Biopesticidal Potential of Noxious Weeds against Leaf Blight of Rice Caused by *Xanthomonas oryzae*

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Bacterial leaf blight (BLB), caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), threatens global rice production. The biopesticidal potential of six weed species, namely *Parthenium hysterophorus*, *Ammi visnaga*, *Chenopodium album*, *Cannabis sativa*, *Amaranthus viridis*, and *Dysphania ambrosioides*, was evaluated against Xoo. Crude extracts and their ethyl acetate and n-hexane fractions were tested using agar well diffusion, minimum inhibitory concentration (MIC) assays, and *in vivo* pot experiments under a factorial completely randomized design. *C. sativa* (1.13 g) and *A. viridis* (1.03 g) yielded the highest crude extracts. *Parthenium hysterophorus* n-hexane extract (63.7% inhibition at 100 ppm), *D. ambrosioides* n-hexane (55.2%), and *A. visnaga* n-hexane (167% at 25 ppm) showed significant antibacterial activity. Ethyl acetate fractions, particularly *D. ambrosioides*, reduced Xoo infection most effectively *in vivo*. *Parthenium hysterophorus* (31 to 70%) and A. *viridis* (59 to 65%) ethyl acetate extracts promoted seed germination and growth, while *A. visnaga* and *D. ambrosioides* n-hexane extracts reduced growth by 24 to 29% and 19 to 25%, respectively. *Chenopodium album* ethyl acetate extract increased chlorophyll content (61 to 68%). Electrolyte leakage was highest in *P. hysterophorus* crude extract (75%) and lowest in *D. ambrosioides* n-hexane (17%). These weed-derived extracts show promise for sustainable BLB management, warranting further compound isolation and field validation.

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INTRODUCTION

Rice (*Oryza sativa* L.), a prominent member of the Poaceae family, holds an unparalleled position as a staple food crop. It is cultivated extensively across tropical and subtropical regions worldwide (Mohapatra *et al.* 2022). It serves as a primary dietary component for 2.7 billion people globally, with Asia alone accounting for 90% of the

world's rice production, particularly in the countries Pakistan, Bangladesh, China, Vietnam, and Korea (Sarfaraz 2023). Beyond its role as a fundamental food source, rice significantly contributes to national economies; for instance, in Pakistan, it stands as the second most widely grown cereal, representing 17% of the nation's total cereal grain output and playing a vital role in both domestic consumption and exports (Maqbool *et al.* 2020). Approximately 10% of Pakistan's arable land is dedicated to rice cultivation. The crop's diverse genetic landscape includes over 40,000 varieties, distinguished by characteristics, such as grain length, color, thickness, stickiness, aroma, and cultivation methods, with Asian rice (*Oryza sativa*) being the most widely cultivated and extensively studied species, alongside African rice (*Oryza glaberrima*) and various wild rice species (genus *Zizania*). Its cultivation is typically suited to regions with high rainfall and readily available labor, though sophisticated water-controlling terrace systems enable its growth in challenging steep, rocky terrains (Elahi *et al.* 2020).

Despite its critical role in global food security, rice cultivation is continually hampered by a myriad of biotic stresses, among which the diseases caused by bacterial pathogens are particularly devastating. One of the most significant yield-reducing pathogens is *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causative agent of bacterial leaf blight (BLB) in rice. Xoo is characterized as a rod-shaped, round-ended, Gram-negative bacterium, with individual cells typically ranging from 0.7 to 2.0 µm in length and 0.4 µm to 0.7 µm in width (Benjamin *et al.* 2024). These cells are motile, propelled by a single polar flagellum. The broader *Xanthomonas* genus is notoriously known for its ability to infect at least 350 different plant species, leading to considerable economic losses in agricultural sectors worldwide. As a critical rice pathogen, Xoo specifically causes bacterial blight, resulting in significant reductions in rice yield. (Timilsina *et al.* 2020).

The challenge posed by BLB is further exacerbated by the escalating global demand for rice. Projections indicate a substantial increase in demand, from 439 million tonnes (milled rice) in 2010 to 498 million tonnes in 2020, and potentially reaching 506 to 607 million tonnes by 2050, underscoring the vital need to protect this crop (Seck *et al.* 2012; Darko *et al.* 2025). However, BLB, driven by Xoo, currently causes significant productivity losses globally. At the peak of tillering, the disease can lead to severe yield losses ranging from 20% to 30%, and in some instances, up to a devastating 80%. Beyond yield reduction, Xoo infection at the plant's booting stage degrades grain quality, often resulting in broken kernels (Chuwa *et al.* 2015). The pathogen demonstrates susceptibility to both tropical and temperate climates, particularly flourishing in irrigated and rainfed lowland areas where weeds and infected plant debris provide conducive environments, especially under optimal conditions of 25 to 34 °C and relative humidity exceeding 70% (Naqvi 2019).

Historically, various strategies have been employed to control BLB caused by Xoo, including cultivation techniques, germicidal substances, antibiotics, and the use of antagonistic microorganisms. However, these conventional methods frequently demonstrate limitations in terms of sustained efficacy. Furthermore, the widespread reliance on agrochemicals and antibiotics has given rise to profound environmental concerns, such as pollution and the alarming emergence of antibiotic-resistant bacterial strains.

The application of conventional pesticides, in particular, is associated with numerous negative externalities, including environmental degradation, the development of pest resistance, bioaccumulation of chemicals in the food chain post-harvest, significant biodiversity losses, the resurgence of secondary pests, and the eradication of beneficial

non-target organisms. This extensive and prolonged use of synthetic pesticides, notably during the Green Revolution era, has led to stringent regulatory limits on their commercial farming application, contributing to an annual decline in synthetic pesticide use and a concurrent 10% annual growth in the adoption of biopesticides as substitute agrochemicals. Given the escalating global economy and critical food security issues, there is an urgent need for alternative, effective, and sustainable approaches to manage this pervasive pathogen (Sanya *et al.* 2022; Thakur 2024; Teja *et al.* 2025).

Keeping in mind these challenges, biopesticides present a compelling alternative. Characterized by their low toxicity, environmental friendliness, and precise mode of action that minimizes harm to non-target organisms, biopesticides offer an effective solution to combat the insect resistance problems associated with synthetic chemicals. Biopesticides function through diverse mechanisms, including direct antimicrobial activity, induction of systemic resistance, and disruption of pathogen signaling pathways (Mirian *et al.* 2025). These agents, derived from natural sources such as plants, microbes, and minerals, offer targeted pest control with minimal environmental impact. In the context of rice bacterial leaf blight (BLB), several plant-derived compounds have demonstrated potent activity against *Xanthomonas oryzae* pv. oryzae (Xoo). Notably, essential oils and extracts from species such as *Trachyspermum ammi*, *Azadirachta indica*, and *Mentha spicata* have shown significant inhibition of Xoo growth and biofilm formation. These effects are attributed to bioactive constituents including thymol, γ-terpinene, flavonoids, alkaloids, and sesquiterpene lactones, which interfere with bacterial cell integrity and virulence factor expression (Sahoo *et al.* 2025). The integration of such plant-based biopesticides into disease management strategies not only reduces reliance on synthetic chemicals but also aligns with sustainable agricultural practices.

Moreover, the development of biopesticides from plant leftovers and agricultural waste holds particular promise, offering valuable benefits to local farmers and developing nations (Fenibo *et al.* 2022). This shift towards biopesticide-fueled agriculture is intrinsically linked to socially acceptable practices, fosters economic productivity, and champions environmental stewardship, aligning directly with the tripartite concept of sustainable development embodied within the United Nations 2030 agenda and its Sustainable Development Goals (SDGs). Despite the growing recognition of natural products as sources of biopesticides against various economically important pests, including fungi, bacteria, nematodes, and insects, a notable research gap exists in comprehensively evaluating the specific potential of many weed species against critical crop diseases including rice BLB. While weeds are often perceived as unwanted competitors for resources in agriculture, recent studies underscore their untapped reservoir of bioactive compounds (Katti 2013; Ramli *et al.* 2018).

This study aimed to investigate the biopesticidal potential of six plant species commonly regarded as weeds due to their unintentional growth in agricultural fields and competition with crops for nutrients and space. The selected species *Parthenium hysterophorus*, *Ammi visnaga*, *Chenopodium album*, *Cannabis sativa*, *Amaranthus viridis*, and *Dysphania ambrosioides* were chosen on account of their abundance in rice-growing regions and their documented phytochemical profiles, including flavonoids, alkaloids, terpenoids, and phenolics with reported antimicrobial activity.

EXPERIMENTAL

Collection and Preparation of Weed Samples

Weeds were collected from Malakander Farm (GPS coordinates: 34.020746, 71.468989) using hand gloves and sterile tools to prevent contamination. Collected samples included *Parthenium hysterophorus*, *Ammi visnaga*, *Chenopodium album*, *Cannabis sativa*, *Amaranthus viridis*, and *Dysphania ambrosioides*. The collected weeds were thoroughly washed with distilled water to remove all adhering debris and then shade-dried at controlled room temperature (25 ± 2 °C) for 15 days until completely brittle. Once fully dried, the plant material was ground into a fine powder using a laboratory grinder and immediately stored in airtight, labeled, HDPE plastic containers (250 mL screw-cap bottles) at 4 °C to preserve their chemical integrity until further use.

Extraction of Plant Material

For extraction, 50 g of powdered plant material from each distinct weed species was precisely weighed and mixed with 500 mL of 95% methanol/5% water. The mixture was then subjected to continuous stirring using a magnetic stirrer at room temperature for 24 h to ensure maximum extraction of active compounds. The resulting crude extract was first filtered through multiple layers of muslin cloth to remove large particulate matter, followed by fine filtration using Whatman No. 1 filter paper to obtain a clear filtrate. The filtrate, representing the aqueous weed extract containing the full spectrum of methanol-soluble compounds from the plant material, was then stored at 4 °C for subsequent experimental applications.

Fractionation of Plant Material

Following the initial methanolic extraction and concentration, the obtained crude methanolic residue was re-dissolved in a minimal volume of distilled water (if necessary for partitioning) and then subjected to a sequential liquid-liquid extraction to separate compounds based on their polarity. The suspension of the crude extract was first partitioned with an equal volume of n-hexane in a separating funnel, vigorously shaken for 10 min, and the n-hexane layer (n-hexane fraction) was collected. This process was repeated three times to ensure maximum extraction of non-polar compounds. The remaining aqueous layer was then successively extracted with an equal volume of ethyl acetate, following the same procedure of shaking and collecting the respective solvent layers. This process was also repeated three times. Each solvent fraction (n-hexane and ethyl acetate) was then concentrated using a rotary evaporator under reduced pressure at temperatures below 40 °C to obtain the dried crude fractions. These concentrated fractions were then stored in airtight, labeled containers at 4 °C until further use for experimental applications.

Isolation and Purification of *Xanthomonas oryzae* pv. *oryzae* (Xoo)

Infected rice leaves exhibiting typical bacterial leaf blight symptoms were carefully collected from diseased plants. The collected leaf samples were surface-disinfected by immersing them in 0.1% sodium hypochlorite solution for 30 s, followed by three rinses with sterile distilled water to remove any residual disinfectant. The disinfected leaf tips (~1 cm) were clipped and streaked directly onto freshly prepared Nutrient Agar (NA) medium. Plates were incubated at 28 °C for 48 h. Distinct yellow, mucoid colonies, characteristic of Xoo, were selected and further purified by repeated streaking on fresh NA plates until a pure culture was obtained.

Confirmation of Xoo

The identity of the isolated bacterial strain was rigorously confirmed using a combination of morphological, biochemical, and physiological tests. Gram staining was performed, consistently revealing Gram-negative, rod-shaped bacteria. This was further corroborated by a positive potassium hydroxide (KOH) solubility test. Biochemical confirmation included standard tests, such as gelatin liquefaction, starch hydrolysis, and catalase tests, all performed according to the protocols outlined in Bergey's *Manual of Systematic Bacteriology*.

Preparation of Inoculum

A single, pure colony of Xoo was aseptically inoculated into 50 mL of sterile Nutrient Broth. The culture was incubated at 28 °C in a rotary shaker set at 150 rpm for 24 h to achieve optimal bacterial growth. After incubation, the bacterial culture was centrifuged at 5000 rpm for 10 min to pellet the bacterial cells. The supernatant was discarded, and the bacterial pellet was gently resuspended in sterile distilled water. The bacterial suspension was then adjusted to a final concentration of 108 CFU/mL (Colony Forming Units per milliliter) using a spectrophotometer (N6000 Double Beam UV Visible; Shanghai Yoke, Shanghai, China), with optical density measured at 600 nm.

Evaluation of Antibacterial Activity

Agar well diffusion assay

The antibacterial activity of weed extracts against Xoo was evaluated using the agar well diffusion method. Nutrient Agar plates were uniformly seeded with 100 µL of the Xoo suspension (108 CFU/mL). Wells (6 mm in diameter) were then aseptically punched into the agar using a sterile cork borer. Each well was filled with 100 µL of the respective weed extract. Streptomycin (0.1% w/v) served as the positive control, while sterile distilled water served as the negative control. Plates were incubated at 28 °C for 24 h. The diameter of the clear zone of inhibition around each well was measured in millimeters. All treatments were performed in triplicate to ensure reliability.

Minimum inhibitory concentration

The Minimum Inhibitory Concentration (MIC) of each weed extract against Xoo was determined using a two-fold serial dilution method in Nutrient Broth. Extract concentrations ranged from 100 mg/mL down to 1.56 mg/mL. Each tube containing the diluted extract received 100 µL of the Xoo inoculum (108 CFU/mL). Tubes were incubated at 28 °C for 24 h. The MIC was defined as the lowest concentration of the extract that exhibited no visible bacterial growth, indicating complete inhibition.

In Vivo Evaluation in Pot Experiments

Seed germination assay

Rice seeds (*Oryza sativa* cv. Super Basmati) were surface-sterilized before the germination assay. Seeds were first rinsed thoroughly with sterile distilled water, then immersed in 70% ethanol for 1 min, followed by a 10-min soak in 2.5% sodium hypochlorite solution. Finally, seeds were rinsed five times with sterile distilled water to remove all traces of chemicals. The sterilized seeds were then soaked in plant extracts (1000 mg/mL concentration) for 12 h. Treated seeds were subsequently placed uniformly in petri dishes lined with sterile moist filter paper and incubated in a controlled growth

chamber set at 28 °C with 70% relative humidity (RH). Germination percentage was recorded after 7 days by counting the number of germinated seeds.

Infection Experiment (Leaf Clipping Method)

Rice seedlings at the four-leaf stage were used for Xoo inoculation. These seedlings were transplanted into 2-L volume plastic pots containing sandy loam soil collected from Malakander farm, The University of Agriculture, Peshawar that contained 5% decomposed organic matter. The soil was sterilized prior to use by autoclaving at 121 °C for 30 min to eliminate any pre-existing pathogens that could hinder the experiment. Three plants per pot were transplanted. Plants were maintained under natural light conditions in a greenhouse, watered every two days, and fertilized every two weeks with a standard nutrient solution. After two weeks of plant growth, foliar treatment with extracts was done 24 hours post inoculation. Pathogen inoculation was performed using the leaf clipping method. Sterile scissors were dipped into the prepared Xoo suspension (108 CFU/mL) and used to clip the tips (~1 to 2 cm) of the uppermost leaves. Disease severity, assessed by measuring the lesion length on inoculated leaves, was recorded 14 days post-inoculation.

Experimental Design

The study employed a factorial experimental design to assess the effects of various plant extracts on rice blight. This design involved two main factors namely Factor A = Weeds, which included six different plant species (*Parthenium hysterophorus*, *Ammi visnaga*, *Chenopodium album*, *Cannabis sativa*, *Amaranthus viridis*, and *Dysphania ambrosioides*) and Factor B = Fractions comprising three types of extracts (ethyl acetate, and n-hexane). All the treatments combinations used in the study are given in Table 1.

Table 1. Treatment Combinations Used In the Experiment

T1=PH+ MeOH	T2=AVG+ MeOH	T3=CA+ MeOH	T4=CS+ MeOH	T5=AVI+ MeOH	T6=DA+ MeOH
T7=PH+ EtoAc	T8=AVG+ EtoAc	T9=CA+ EtoAc	T10=CS+ EtoAc	T11 AVI+ EtoAc	T12=DA+ EtoAc
T13=PH+n-Hexane	T14=AVG+n-Hexane	T15=CA+ n-Hexane	T16=CS+ n-Hexane	T17=AVI+ n-Hexane	T18=DA+ n-Hexane

where T= Treatment, PH = *Parthenium hysterophorus*; AVG = *Ammi visnaga*; CA = *Chenopodium album*; CS = *Cannabis sativa*; AV = *Amaranthus viridis*; DA= *Dysphania ambrosioides*; MeOH = Methanol; EtoAc = Ethyle acetate; n-Hexane = n-hexane

Statistical Analysis

All collected data were subjected to one-way analysis of variance (ANOVA) to determine significant differences among treatment means. Statistical analysis was performed using GraphPad Prism v9.3.1. Following ANOVA, Tukey's Honestly Significant Difference (HSD) *post hoc* test was applied to identify specific significant differences between individual treatment means at a significance level of $P < 0.05$. All experiments were conducted in triplicate, and results are consistently presented as the mean ± standard error (SE) of these replicates.

RESULTS AND DISCUSSION

Crude extract yields varied among weed species, with *Cannabis sativa* (1.13 g) and *Amaranthus viridis* (1.03 g) showing the highest quantities, followed by *Chenopodium album* (0.95 g), *Dysphania ambrosioides* (0.73 g), *Parthenium hysterophorus* (0.57 g), and *Ammi visnaga* (0.43 g). These differences are possibly due to inherent biochemical composition, growth conditions, and extraction methods. The higher yields from *Cannabis sativa* and *Amaranthus viridis* suggest their potential as sources of bioactive compounds. This observation on the varying potential and bioactive compounds from these weed species is supported by literature. The current study's findings regarding *Dysphania ambrosioides* align with Zohra *et al.* (2019), who investigated extraction optimization, total phenolic and flavonoid contents and diverse pharmacological evaluations of this species. Similarly, Sarker and Oba (2019) conducted research on the nutraceuticals, antioxidant pigments, and phytochemicals in the leaves of *Amaranthus spinosus* and *Amaranthus viridis* weedy species, which reinforces the current findings on *Amaranthus viridis*. The broader concept of utilizing weeds as a valuable resource is also consistent with the work of Gupta *et al.* (2023). Furthermore, this study's investigation into *Cannabis sativa*'s potential echoes the research by Pino *et al.* (2023), who studied *C. sativa* oils obtained from various varieties and extraction methods, examining their phytochemical characterization and biological activities.

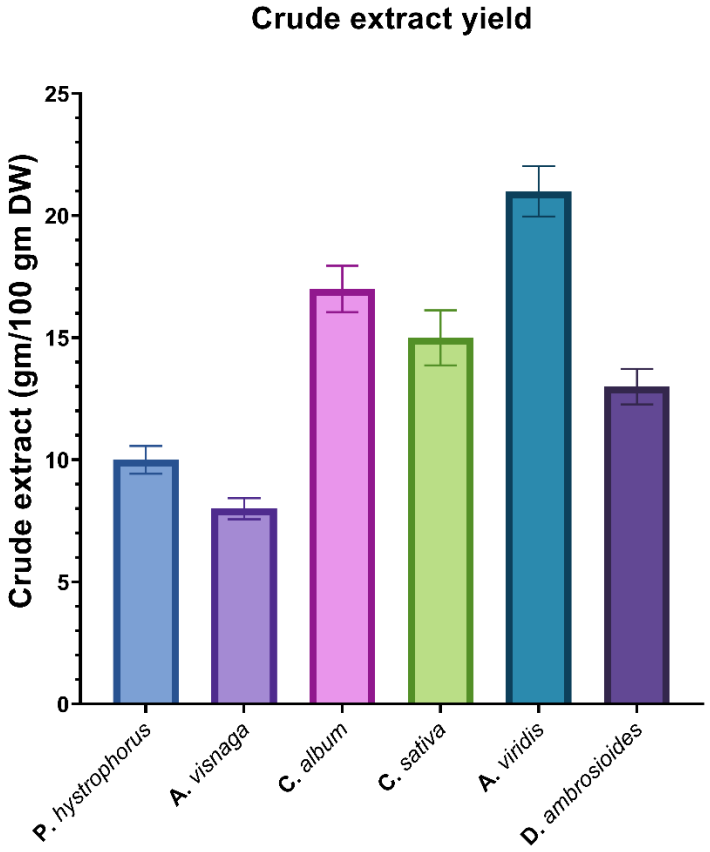

Fig. 1. Crude methanolic extracts yield

Various plant extracts from *Parthenium hysterophorus*, *Ammi visnaga*, *Chenopodium album*, *Cannabis sativa*, *Amaranthus viridis*, and *Dysphania ambrosioides*

demonstrated concentration-dependent antibacterial activity against *Xanthomonas oryzae*. Efficacy varied by plant species and extraction solvent, with specific extracts (*e.g.*, *P. hysterophorus* n-hexane, *A. viridis* ethyl acetate, and *D. ambrosioides* n-hexane) showing potent inhibition. These findings highlighted the presence of diverse bioactive compounds and suggest the promising potential of these natural resources as sustainable alternatives for bacterial disease management in agriculture.

The antibacterial activity of the selected plants were comprehensively evaluated against *Xanthomonas oryzae* (Xoo). The findings are expressed in Fig. 2 and consistently demonstrated that different solvent extracts *i.e.*, crude (methanol), ethyl acetate, and n-hexane exhibited varying degrees of concentration-dependent growth inhibition against Xoo, indicating the presence of diverse bioactive compounds.

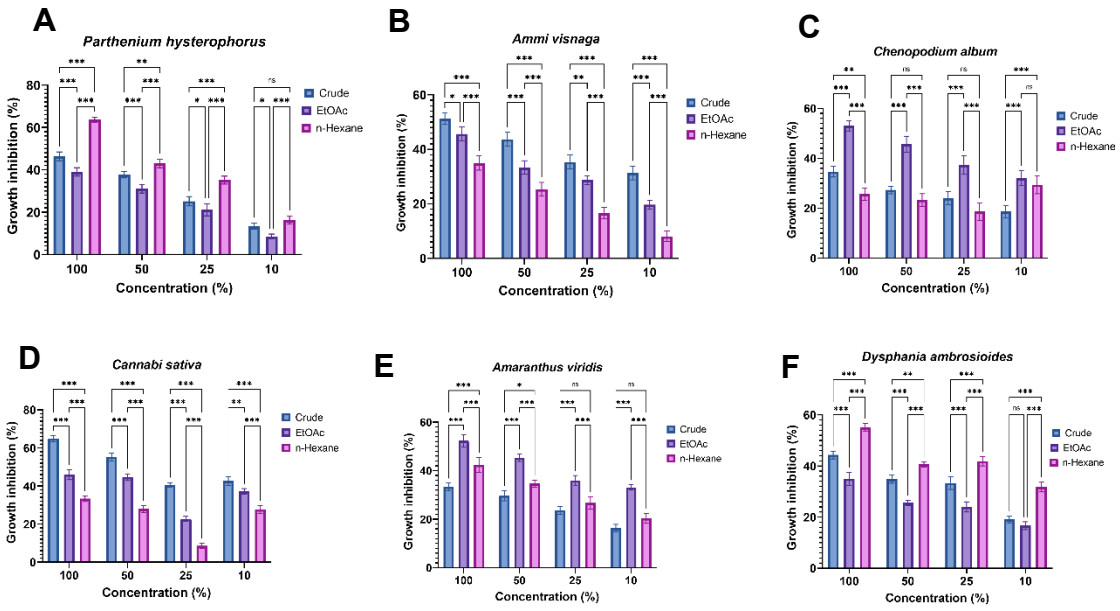

Fig. 2. Antibacterial activity of (A) *Parthenium hysterophorus*, (B) *Ammi visnaga*, (C) *Chenopodium album*, (D) *Cannabis sativa*, (E) *Amaranthus viridis*, and (F) *Dysphania ambrosioides* against *Xanthomonos oryzae* at different concentrations; > 0.005 (ns), 0.033 (*), 0.002 (**), and < 0.001 (***)

Parthenium hysterophorus extracts showed strong antibacterial potential, with the n-hexane extract being the most potent, achieving 63.7% inhibition at 100 ppm, suggesting effective extraction of sesquiterpene lactones and flavonoids. *Ammi visnaga* extracts also displayed significant activity, though the AVG-n-hexane extract presented an exceptionally high and unusual inhibition of 167% at 25 ppm, warranting further investigation into potential cytotoxic mechanisms. *Chenopodium album* extracts exhibited variable inhibition patterns across different solvents, with the ethyl acetate extract generally showing stronger effects, highlighting the influence of extraction method on efficacy. Similarly, *Cannabis sativa* extracts, which are rich in cannabinoids and terpenoids, demonstrated varying levels of antibacterial activity, with the crude (methanol) extract showing a potential dose-response. *Amaranthus viridis* extracts, particularly the ethyl acetate fraction, proved to be potent, with inhibition increasing up to 48.5% at 50 ppm, suggesting its promise as a source of antibacterial agents. Finally, *Dysphania ambrosioides* extracts, especially the n-hexane fraction, exhibited robust concentration-

dependent inhibition, reaching 55.16% at 100 ppm, attributed to compounds like alkaloids and flavonoids.

The current study's findings align with previous research, demonstrating that various plant species possess significant antibacterial activity against *Xanthomonas oryzae*, a key pathogen causing bacterial blight in rice. For instance, *P. hysterophorus* contains sesquiterpene lactones, flavonoids, alkaloids, and phenolic compounds (Adhikari *et al.* 2016), and its extracts show concentration-dependent inhibition of *X. oryzae* while being safe for beneficial microbes (Phatangare *et al.* 2015). Similarly, *A. visnaga* is rich in flavonoids and coumarins (Al-Snafi 2013), and its extracts effectively inhibit *X. oryzae*, suggesting a natural alternative for blight management (Nirmala *et al.* 2017). *Chenopodium album*, with its phytol, linalool, α-terpineol, and linolenic acid content (Khomarlou *et al.* 2018), also exhibits variable but often concentration-dependent growth inhibition of *X. oryzae*, highlighting its potential for disease control (Dubey *et al.* 2014). *C. sativa*, known for cannabinoids, flavonoids, and terpenoids (Liu *et al.* 2022), shows moderate to high antibacterial activity against *X. oryzae*, positioning it as a potential eco-friendly pesticide (Ranjan *et al.* 2017). Furthermore, *Amaranthus viridis* L., containing quercetin, kaempferol, and various hydroxycinnamic acids (Kumar *et al.* 2022), demonstrates considerable antibacterial efficacy against *X. oryzae*, making it a promising source for antibacterial agents (Iqbal *et al.* 2012). Lastly, *Dysphania ambrosioides*, rich in alkaloids, sesquiterpenes, flavonoids, and coumarins (Mwanauta *et al.* 2014), also significantly inhibits *X. oryzae*, supporting its use in crop defense (Zefzoufi *et al.* 2020). These consistent findings underscore the potential of these plant extracts as sustainable solutions for agricultural disease management.

Plant Growth

The plant growth percentages for the six weed species, as influenced by treatments with methanolic, ethyl acetate, and n-hexane extracts, are depicted in Fig. 3, showing variability across three replicates. The plant growth percentages, influenced by methanolic, ethyl acetate, and n-hexane extracts, demonstrated considerable variability across weed species. While crude extracts exhibited a broad range of effects, with *Parthenium hysterophorus* showing significant growth (31 to 70%) and *Ammi visnaga* displaying moderate growth (29 to 48%), it was observed that ethyl acetate extracts generally produced comparable or even slightly enhanced growth outcomes across most species. This suggests a potential growth-promotive effect for these fractions. Conversely, n-hexane extracts showed selective efficacy, notably reducing plant growth in *Ammi visnaga* (24 to 29%) and *Dysphania ambrosioides* (19 to 25%). These differentiated responses highlight the complex phytochemical composition of the weed extracts, encompassing bioactive compounds capable of exerting both growth-inhibitory (*e.g.*, n-hexane fractions on *A. visnaga* and *D. ambrosioides*) and potentially growth-promotive effects (*e.g.*, ethyl acetate extracts). This dual activity underscores their potential utility as natural agents in either weed management or crop growth modulation, respectively, depending on the specific extract and desired outcome. Such compounds could underpin the development of eco-friendly herbicidal or growth-enhancing formulations, supporting integrated pest management strategies (Sultana *et al.* 2024).

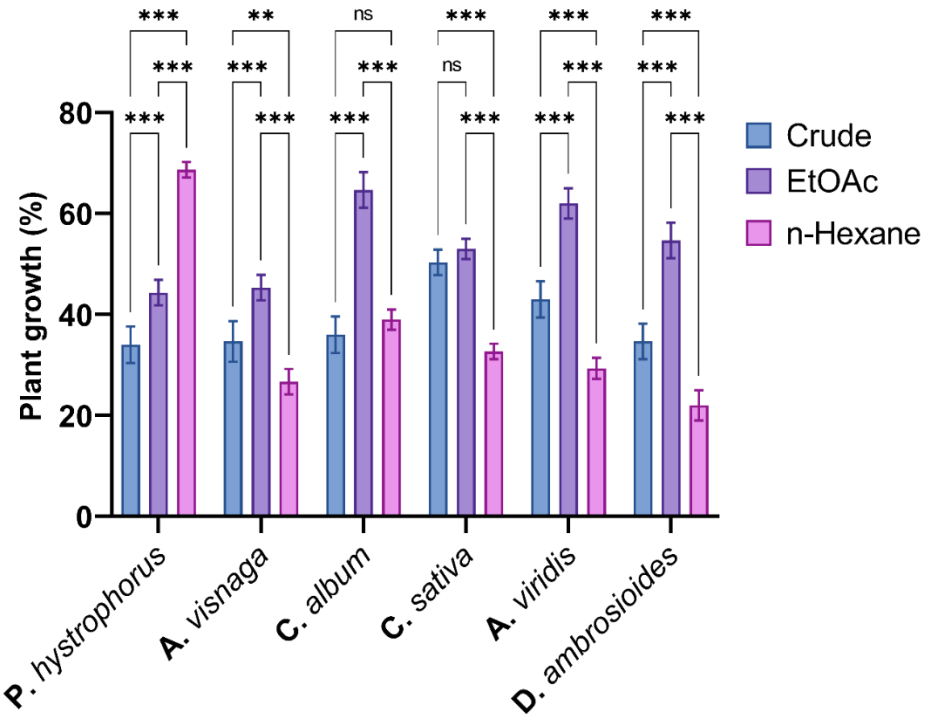

Fig. 3. Plant growth at different concentrations, 0.12 (ns), 0.033 (*), 0.002 (**), and < 0.001 (***)

Xanthomonas oryzae Infection

The impact of various weed extracts (crude, ethyl acetate, and n-hexane) on *Xanthomonas oryzae* infection rates in rice was assessed across three replicates (R1 to R3), as shown in Fig. 4. Infection levels varied significantly, depending on both the weed species and the extraction solvent used. Crude extracts exhibited fluctuating infection rates, with *Parthenium hysterophorus* and *Amaranthus viridis* generally associated with higher infection percentages, while *Dysphania ambrosioides* and *Cannabis sativa* demonstrated comparatively lower infection levels. Notably, treatments with ethyl acetate extracts consistently reduced infection rates relative to crude extracts across all species, highlighting their potential efficacy. Similarly, n-hexane extracts also suppressed infection in several species, particularly *Dysphania ambrosioides*, which showed the lowest infection levels throughout the experiment (Poonpaiboonpipat *et al.* 2021). These compelling results strongly indicate the presence of potent bioactive compounds within these weed extracts with significant antagonistic effects against *Xanthomonas oryzae* infection. The observed suppression of bacterial blight underscores the promising potential of these natural products as alternative or complementary agents in integrated disease management strategies for rice, a crop critically threatened by bacterial blight (Frayco *et al.* 2021; Xue *et al.* 2021).

These findings strongly indicated that weed extracts harbor potent bioactive compounds with significant antagonistic effects against *Xanthomonas oryzae* infection. The suppression of bacterial blight underscored the promising potential of these natural products as alternative or complementary agents in integrated disease management. To fully engage this potential, comprehensive *in vitro* investigations coupled with rigorous isolation and characterization of the active constituents are essential (Frayco *et al.* 2021). Such efforts are critical for advancing sustainable and environmentally friendly strategies

to protect rice, one of the world's most essential staple crops, from the devastating impacts of bacterial blight (Xue *et al.* 2021).

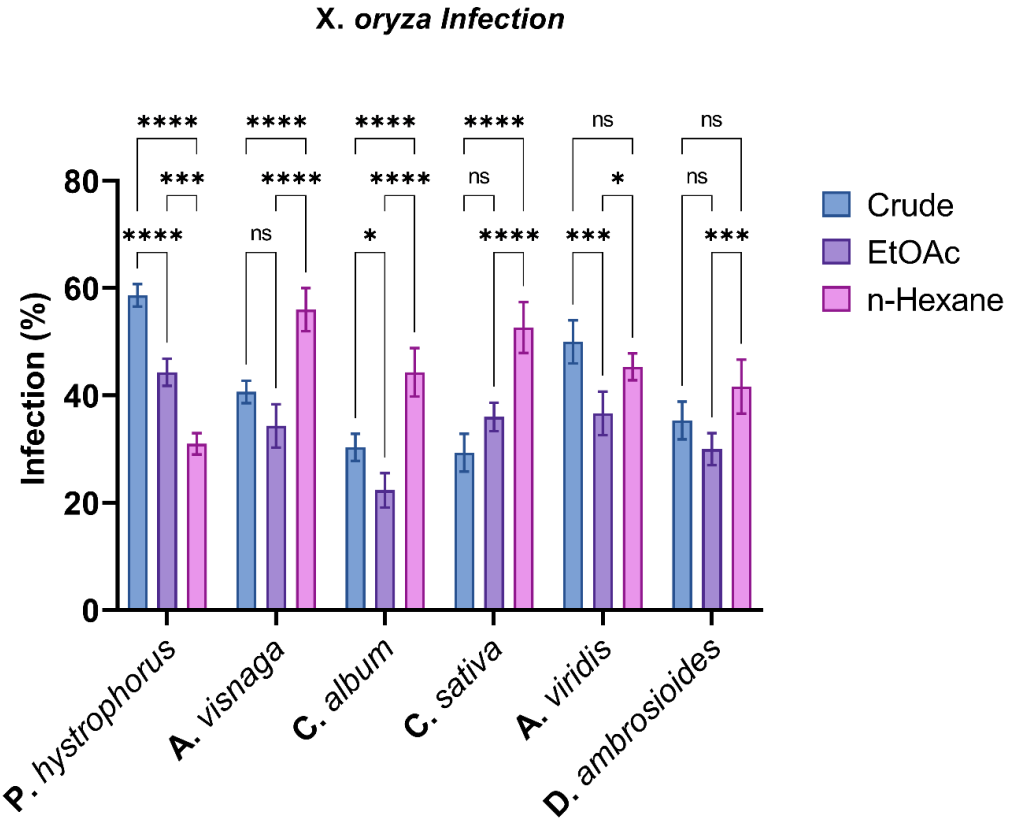

Fig. 4. Percent *Xanthomonas oryzae* infection in rice crop at different concentrations, 0.12 (ns), 0.033 (*), 0.002 (**), and < 0.001 (***)

Chlorophyll Content (%)

The chlorophyll content, which is a vital indicator of photosynthetic efficiency and plant health, was monitored across all the selected extracted. Variation was observed among weed species and solvent types, as depicted in Fig. 5. *Parthenium hysterophorus* consistently exhibited high chlorophyll levels across all extraction solvents (31 to 70%). *Ammi visnaga* showed moderate to low concentrations (24 to 48%), while *Chenopodium album* recorded moderate levels, with notably higher values in the ethyl acetate extract (61 to 68%). *Cannabis sativa* demonstrated moderate to high chlorophyll content (31 to 55%), and *Amaranthus viridis* displayed moderate levels, again with elevated values observed in ethyl acetate (59 to 65%). In contrast, *Dysphania ambrosioides* exhibited comparatively lower chlorophyll content, particularly in the n-hexane extract (19 to 25%).

The observed variation underscores the significant impact of solvent polarity on chlorophyll extraction efficiency, with ethyl acetate demonstrating superior efficacy compared to other solvents. These findings reveal distinct interspecies differences in pigment accumulation, reflecting the combined influence of species-specific physiological traits and solvent chemistry on chlorophyll retention and extractability. Consequently, the results emphasize the necessity of careful solvent selection to optimize pigment recovery and suggest that extraction protocols should be tailored to individual plant species.

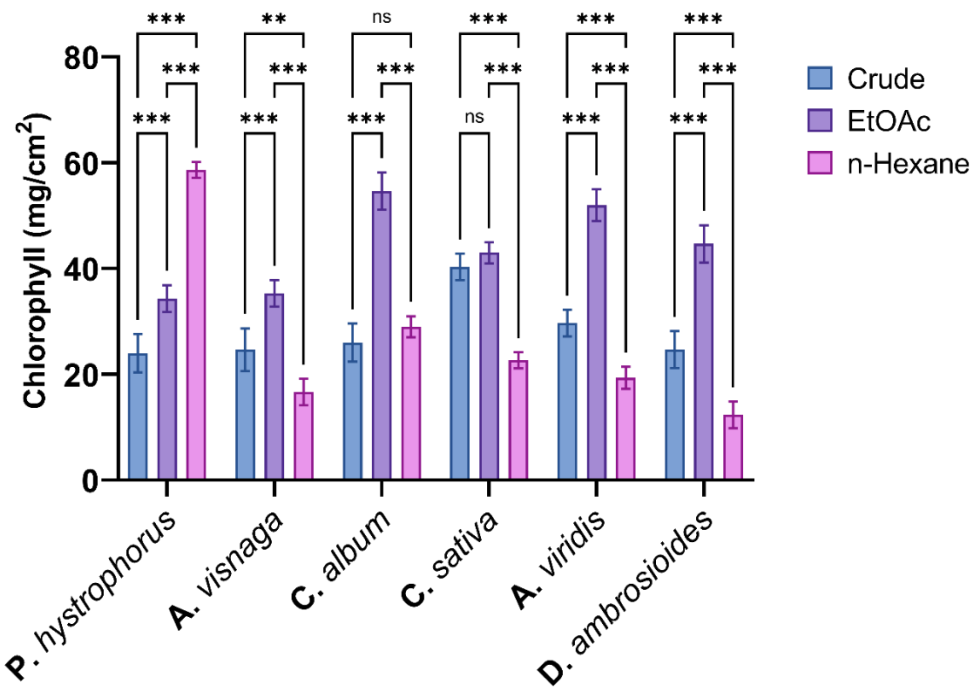

Fig. 5. Chlorophyll content at different concentrations. 0.12 (ns), 0.033 (*), 0.002 (**), and < 0.001 (***)

Relative Electrolyte Leakage

In this study, three various extracts, *i.e.*, crude, ethyl acetate, and n-hexane, were evaluated for their effect on relative electrolyte leakage, which is an indicator of membrane integrity, as shown in Fig. 6. *Parthenium hysterophorus* exhibited the highest leakage in the crude extract (67 to 75%), moderate leakage with ethyl acetate (62 to 69%), and the lowest with n-hexane (42 to 50%). *Ammi visnaga* showed consistently low leakage: crude (32 to 39%), ethyl acetate (38 to 45%), and n-hexane (30 to 35%). *Chenopodium album* displayed moderate leakage in crude (42 to 50%), which decreased with ethyl acetate (25 to 31%) and was lowest in n-hexane (16 to 22%). For *Cannabis sativa*, leakage was moderate to high in crude (58 to 65%) and declined in ethyl acetate (35 to 45%) and n-hexane (28 to 30%). *Amaranthus viridis* also showed a decreasing trend from crude (38 to 45%) to ethyl acetate (25 to 31%) and n-hexane (20 to 23%). In contrast, *Dysphania ambrosioides* exhibited low leakage in crude (15 to 20%), which was slightly higher in ethyl acetate (29 to 35%), and comparable levels in n-hexane (17 to 26%). These results highlight species-specific responses and suggest differential membrane stability effects associated with each extract.

These findings indicated that the type of solvent employed plays a significant role in influencing membrane stability, supporting the potential use of weed-derived extracts as eco-friendly agents for biomanagement, as also suggested by Ahmad *et al.* (2020). The extent of electrolyte leakage varied with solvent type, with ethyl acetate and n-hexane generally inducing lower leakage levels compared to crude extracts. This suggests a possible protective effect of these solvents on cellular membranes. The observed species-specific responses provide valuable insights into variations in membrane integrity among different weed species. However, further investigation is required to elucidate the biochemical mechanisms involved and to assess the practical applicability of these findings

in integrated weed management and crop protection strategies. It should also be noted that these results are context-specific and may not be directly extrapolated to all weed populations or environmental conditions. Furthermore, developing stable and effective biopesticide formulations with controlled release mechanisms is crucial. Finally, extensive field trials are necessary to compare the long-term effectiveness of these weed extracts against conventional synthetic pesticides, assessing their impact on crop health, yield, and overall environmental sustainability.

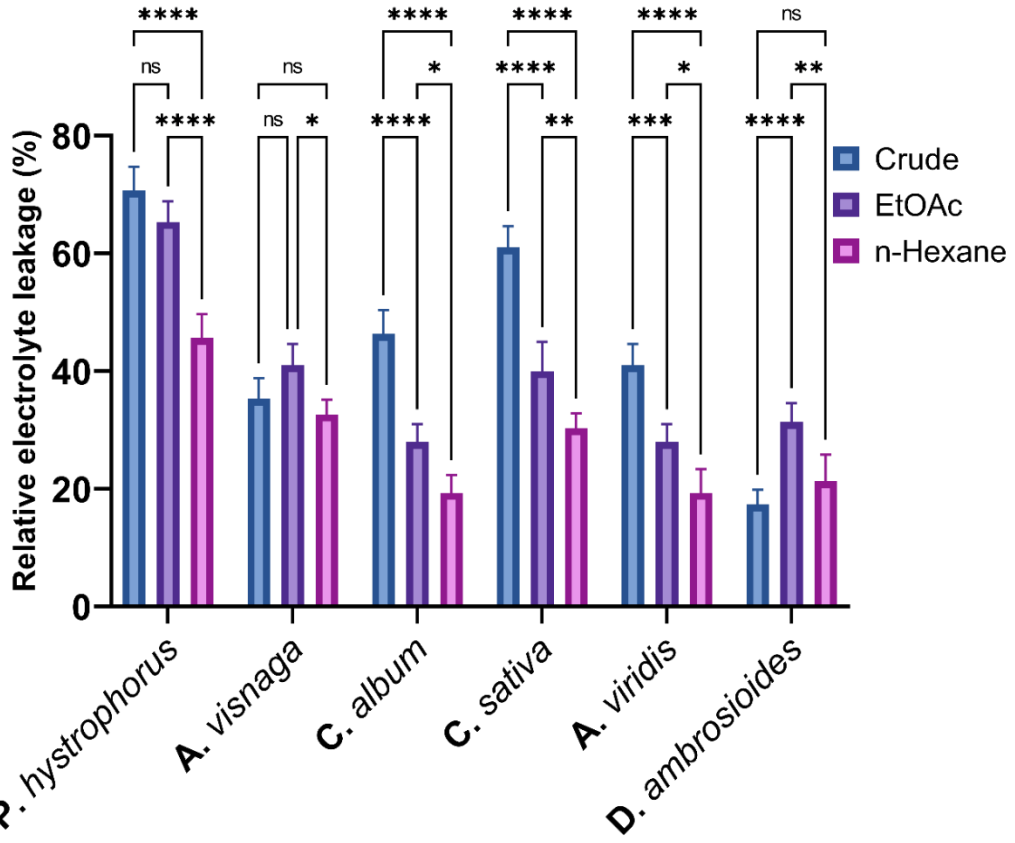

Fig. 6. Relative electrolyte leakage at different concentrations, 0.12 (ns), 0.033 (*), 0.002 (**), and < 0.001(***)

Recommendations

1. Further study is advised, as one of the key limitation of this study is the absence of comprehensive chemical profiling to identify and isolate the active constituents responsible for the antibacterial activity observed in the weed extracts, particularly those from *Parthenium hysterophorus*, *Ammi visnaga*, and *Dysphania ambrosioides*.
2. These weed extracts should also be applied on other cash crops such as wheat that are susceptible to microbial diseases *e.g.* powdery mildew (Ahmad *et al.* 2022) to assess its wider impacts.
3. Exploration is needed of advanced extraction techniques (*e.g.*, ultrasound-assisted or supercritical CO_2 extraction) to enhance the yield and potency of bioactive compounds.

4. It is important to optimize extraction parameters (*e.g.*, solvent concentration, temperature, and time) to maximize efficacy and scalability of promising weed-derived biopesticides.
5. The extracts should be screened against other pathogen in different crops.
6. It is important to investigate formulation strategies to develop stable, cost-effective biopesticidal products for sustainable bacterial leaf blight management.

CONCLUSIONS

This study comprehensively explored the potential of various weed extracts as sustainable biopesticides for controlling bacterial leaf blight in rice, caused by *Xanthomonas oryzae* (Xoo).

1. Significantly, extracts from *Parthenium hysterophorus* (particularly its n-hexane fraction) and *Ammi visnaga* (with its notably high inhibition in the n-hexane extract) demonstrated potent antibacterial inhibition against Xoo.
2. Assessment of membrane damage indicated species-specific responses, with *Parthenium hysterophorus* crude extract exhibiting moderate to high damage, suggesting a mechanism of action.
3. The study observed varied effects on plant growth, with ethyl acetate extracts generally showing beneficial outcomes, indicating their potential for crop growth modulation

Conflict of Interest

The authors declare no conflict of Interest.

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