml)	Extract (ml)	Total
100	15	5	20
50	15	5(2.5 E+2.5DW)	20
25	15	5(1.25 E+3.75DW)	20
Control	15	0	15

Mycelial growth inhibition (%) = $(G_C – G_T / G_C) \times 100\%$

Where,

G_C = Growth of mycelial colony in the control set

G_T = Growth of mycelial colony in the treatment set

Results and Discussion

Fungi Isolation

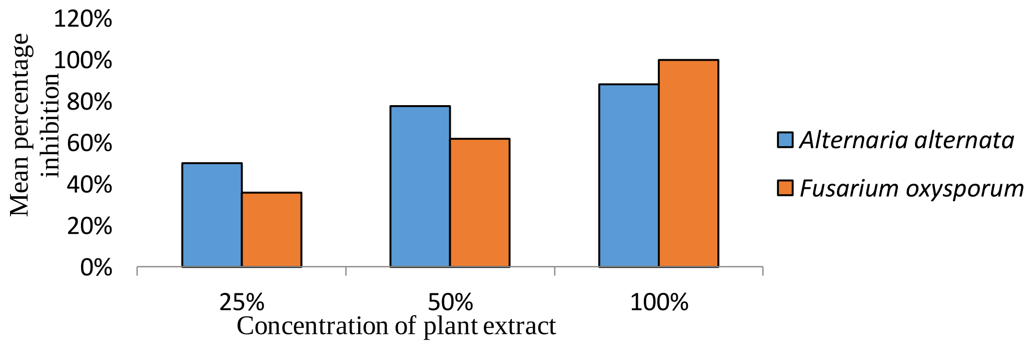
From 25 wheat seeds collected from storage in Kathekhola-8, Baglung, five fungi were isolated using the Standard Blotter Technique (De Tempe, 1953). *Aspergillus niger*, *Fusarium oxysporum*, *Aspergillus flavus*, *Alternaria sp.*, and *Alternaria alternata*. Among these, *A. niger* was the most prevalent, indicating its dominance in stored wheat seeds under local conditions.

Colonial Morphology of Isolated Fungi

The colony of *Aspergillus niger* was initially white, turning to black sporulation; the Fusarium oxysporum colony was initially pinkish white from above but had a yellowish underside. Simillarly *Alternaria alternata* has blackish colonies with cracked surfaces underneath and *Aspergillus flavus* showed yellowish- green colonies on the upper surface with reddish gold below.

Figure 1

Antifungal activity (Mean percentage inhibition) of Ageratum houstonianum extract.



Two fungi, *F. oxysporum* and *A. alternata*, were selected for antifungal assay. The aqueous extract of *A. houstonianum* showed clear inhibitory effect. The extract was more effective against *A. alternata* at lower concentrations, while *F. oxysporum* required full crude extract for complete inhibition.

Mean percentage inhibition of Alternaria alternata

At 25% of *Ageratum houstonianum*, the mean percentage inhibition of the fungus was 50, and at 50%, the inhibition was 77.78%. While at 100% the extract inhibited the fungi by 88.09.

Mean percentage inhibition of Fusarium oxysporum

At 25% of *Ageratum houstonianum*, the mean percentage inhibition of the fungus was 36, at 50%, the inhibition was 62%, but at 100%, the fungus was completely inhibited.

Discussion

The blotter method proved effective for fungal isolation, supporting previous studies that identified it as the most reliable technique for seed-borne fungi detection (Agrawal et al., 1971). The inhibition shown by *Ageratum haustonianum* clearly shows the strong antifungal properties, which were similar to earlier studies (Devkota & Sahu, 2020; Khaled, 2012). The difference in inhibition observed between *A. alternata* and *F. oxysporum* highlights the importance of pathogen-specific responses to plant- derived compounds. This finding supports the potential of invasive plants that are considered problems and threats as eco- friendly biocontrol agents, offering a very good alternative to the commercial fungicides that pose risks to health and the environment. Aqueous extract of *Ageratum haustonianum* has shown strong antifungal activity with complete inhibition of *F. oxysporum* and *A. alternata* at 100 % crude extraction.

This result shows a similar result to that of Chhetri and Jha (2014), who found *A. houstonianum* effective against *Alternaria alternata* and *Fusarium oxysporum*. According to them, at 25% extract, A. alternata was inhibited by 52.92%; at 50% concentration, the inhibition was 72.92%; and at 100%, the inhibition was 87.92%. But at 25 %, 50%, and 100% concentrations, the Fusarium oxysporum was inhibited by 25.82%, 45.37%, and 77.13%, respectively, in lentil seeds, and Kumar (2014 also reported the fungicidal properties of the plant's oil against other pathogens. The antifungal potential is attributed to phytochemicals such as alkaloids, flavonoids, and tannins (Chandrakar et al., 2020). These chemicals are actually the factors for antifungal assay, and perhaps this research is seeking the phytochemical analysis of the aqueous extract of *A. houstoninanum*.

Overall, the finding confirms that *A. houstoninaum* can serve as an eco-friendly biocontrol agent against seed- borne fungi in wheat, offering a sustainable alternative to synthetic fungicides. However, further molecular identification, field trials, and comparisons with commercial fungicides are needed to validate its practical application.

Limitation

Limitations of this study include the dependence on morphological identification and a lack of molecular confirmation regarding the identification of fungi, as well as a lack of field trials for control. Further research should include the identification on a molecular basis, compare the inhibition between plant extract and commercial fungicides, and conduct field experiments to validate laboratory findings. The aqueous extract was not tested for field trials.

Conclusion

Ageratum houstonianum leaf extracts effectively inhibited seed- borne fungi in stored wheat seeds, suggesting its potential as a biocontrol agent. Future research should include molecular identification of fungi, comparison with commercial fungicides, and field trials to validate the laboratory findings.

Ethical approval for the research: Not applicable

Consent for publication: Not applicable

Conflict of interest: The author does not have any conflict of interest with any institutions concerning this research

Ethical conduct of research: This paper is written ethically

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