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Quantitative analysis of phenolic, tannin and flavonoid content and *in vitro* antifungal activity of wild plant extracts against soil-borne phytopathogenic fungi affecting broccoli

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ABSTRACT

The present study investigates the phytochemical profiles and antifungal activity of six wild plant species: *Solanum nigrum* L., *Martynia lutea* Lindl, *Argyreia speciosa* (L.f.) Sweet, *Barleria cristata* L., *Acalypha wilkesiana* Müll and *Vitex trifolia* L. The leaves were extracted with ethanol and evaluated for bioactive components, such as phenolics, tannins, alkaloids, flavonoids, and saponins, using qualitative techniques. The total phenolic content (TPC), tannin content (TC), and total flavonoid content (TFC) were quantified using spectrophotometric methods. The antifungal activity of plant extract has been evaluated against soil-borne pathogens, *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium oxysporum* f. sp. *conglutinans*, and *Sclerotinia sclerotiorum* using the poisoned food technique at doses ranging from 5% to 20%. *Argyreia speciosa* demonstrated a significant inhibition of mycelial growth (20%), followed by *Acalypha wilkesiana* and *Vitex trifolia*. The synthetic fungicide Bavistin, employed as a control, outperformed the plant extracts. The plant extracts were investigated for their effect on fungal spore germination and enzyme activity, including α-amylase and protease, which are essential to fungal pathogenicity. Treatments with *A. speciosa* and *A. wilkesiana* revealed significant suppression of spore germination and enzyme activity, indicating that they have the potential to be effective as fungal biocontrol agents. The findings of this study reveal that the antifungal activity of various plants is affected by their distinct phytochemical profiles, notably their phenolic and flavonoid content. It implies that the plants might be used in sustainable agriculture techniques to control soil-borne plant diseases.

KEYWORDS: Antifungal Activity, Phytochemicals, Wild Plants, Soil-Borne Pathogens, Enzyme Inhibition, Sustainable Agriculture

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INTRODUCTION

Sustainable agriculture practices have become a global imperative due to the increasing demand for food production and the need to mitigate the environmental impact of conventional agricultural methods (Rockström *et al.*, 2017). Through the incorporation of Integrated Disease Management strategies, horticultural crop health and productivity can be significantly enhanced, as they emphasise the use of biological control methods that reduce reliance on harmful chemical fungicides, thereby promoting both crop resilience and environmental sustainability (Vinogradova *et al.*, 2023).

One key aspect of IDM is the identification and utilization of natural plant-derived compounds with potent antifungal activity against soil-borne phytopathogens (De Senna & Lathrop, 2017). Previous research has demonstrated the effectiveness of various phenolic compounds and tannins extracted from medicinal and aromatic plants, which have shown promise in inhibiting the growth of several phytopathogenic fungi, including *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium oxysporum f. sp. conglutinans*, and *Sclerotinia sclerotiorum* (Javaid & Shoaib, 2012; Gade *et al.*, 2020; Naghman *et al.*, 2023). The efficacy of these plant-derived compounds can be attributed to their diverse phytochemical constituents, which exhibit antifungal properties by disrupting

fungal cell membranes and inhibiting key enzymatic activities, thereby providing a compelling argument for their application in sustainable agricultural practices to combat soil-borne fungal diseases (Park *et al.*, 2021; Tian *et al.*, 2022). Furthermore, research has demonstrated that the incorporation of these biological agents not only aids in combating fungal pathogens but also enhances soil health and biodiversity, fostering a more resilient agricultural ecosystem. Leveraging such biological alternatives can significantly reduce the chemical load on the environment, thus addressing concerns related to the toxicity of synthetic fungicides and their long-term impact on human health and ecosystems (Rajput *et al.*, 2020).

Rhizoctonia solani, *Pythium ultimum*, *Fusarium oxysporum f. sp. Conglutinans* and *Sclerotinia sclerotiorum* in particular, are highly problematic soil-borne fungal pathogen that causes wilt, blight, damping off and root rot in a wide range of horticulture plants, including broccoli (Sihag *et al.*, 2022). The lack of curative control methods against this pathogen underscores the urgent need for the development of alternative, eco-friendly strategies to mitigate its impact on crop production (Wong *et al.*, 2024). In this context, plant extracts rich in bioactive compounds have emerged as promising candidates for sustainable disease management, as they not only exhibit antifungal properties but also contribute to soil health and biodiversity preservation in agricultural systems. In addition to these biocontrol agents, the exploration of wild plant extracts presents a valuable opportunity to harness the antifungal properties of naturally occurring compounds, which can serve as a sustainable alternative to synthetic fungicides and align with the broader goals of eco-friendliness. The present study aims to quantify the phenolic and tannin content of several wild plant extracts and evaluate their *in vitro* antifungal efficacy against a panel of soil-borne fungal phytopathogens affecting broccoli, a nutrient-dense and widely cultivated Brassica vegetable.

MATERIALS AND METHODOLOGY

Plant Material Collection

The species selected for this study include *Solanum nigrum* L., *Martynia lutea* Lindl, *Argyreia speciosa* (L.f.) Sweet, *Barleria cristata* L., *Acalypha wilkesiana* Mull. Arg., *Vitex trifolia* L. were collected from different regions of the forest area, Jalna District of Maharashtra, India. The plant materials were taxonomically identified by using the flora of Marathwada (Naik *et al.*, 1998). Leaves were carefully detached, placed in polyethene bags, and transported to the laboratory for immediate processing.

Preparation of Plant Samples and Extraction of Phytochemicals

The collected leaves were washed with tap water, air-dried under shade for one week, and then finely powdered using an electric blender. The powdered samples were stored at 10 °C in airtight brown bottles until further analysis. 50 g of each powdered leaf sample was subjected to extraction with 250 mL of 90% ethanol using a Soxhlet apparatus for 72 hours at 65 °C. The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were stored in clean bottles at 4 °C for further analysis (Adam *et al.*, 2019).

Qualitative Phytochemical Analysis

The phytochemical analysis of each sample extract was tested for the presence of bioactive compounds by following the procedures as described in many reports (Hussain *et al.*, 2011; Yadav & Agarwala, 2011; Shaikh & Patil, 2020).

Test for phenol

To detect phenols, 2 mL of distilled water was added to 1 mL of the extract, followed by a few drops of 10% ferric chloride solution. The appearance of a blue or green colour indicated the presence of phenols.

Test for tannin

To detect the tannin content, 2 mL of 5% ferric chloride solution was added to 1 mL of the extract. A greenish-black or dark blue colour confirmed the presence of tannins.

Test for alkaloids

Crude extract was mixed with 2 mL of 1% HCl and heated gently. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Test for saponins

Crude extract was mixed with 5 mL of distilled water in a test tube, and it was shaken vigorously. The formation of stable foam was taken as an indication of the presence of saponins.

Test for flavonoids (Alkaline reagent test)

Crude extract was mixed with 2 mL of 2% solution of NaOH. An intense yellow colour was formed, which turned colourless in the addition of a few drops of diluted acid, which indicated the presence of flavonoids.

Quantification of Total Phenolics, Tannins and Total Flavonoid Content (TFC)

100 g leaves of each selected plant were separately washed in tap water and air dried under shade for a period of one week. The dried leaf component of each plant was ground into powder using a clean and dry electric blender. Each powdered sample was extracted with 15 mL of acidified ethanol (0.1% HCl) to enhance the extraction of phenolic compounds and prevent oxidation. The extraction process was performed at room temperature in the dark for 12 hours. The resulting infusion was filtered using Whatman No. 4 filter paper and stored at 20 °C until analysis (Samatha *et al.*, 2012).

Determination of total phenols

An aliquot (0.5 mL) of the ethanol extract was diluted and mixed with 35 mL of deionized water. 2.5 mL of Folin-Ciocalteu reagent was added to the mixture and allowed to incubate for 3 minutes. 5 mL of 20% sodium carbonate solution was then added. The solution was incubated at 70 °C for 20 minutes. The volume was adjusted to 50 mL with deionized water. Absorbance was measured at 750 nm using a UV-VIS spectrophotometer. The phenolic content was estimated in gallic acid equivalents (GAE/g) using the gallic acid standard curve. The results were expressed as gallic acid equivalents (GAE) per gram of plant material. All determinations were carried out six times (Singleton *et al.*, 1999).

Determination of tannins

The Tannin content was determined following the method described by Fadda and Mulas (2010). The 4 mL of the diluted extract was mixed with 2 mL of ethanol and 4 mL of vanillin solution. The mixture was incubated at room temperature for 30 minutes. Absorbance was measured at 500 nm. Tannin content was expressed as mg catechin equivalent (CE)/g, based on a calibration curve (R^2=0.99).

Determination of total flavonoid content (TFC)

The total flavonoid content was determined according to (Zhishen *et al.*, 1999). A 0.5 mL aliquot of each plant extract was transferred into separate test tubes. 2 mL of distilled water was added to each tube. Then, 0.15 mL of 5% sodium nitrite ($NaNO_2$) solution was added and allowed to stand for 6 minutes. Subsequently, 0.15 mL of 10% aluminum chloride ($AlCl_3$) was added, followed by another 6-minute incubation. 2 mL of 4% sodium hydroxide (NaOH) solution was then added, and the total volume was adjusted to 5 mL with distilled water. After 15 minutes of incubation, the mixture turned pink, and its absorbance was measured at 510 nm using a colorimeter. Distilled water served as the blank control. The TFC was expressed in milligrams of catechin equivalents (mg CE) per gram of extract.

Isolation of Soil-borne Pathogens

The pathogen was isolated from the Broccoli plant, showing typical symptoms of damping off, stem rot and wilt by using potato dextrose agar (PDA) medium and identified as *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium oxysporum f. sp. Conglutinans*, and *Sclerotinia sclerotiorum* according to Ellis and Martin (1882).

Antifungal Activity of Botanicals on the Growth of Fungi using the Poisoned food Method

Leaf extracts from selected plants were evaluated for their antifungal activity against the mycelial growth of *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium oxysporum* f. sp. *conglutinans*, and *Sclerotinia sclerotiorum*. The plant extracts were tested at concentrations of 5%, 8%, 12%, 16%, and 20%, respectively. The synthetic fungicide (Bavistin) was used as a positive control. To prepare the media, 5, 8, 12, 16, and 20 mL of stock solution of each plant extract were mixed with 95, 92, 88, 84, and 80 mL of sterilized molten Potato Dextrose Agar (PDA) media, respectively. The mixture was thoroughly shaken to ensure uniform distribution of the leaf extract. Subsequently, 20 mL of the agar medium was poured into sterile Petri plates and allowed to solidify. Agar disks (5 mm in diameter) of the test fungi were cut from 7-day-old culture plates using a sterile cork borer and placed in the center of the Petri plates containing different concentrations of the plant extracts. Each treatment was replicated three times. Control plates, which contained PDA without any plant extract, were also prepared. The inoculated plates were incubated at 22±2 °C under a 12/12-hour light/dark cycle for seven days (Grover & Moore, 1962).

The percentage inhibition of mycelial growth was calculated using the formula provided by Vincent: I (% Inhibition) = C−T/C x 100

EVALUATING THE INHIBITORY EFFECTS OF PLANT EXTRACTS ON FUNGAL SPORE GERMINATION

The fungal pathogens were cultivated to produce viable spores in sufficient numbers (e.g., 50,000 spores/mL), and the spores were obtained by flooding 1-2-week-old cultures with 5 mL of sterile distilled water. The concentrations of spores were diluted to approximately 50,000/mL with sterile distilled water (Dhingra & Sinclair, 1985). The spore suspensions of *R. solani*, *F. oxysporum* f. sp. *conglutinans* and *P. ultimum* were prepared using sterile distilled water, with the spore concentration adjusted to 1.0×10^4 spores/mL. A 90 µL aliquot of the conidial suspension was pipetted into each cavity of a sterile cavity slide. The slides were placed in large Petri dishes lined with moist blotter paper to maintain a humid environment. Aqueous extracts from the leaves of selected plants were prepared at concentrations of 2%, 5%, 8%, 12%, 16% and 20%. Ten microliters of each plant extract were added to the conidial suspension in separate cavity slides and mixed thoroughly. For the control, 10 µL of sterile distilled water was added to 90 µl of the conidial suspension. The prepared slides were incubated at 22±1 °C for 12 hours. The incubation setup ensured adequate moisture and temperature control, critical for accurate spore germination. After incubation, spore germination was assessed under a compound microscope. The percentage of inhibition of spore germination was calculated using the following formula:

$$\text{Inhibition of spore germination (\%)} = C-T/C \times 100$$

Where C = Number of spores germinated in the control (average of 10 microscopic fields), T = Number of spores germinated in the treatment (average of 10 microscopic fields).

Data were subjected to statistical analysis using Tukey's Honest Significant Difference (HSD) test at a 0.05 significance level to determine the efficacy of the plant extracts.

α-Amylase Screening and Assay

The fungal pathogens (*Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *conglutinans* and *Pythium ultimum*) were screened for α-amylase activity on starch agar medium (0.5 g peptone, 0.15 g beef extract, 0.15 g yeast extract, 0.5 g NaCl, 1 g starch, 2 g agar per liter). Sterilized media with 100% selected plant extract (Aqueous) were inoculated with 5 mm discs of 8-day-old fungal cultures and incubated at 22±2 °C for 3 days. A clear zone following iodine staining indicated amylase activity (Saleem & Ebrahim, 2014). For quantitative analysis, fungi were grown in liquid medium (1.4 g KH_2PO_4, 10 g NH_4NO_3, 0.5 g KCl, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 20 g starch per liter) mixed with 100 mL extract. After 3 days, the cultures were centrifuged at 2000 rpm for 5 minutes, and the supernatant served as crude enzyme (Oyeleke *et al.*, 2010). Amylase activity was determined using the DNSA method (Bertrand *et al.*, 2004). A 0.5 mL crude enzyme sample was incubated with 1 mL of 1% soluble starch in citrate-phosphate buffer (pH 6.4) at 40 °C for 30 minutes. The reaction was terminated with 1 mL DNSA, heated in a boiling water bath, cooled, and diluted with 5 mL of distilled water. Absorbance was measured at 540 nm, and reducing sugars were quantified against a maltose standard curve (Ramakrishna *et al.*, 1982).

Protease Production and Assay

Fungal isolates were cultured in protease-specific broth (1.0 g yeast extract, 0.02 g $MgSO_4$, 2.0 g glucose, 0.1 g K_2HPO_4 per liter, pH 7.0) at 28 °C for 5-6 days in a shaker. The culture filtrates were centrifuged at 8,000 rpm for 10 minutes, and the supernatant was collected for protease assays (Josephine *et al.*, 2012). Protease activity was measured using casein as a substrate (Tsuchida *et al.*, 1986). A mixture of 500 µL 1% casein in 50 mM phosphate buffer (pH 7) and 200 µL crude enzyme extract was incubated at 40 °C for 20 minutes. After terminating the reaction with 1 mL 10% TCA, the supernatant was mixed with 2.5 mL 0.4 M Na_2CO_3 and 1 mL Folin-Ciocalteu reagent. The absorbance of the resulting solution was measured at 660 nm. One unit of protease activity was defined as the amount of enzyme releasing 1 µg of tyrosine per mL per minute (Alnahdi, 2012). Control experiments included cultures without plant extracts and those supplemented with 0.2% Bovistin as a positive reference.

Statistical Analysis

All experiments were performed in triplicate. Data was analysed using standard error (SE), and statistical significance was assessed using appropriate statistical tests.

RESULT

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis of six wild plant species revealed diverse profiles with potential medicinal applications (Table 1). *S. nigrum* exhibited moderate levels of tannins, phenols, and alkaloids, though lower flavonoid and saponin content. *M. lutea* showed a high presence of tannins, phenols, and saponins, while the low levels of alkaloids and flavonoids. *A. speciosa* was rich in phenols, with moderate levels of tannins, alkaloids, and flavonoids. *B. cristata* had moderate phenols and flavonoids but lacked saponins. *A. wilkesiana* stood out for its high saponin content, while moderate levels of other phytochemicals. *V. trifolia* showed a strong presence of flavonoids, with moderate tannins, phenols, and alkaloids, though its low saponin content might be a limiting factor. Overall, the varying phytochemical profiles suggest that these plants may serve different medicinal purposes.

Analysis of Phenolic, Tannin, and Flavonoid Content in Selected Plant Extracts

The quantitative assessment of Total Phenolic Content (TPC), Tannin Content (TC), and Total Flavonoid Content (TFC) across a spectrum of ethanolic and aqueous extracts from six selected wild plant species reveals significant heterogeneity, underscoring the differential bioactive compound extraction efficiency associated with solvent polarity. Table 2 systematically delineates these findings, offering a comprehensive view of the phytochemical landscape within the studied taxa. Among the evaluated extracts, *A. speciosa* demonstrated a remarkable affinity for phenolic compounds, with its ethanolic extract exhibiting the highest TPC at 170.24 mg GAE/g. followed by in its aqueous extract, which is a substantial 145.15 mg GAE/g. Conversely, *S. nigrum* recorded the lowest TPC in its aqueous extract (88.67 mg GAE/g), reflecting a more modest phenolic profile in this extraction medium. A comparison across all species reveals that *B. cristata* and *V. trifolia* also show significant phenolic content in their ethanolic extracts, 140.78±2.92 and 135.34 mg GAE/g, respectively. Notably, the lowest phenolic content was observed in the ethanolic extract of *S. nigrum* (98.21 mg GAE/g), suggesting a less efficient extraction. In terms of Tannin contents followed a similar trend, with *A. speciosa*, as its ethanolic extract (78.65 mg/g) followed by in its aqueous extract (72.34 GAE/g), and least amount of TC were found in ethanolic extract of *S. nigrum* (42.18±2.04 GAE/g) followed by aqueous extract of same species (35.67±1.95 mg GAE/g), whereas

Table 1: Qualitative analysis of phytochemicals of the ethanolic extract of selected plants

S. No.	Wild Plant Species	Tannins	Phenols	Alkaloids	Flavonoids	Saponins
1	*S. nigrum*	+ (++)	+ (+++)	+ (++)	+ (+)	+ (+)
2	*M. lutea*	+ (+++)	+ (+++)	+ (+)	+ (+)	+ (+++)
3	*A. speciosa*	+ (++)	+ (+++)	+ (++)	+ (++)	+ (+)
4	*B. cristata*	+ (+)	+ (++)	+ (+)	+ (++)	-
5	*A. wilkesiana*	+ (+)	+ (++)	+ (+)	+ (++)	+ (+++)
6	*V. trifolia*	+ (++)	+ (++)	+ (++)	+ (+++)	+ (+)

(-) The absence of the compound; (+) the presence of the compound; (++), (+++) Increased intensity of color.

moderate amounts were recorded in *V. trifolia* and *M. lutea* also showing considerable tannin levels (65.45±2.25 mg/g and 63.12±2.19 mg/g, respectively). Similarly, as TFC was found to be highest in the ethanolic extract of *V. trifolia* (92.3±3.2 mg CE/g), followed by *A. speciosa* (89.4±3.1 mg CE/g), and *M. lutea* demonstrated a notable TFC in its ethanolic extract (78.6±2.9 mg CE/g), *S. nigrum* and *B. cristata* also displayed considerable flavonoid content, especially in the ethanolic extracts (75.2±2.4 mg CE/g and 62.5±2.7mg CE/g, respectively) when compared to the aqueous extract of *V. trifolia* (68.4±2.8 mg CE/g), followed by *M. lutea* (57.8±2.6 mg CE/g) and the least amount was found in *A. wilkesiana* aqueous and well as ethanolic extract (33.9±1.7 mg GAE/g). These findings not only elucidate the differential phytochemical profiles of the selected plant species but also provide critical insights into the solvent-dependent extraction efficiencies.

Antifungal Efficacy of Selected Plant Extracts against Soil-borne Pathogenic Fungi

The data presented in Table 3 demonstrate that the mycelial growth of the tested fungus was effectively inhibited by the antifungal compounds present in the plant materials across all tested extracts, with varying levels of inhibition observed. The results were compared with a control treatment using Bavistin at 0.2%, a standard synthetic fungicide. The extract of *A. speciosa*

Table 2: Determination of phenolics, tannin and flavonoid content in selected plants

S. No.	Plant used for extraction	Total Phenolic Content (TPC) (mg GAE/g)	Total Phenolic Content (TPC) (mg GAE/g)	Tannin Content (TC) (mg/g)	Tannin Content (TC) (mg/g)	Total Flavonoid Content (TFC) (mg CE/g)	Total Flavonoid Content (TFC) (mg CE/g)
		Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract	Aqueous Extract
1	*S. nigrum*	98.21±3.12	88.67±3.04	42.18±2.04	35.67±1.95	75.2±2.4	52.7±2.5
2	*M. lutea*	110.45±2.85	101.76±2.97	63.12±2.19	45.15±2.04	78.6±2.9	57.8±2.6
3	*A. speciosa*	170.24±3.10	145.15±2.75	78.65±1.92	72.34±1.79	89.4±3.1	37.4±2.2
4	*B. cristata*	140.78±2.92	130.12±2.89	52.30±2.10	60.34±2.18	62.5±2.7	43.1±1.8
5	*A. wilkesiana*	125.67±2.73	115.89±2.94	58.45±2.12	50.78±2.21	54.8±2.3	33.9±1.7
6	*V. trifolia*	135.34±2.71	120.24±2.65	65.45±2.25	55.67±2.16	92.3±3.2	68.4±2.8

*Data based on the average triplicate±S. E. (S. E. = Standard Error)

Table 3: Inhibition percentage of fungal growth by plant extracts

S. No.	Plant Species/Control	Concentration of Extract (%)	*Rhizoctonia solani*	*Pythium ultimum*	*Fusarium oxysporum*	*Sclerotinia sclerotiorum*
1	*S. nigrum*	5	9.4±0.45	21.1±0.19	7.32±0.23	13.4±0.12
		8	30.1±0.7	54.2±0.22	10.8±0.14	23.8±0.05
		12	40.1±0.4	60±0.12	15.23±0.19	30.3±0.07
		16	52.13±1.84	62±0.12	20±0.28	38.5±0.3
		20	32.4±0.26	64.1±0.1	45.6±0.05	52.13±1.84
2	*M. lutea*	5	28.34±2.05	50±0.15	16.6±0.09	19.3±0.08
		8	35.48±2.01	52.1±0.12	21.8±0.09	22.1±0.82
		12	44.23±1.97	55±0.06	32±0.03	30±0.74
		16	63.14±1.75	60±0.11	53±0.09	38.5±0.3
		20	74.15±1.82	75.1±0.07	74.15±1.82	59.13±1.84
3	*A. speciosa*	5	19±0.2	27±0.2	18.6±0.07	15.3±0.19
		8	30±0.2	60.3±0.15	26.7±0.15	22.1±0.82
		12	35.2±0.3	64.1±0.1	35±0.03	30±0.74
		16	72.45±1.68	70.18±1.67	46.3±0.03	50.12±1.90
		20	85.23±1.65	79.84±1.52	63.14±1.75	78.84±1.72
4	*B. cristata*	5	11.6±0.3	22.3±0.17	11.4±0.07	10.5±0.21
		8	12±0.81	49.2±0.16	19±0.09	16.21±0.41
		12	15.45±0.65	54.2±0.12	21.8±0.06	21.5±0.42
		16	38.5±0.3	55±0.06	39.2±0.19	30±0.74
		20	46.7±0.17	72±0.12	54.2±0.12	39.2±0.19
5	*A. wilkesiana*	5	11.4±0.07	22.3±0.17	11.2±0.32	13.8±0.45
		8	19±0.09	37.1±0.23	22.3±0.17	14.4±0.64
		12	21.8±0.06	62±0.12	39.2±0.19	20.7±0.29
		16	39.2±0.19	70.18±1.67	62±0.12	57.98±0.74
		20	54.2±0.12	78.84±1.72	75.1±0.07	39.2±0.19
6	*V. trifolia*	5	20.8±0.5	10.6±0.61	15.2±0.09	11.12±0.71
		8	14.3±0.63	15.32±0.42	17.1±0.08	13.9±0.44
		12	20.5±0.521	20.6±0.45	29±0.09	20.6±0.21
		16	62.75±1.72	55.18±1.76	38.7±0.05	30.3±0.51
		20	60±0	60±0.11	44.9±0.03	38.2±0.19
7	Bavistin/Control	0.2%	92±0.19	87±0.33	83±0.12	88±0.98

Data based on the average triplicate±S.E. (S.E. = Standard Error)

at a 20% concentration exhibited the highest inhibition rate (85.23±1.65%), underscoring its strong antifungal activity. This was followed by *M. lutea* at 20% (74.15±1.82%) and *V. trifolia* at 20% (62.75±1.72%). Conversely, the least effective was *S. nigrum* at 5% (9.4±0.45%), which showed minimal inhibitory effects. The control treatment, Bavistin, demonstrated the greatest efficacy, achieving a 92±0.19% inhibition against *R. solani*. In a similar pattern, *A. speciosa* at 20% concentration showed the highest inhibition (79.84±1.52%), closely matched by *A. wilkesiana* at 20% (78.84±1.72%). *M. lutea* at 20% also displayed significant antifungal properties (75.1±0.07%), followed by *B. cristata* (72±0.12) at 20%. The lowest inhibition was observed with *V. trifolia* at 5% (10.6±0.61%). Bavistin again proved to be highly effective, with a control efficacy of 87±0.33% against the *Pythium ultimum*. Regarding efficacy against *Fusarium oxysporum*, *A. wilkesiana* at 20% was the most potent, with an inhibition rate of 75.1±0.07%, followed closely by *M. lutea* at 20% (74.15±1.82%). *A. speciosa* at 20% also showed notable activity (63.14±1.75%). The least effective was *S. nigrum at* 5% (7.32±0.23%). Bavistin continued to demonstrate superior performance, achieving an inhibition rate of 83±0.12%. Similarly, the efficacy against *Sclerotinia sclerotiorum*, *A. speciosa* at a 20% concentration demonstrated the highest inhibition, with a rate of 78.84±1.72%. This was followed by *M. lutea* and *A. wilkesiana* at 20% and 16%, which exhibited an inhibition rate of 59.13±1.84% and 57.98±0.74 at 16% respectively. In terms of the least effective extract was *B. cristata* at 5% showed an inhibition rate of just 10.5±0.21%. Bavistin, used as the control, exhibited the greatest efficacy, achieving an inhibition rate of 88±0.98%. As per the results obtained, the *A. speciosa* at 20% concentration consistently demonstrated the highest antifungal activity across most fungi, particularly against *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, making it the most potent among the tested extracts. *M. lutea* and *A. wilkesiana* also showed strong antifungal properties at higher concentrations, particularly against *Fusarium oxysporum* and *Pythium ultimum*. Conversely, *V. trifolia* was the least effective across all concentrations, with minimal inhibitory effects against all tested fungi.

Efficacy of Plant Extracts against Spore Germination of Soil-borne Fungi

The data presented in the bar charts illustrate the inhibitory effects of various concentrations of plant extracts on the spore germination of soil-borne fungal isolates: *R. solani* (Figure 1), *P. ultimum* (Figure 2) and *F. oxysporum* (Figure 3). The plant extracts tested include those from *S. nigrum* L., *M. lutea* Lindl., *A. speciosa*, *B. cristata* L., *A. wilkesiana*, and *V. trifolia* L. The concentrations used in the study ranged from 2 mg/mL to 20 mg/mL. In the case of *R. solani* (Figure 1), *V. trifolia* showed the highest inhibitory effect (80.4) at a 20% concentration, followed closely by *B. cristata* and *M. lutea* (78.9 and 78.2) inhibition at the same concentration. Meanwhile, *A. wilkesiana* and *A. speciosa* exhibited substantial inhibition, i.e. 77.1 and 76.3, respectively, at 20% and 16% concentration. *S. nigrum* exhibited the lowest inhibition, 20.4 % at the minimum tested concentration of 2%.

Similarly, in the case of *P. ultimum* (Figure 2), among the plant species evaluated, *A. speciosa* displayed the maximum inhibitory activity (79.8) at the 20% concentration and was closely followed by *V. trifolia* (78.8) at the same concentration. Along with this, *B. cristata* and *A. wilkesiana* exhibited moderate antifungal effects (72.4 and 70.2) on spore germination at the 16% concentration. At a concentration of 2% the *S. nigrum*

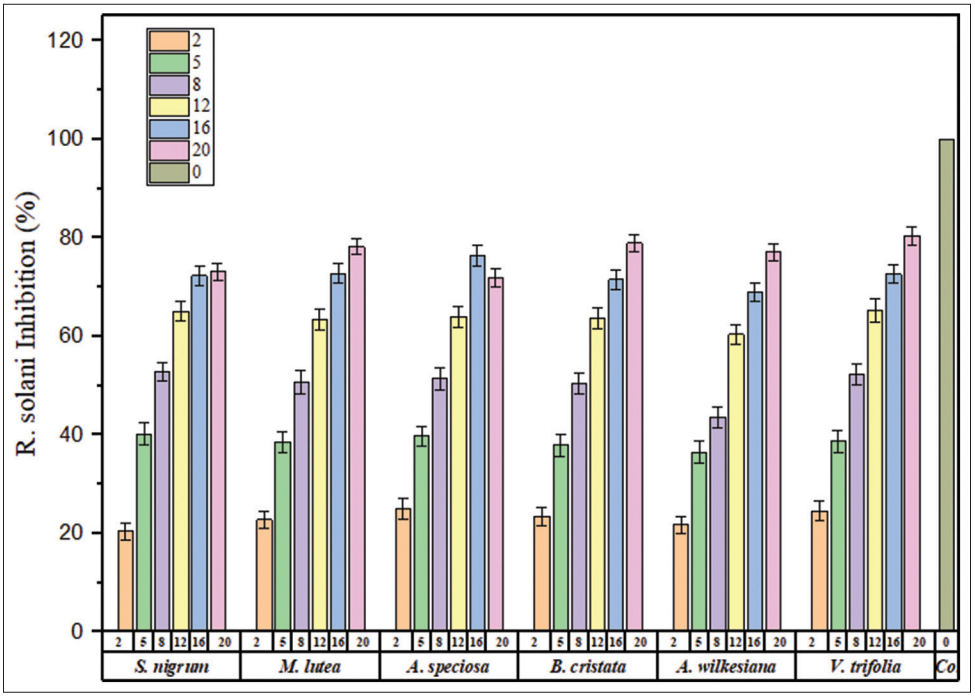

Figure 1: Efficacy of selected plant extract on the inhibition of spore germination of *R. solani*, the vertical bars indicate the standard error of three replications

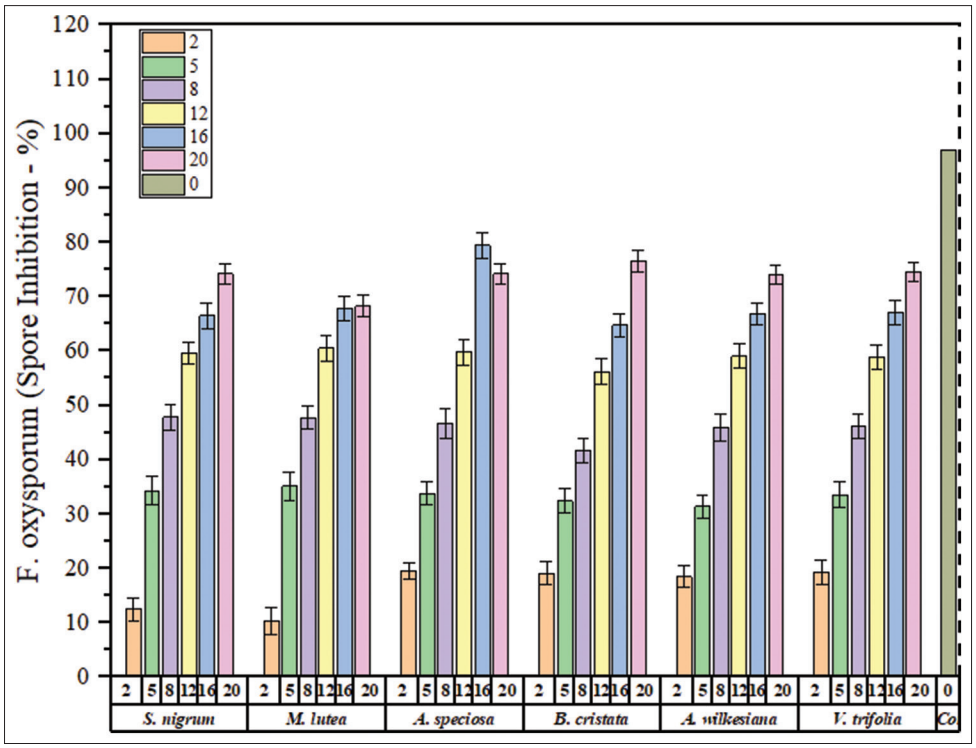

Figure 2: Efficacy of selected plant extract on the inhibition of spore germination of *F. oxysporum,* the vertical bars indicate the standard error of three replications

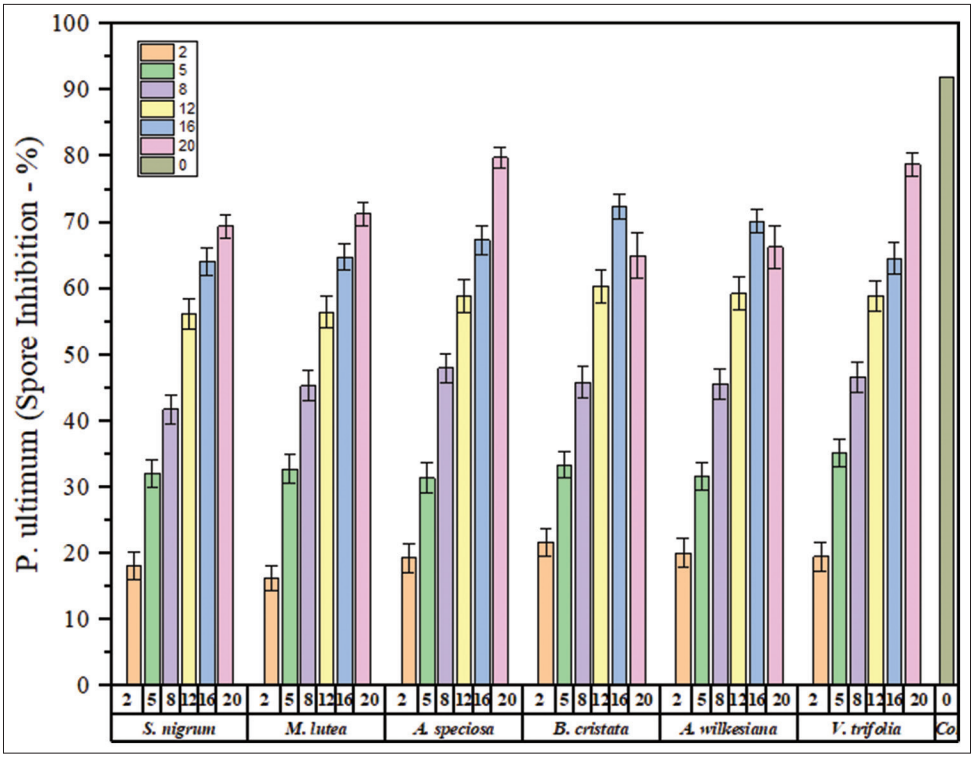

Figure 3: Efficacy of selected plant extract on the inhibition of spore germination of *P. ultimum*, the vertical bars indicate the standard error of three replications

exhibited 18.2 percent of inhibition, followed by *M. lutea* 16.2 at the same concentration.

For *F. oxysporum* (Figure 3), the pattern of inhibition was consistent with the previous fungi, where the inhibitory

effect increased with higher concentrations of plant extracts. A. *speciosa* exhibited the maximum inhibitory effect, 79.4% at the 20% concentration, followed by *B. cristata*, and showed 76.5 % inhibition at the same concentration. Similarly, A. *wilkesiana* and V. *trifolia* also demonstrated a notable inhibitory effect (74.5 and 74.1) at 20%. In contrast, *M. lutea* showed the lowest inhibitory effect (68.3%) at 20% concentration. At the lowest concentration of 2%, the least inhibition was observed in the treatment of S. *nigrum* and *M. lutea* (12.5 and 10.3), respectively.

In-vitro Assessment of Amylase Activity in Pathogenic Fungi Treated with Plant Extracts

The study of the impact of different plant extracts on the α-amylase activity in various pathogenic fungi revealed that suppression of α-amylase production, which is crucial for the fungi's ability to hydrolyse starch and obtain energy, The observations were summarised in Table 4. The control shows the baseline α-amylase activity in the fungi without any treatment, and the treatment columns present the enzyme activity after plant extract treatments, highlighting the inhibitory effects. In terms of *R. solani*, the control activity ranges from 120.34 U/mL to 127.78 U/mL across the treatments, with the maximum reductions observed in *S. nigrum* (reduced to 75.45 U/mL), followed by *M. lutea* (reduced to 82.34 U/mL) and the minimum reductions observed in *V. trifolia* (reduced to 85.45 U/mL). Similarly for *P. ultimum*, the activities were found to increase at control conditions However, the activities of enzymes in plants treated a decline was noticed particularly in A. *speciosa* the significant reduction, with the largest drop noted (126.34 U/ml to 64.23 U/ml) followed by the *V. trifolia* (112.56 U/ml to 62.67 U/ml) and minimum reduction of activity noted in *M. lutea* (110.45 U/mL to 74.56 U/mL). However, for the *F. oxysporum*, the control activity is notably higher, after the treatments noted the substantial reductions, especially in *V. trifolia* (138.34 to 62.67 U/mL), followed by *A. speciosa* (136.78 to 87.45 U/mL). Similarly, with *Sclerotinia sclerotiorum*, the highest control activity is observed in *V. trifolia* (122.34 U/mL). After post-treatment, it shows moderate reductions, with *S. nigrum* and *M. lutea* showing the most significant decreases.

In-vitro Evaluation of Variation in Protease Activity of Pathogenic Fungi due to Plant Extracts Treatment

Table 5 reveals a significant decrease in enzyme activity among soil-borne pathogenic fungi treated with various plant extracts. Protease is necessary for pathogenic fungi because it aids in the digestion of proteins, supplying them with vital nutrients. The control column reveals the baseline protease activity in fungi before treatment, whereas the treatment column depicts the enzyme activity after including plant extracts, emphasizing the suppressive effects that have been observed. The control protease activity of *R. solani* varies between 3.10 and 3.25 U/mL depending on the treatment. The greatest notable drop was observed with *A. speciosa* (from 3.25 U/mL to 1.90 U/mL), subsequently followed by *A. wilkesiana* (from 3.22 U/mL to 1.82 U/mL), whereas the smallest reduction was seen with *M. lutea* (from 3.10 U/mL to 1.85 U/mL). Similarly,

Table 4: **α**-Amylase Activity in Pathogenic Fungi Subjected to Plant Extract Treatment *in-vitro*

S. No.	Selected Plants used	Amylase activity (U/mL) in fungi under phyto extracts treatment							
		Rhizoctonia solani		*Pythium ultimum*		*Fusarium oxysporum*		*Sclerotinia sclerotiorum*	
		Control (U/mL)	Treatment (U/mL)	Control (U/mL)	Treatment (U/mL)	Control (U/mL)	Treatment (U/mL)	Control (U/mL)	Treatment (U/mL)
1	*S. nigrum*	120.34±2.11	75.45±1.56	105.45±2.30	70.23±1.88	128.67±2.79	88.12±2.05	117.67±2.45	78.56±1.67
2	*M. lutea*	123.23±2.10	82.34±1.89	108.45±2.05	74.56±1.78	135.56±2.91	89.67±2.10	115.34±2.36	76.23±1.79
3	*A. speciosa*	124.45±2.15	80.12±1.95	109.34±2.06	72.45±1.89	136.78±2.88	87.45±2.12	119.45±2.50	79.34±1.75
4	*B. cristata*	125.34±2.14	83.67±1.90	110.23±2.08	74.56±1.91	137.56±2.85	99.23±2.15	120.34±2.44	81.23±1.85
5	*A. wilkesiana*	126.34±2.12	84.23±1.93	126.34±2.12	64.23±1.93	129.34±2.82	98.56±2.14	121.67±2.39	82.45±1.89
6	*V. trifolia*	127.78±2.13	85.45±1.94	112.56±2.15	62.67±1.92	138.34±2.15	62.67±1.92	122.34±2.42	83.45±1.86

Control=cultures without plant extract treatment, Treatment=cultures with plant extract treatment. Values are the meaning of three replications with the standard error

Table 5: Variation in the protease activity of pathogenic fungi due to plant extract treatment

S. No.	Selected Plants used	Amylase activity (U/mL) in fungi under phyto extracts treatment							
		Rhizoctonia solani		*Pythium ultimum*		*Fusarium oxysporum*		*Sclerotinia sclerotiorum*	
		Control (U/mL)	Treatment (U/mL)	Control (U/mL)	Treatment (U/mL)	Control (U/mL)	Treatment (U/mL)	Control (U/mL)	Treatment (U/mL)
1	*S. nigrum*	3.20±0.12	1.80±0.08	2.75±0.11	1.65±0.07	4.10±0.15	2.90±0.12	2.80±0.10	1.55±0.07
2	*M. lutea*	3.10±0.11	1.85±0.07	2.60±0.10	1.55±0.06	3.95±0.14	2.80±0.11	2.70±0.09	1.50±0.06
3	*A. speciosa*	3.25±0.13	1.90±0.08	2.70±0.10	1.70±0.07	4.20±0.16	3.00±0.13	2.85±0.11	1.60±0.07
4	*B. cristata*	3.15±0.12	1.88±0.07	2.65±0.10	1.60±0.06	4.05±0.15	2.85±0.12	2.75±0.10	1.52±0.06
5	*A. wilkesiana*	3.22±0.13	1.82±0.07	2.72±0.11	1.67±0.07	4.18±0.16	2.95±0.12	2.83±0.11	1.57±0.07
6	*V. trifolia*	3.18±0.12	1.86±0.07	2.68±0.11	1.63±0.06	4.12±0.15	2.88±0.12	2.78±0.10	1.54±0.06

Control=cultures without plant extract treatment, Treatment=cultures with plant extract treatment. Values are the meaning of three replications with the standard error.

the control activity in *P. ultimum* fluctuates between 2.60 and 2.75 U/mL. A. *speciosa* exhibits a substantial drop-in protease activity (from 2.70 U/ml to 1.70 U/ml), followed by *S. nigrum* (from 2.75 U/mL to 1.65 U/mL), while *M. lutea* exhibits the smallest reduction (from 2.60 U/mL to 1.55 U/mL). However, for the *Fusarium oxysporum*, the control protease activity varies from 3.95 to 4.20 U/mL. The most significant decrease was observed with A. *speciosa* (from 4.20 U/mL to 3.00 U/mL), subsequent by A. *wilkesiana* (from 4.18 U/mL to 2.95 U/mL), whereas the smallest reduction is seen with *M. lutea* (from 3.95 to 2.80 U/mL). Furthermore, in *S. sclerotiorum*, the control protease activity is distinct between 2.70 and 2.85 U/mL. *S. nigrum* shows a significant reduction (from 2.80 U/mL to 1.55 U/mL), followed by A. *speciosa* (from 2.85 U/mL to 1.60 U/mL), alongside *M. lutea* exhibits the smallest decrease (from 2.70 U/mL to 1.50 U/mL). These findings suggest that plant extracts exhibit differing levels of efficacy in inhibiting the alpha amylase as well as protease activity, which might be useful in developing bio-control techniques against these pathogenic fungi.

DISCUSSION

Preliminary Screening for Phytochemicals

A preliminary evaluation of ethanolic extracts from different plants revealed the presence of certain phytochemicals in our investigation. Several studies of phytochemical analysis on *S. nigrum* L. have revealed the presence of bioactive compounds, including alkaloids, flavonoids, tannins, coumarins, sterols, triterpenoids, and saponins (Gogoi & Islam, 2012). *Acalypha wilkesiana* contains alkaloids, cardiac glycosides, flavonoids, saponins, steroids, and tannins, which were reported by Madziga *et al*. (2010). *Barleria cristata* leaves exhibit alkaloids, glycosides, flavonoids, carbohydrates, proteins, amino acids, and reducing sugars, as reported by Harini *et al*. (2022). Similar studies on *Vitex trifolia* revealed that leaves contain alkaloids, saponins, tannins, phenols, terpenoids, flavonoids, and steroids, with acetone and ethanol extracts (Saklani *et al*., 2017).

Quantitative Evaluation of Flavonoid, Tannin, and Phenolic Content

The study reported that ethanolic leaf extracts of wild plants such as *S. nigrum* L., *M. lutea* Lindl., A. *speciosa* (L.f.) Sweet, *B. cristata* L., A. *wilkesiana* Müll. Arg., and *V. trifolia* L. revealed the presence of phenol, tannin, and flavonoids. This agrees with several reports of research that have shown that *S. nigrum* leaves contain significant amounts of phenolic compounds and flavonoids in methanol extract, followed by ethyl acetate extract (Najjar *et al*., 2022). Nisa *et al*. (2023) studied the phenomena and flavonoid contents of *Vitex trifolia* and found that the leaves had the highest total phenolic content, followed by twigs and fruit, and the leaves also contained the highest flavonoid content. However, studies on *Argyreia speciosa* reported that the total tannin in the ethanolic extract has been found to be 8.75 and 11.25 mg per gram of dry weight extract, respectively. The total flavonoid content is 23±1.37 and 30±0.52 mg/gm plant

extract, whereas the total flavonol content is 0.49±0.03 and 3.60±0.25 mg/gm plant extract (Sahu *et al*., 2013). Asekunowo *et al*. (2021) reported that the methanolic extract of *Acalypha* had the highest flavonoid content (379.66 mg/mL), the ethanolic extract had the highest phenolics (208.03 mg/mL), and the aqueous extract had the least polyphenolic compounds.

The Effectiveness of Wild Plants Against Soil-borne Pathogens

The results of this investigation demonstrate that *A. speciosa*, at a 20% concentration, exhibits potent antifungal activity against all test fungi, especially *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. In contrast, *M. lutea* and *A. wilkesiana* possess effective antifungal properties at higher concentrations, notably against *Fusarium oxysporum* and *Pythium ultimum*. Meanwhile, *V. trifolia* and *S. nigrum* are less effective, with moderate inhibitory effects against all tested fungi. Plant extracts have demonstrated remarkable antifungal action against *R. solani*, *S. sclerotiorum* and *P. ultimum in vitro*. Recent studies have investigated the antifungal potential of various plant extracts against soil-borne pathogens, including *Ailanthus excelsa*, *Lawsonia inermis*, *Argyreia nervosa*, *Terminalia arjuna*, and *Argemone mexicana*, which have shown significant inhibition of mycelial growth for soil-borne fungal pathogens (Bansode *et al*., 2022; Udasi *et al*., 2023). As an instance, *S. nigrum* has been found to successfully inhibit the growth of *Fusarium oxysporum*, *Macrophomina phaseolina*, and *Rhizoctonia solani* (Shirazi *et al*., 2020). Similarly, *A. speciosa* has shown significant antifungal activity in various solvent extracts, notably against *Candida albicans* and *Aspergillus niger* (Ahlawat *et al*., 2015; Khichi *et al*., 2021). Furthermore, *B. cristata* has been revealed to be effective against pathogenic fungi like Fusarium oxysporum and Rhizoctonia solani (Abubacker & Devi, 2015). The antifungal properties of *A. wilkesiana*, particularly its aqueous and methanolic extracts, have shown inhibitory effects on *C. albicans* and *A. niger* with MICs ranging from 75-100 mg/mL (Katibi *et al*., 2022). Although *V. trifolia* has demonstrated significant antimicrobial properties, especially due to its essential oil composition (Devi & Singh, 2014), its efficacy against the tested soil-borne fungi in this study was limited. Nevertheless, its antifungal activity against *R. solani* and other plant pathogens has been documented (Yilar *et al*., 2016), suggesting that different extraction methods or concentrations may yield more potent results. This study shows that *A. speciosa*, *M. lutea*, and *A. wilkesiana* have antifungal potential against the soil-borne pathogens inciting broccoli plants. However, more study is required to optimize extraction procedures and concentrations to improve the efficacy of less potent extracts such as *V. trifolia* and *S. nigrum* against soil-borne pathogenic fungi.

The Effectiveness of Plant Extracts in Inhibiting Spore Germination

The study underscores the effectiveness of certain plant extracts, mainly *A. speciosa*, *V. trifolia*, and *B. cristata*, in inhibiting spore germination in soil-borne fungi. The constancy of their inhibitory effects across many fungus species indicates an extensive antifungal feature, which might be extremely useful in agricultural activities, particularly for eliminating fungal diseases in crops. Changing efficiency at different concentrations gives useful information for improving the usage of these extracts in practical applications. These findings could eventually guide future research towards the formulation of plant-based antifungal remedies and ultimately result in the development of environmentally friendly alternatives to chemical fungicides. Numerous studies carried out by different researchers agreed on similar results, like Vice, Mishra *et al*. (2024), which highlighted the significant antifungal properties of *Azadirachta indica* (Neem) and *Allium sativum* (Garlic) extracts, demonstrating over 80% inhibition of *Fusarium oxysporum* spore germination at concentrations as low as 15 mg/mL. This is comparable with the substantial efficacy levels observed in *Acalypha speciosa* and *Vitex trifolia* in the current study. However, Kulbat-Warycha *et al*. (2024) observed phenolic compounds in *Thymus vulgaris* (thyme) extract, devastating the cell wall integrity of *Rhizoctonia solani*, inhibiting spore germination. Plant metabolites are not only antifungal, but they have also been shown to demonstrate fungistatic effects by reducing spore and mycotoxin formation. Similar findings were noted by Mohammedi and Atik (2013), who showed that the methanolic extracts of *Tamanxi spp*., *D. gridium*, *C. procera*, *H. scoparium*, *P. argentea*, and *M. caressens* leaves were effective in inhibiting the growth of mycelial cells, spores, and aflatoxin generation by *Aspergillus flavus*. This suggests that certain plant extracts consistently exhibit strong antifungal effects at different dosages. It is similar to the wide-ranging activity exhibited with *M. lutea* and *A. wilkesiana* in the present studies.

Amylase and Protease Activities in Pathogenic Fungi Affected by Plant Extracts

Plant extracts have been shown to reduce the activity of α-amylase and protease in soil-borne pathogens studied in this research, revealing that these natural compounds may have the potential to be effective anti-fungal agents. The potential antifungal effects of certain extracts, such as those from *Vitex trifolia*, *B. cristata*, *A. speciosa*, *A. wilkesiana*, and *Solanum nigrum*, were demonstrated in the present investigation by their capacity to significantly inhibit enzyme activity in test fungi. This result is especially relevant to agricultural endeavours, where preventing the spread of soil-borne fungi such as *Rhizoctonia solani*, *Pythium ultimum*, and *Fusarium oxysporum* is crucial (Sulaiman & Bello, 2024). Plant-derived extracts that target these pathogens' metabolic pathways may diminish their capacity to colonise and have adverse effects on crops, resulting in more sustainable and ecologically friendly biological control strategies (Shang *et al*., 2024). Studies on *in vitro* assessment of amylase and protease activity have reported the potential of plant extracts to inhibit α-amylases as well as protease, an enzyme involved in carbohydrate digestion and protein. Some plant extracts, including ethanolic and aqueous, exhibited notable α-amylase inhibitory action. Furthermore, research has shown that plant extracts possess antifungal properties against pathogenic fungi such as *Alternaria* and *Fusarium*

solani. Extracts from various plants, including cinnamon, ginger, turmeric, and *Prosopis juliflora*, have shown significant inhibitory effects on fungal growth and enzyme activity. The effectiveness of plant extracts generally increased with higher concentrations, with some extracts inhibiting fungal growth by up to 75%. These extracts were found to suppress the growth of common plant pathogens such as *Alternaria alternata*, *Fusarium oxysporum*, and *Pythium ultimum* (Muthomi *et al.*, 2017; Abbas *et al.*, 2022). These findings indicate that plant extracts could be effective alternatives to synthetic fungicides in managing plant diseases and pathogenic microorganisms.

CONCLUSION

The study efficiently evaluated the phenomenon, tannin, and flavonoid contents of several wild plant extracts and investigated their *in-vitro* antifungal activity against soil-borne phytopathogenic fungi affecting broccoli. *Argyreia speciosa* consistently outperformed the other extracts in terms of antifungal activity, notably against *Rhizoctonia solani* and *Sclerotinia sclerotiorum*, making it an effective applicant for sustainable agriculture techniques. The study illustrates the potential of plant-derived chemicals as eco-friendly alternatives to synthetic fungicides, which will help to create integrated disease management (IDM) techniques. These findings support the use of wild plant extracts to improve agricultural resilience and environmental sustainability.

AUTHORS' CONTRIBUTION

Material preparation, data collection, analysis and drafting of manuscript were performed by Yogesh Urdukhe and Dr. Umesh Mogle have made substantial contributions to conception, supervision and design of research.

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