PLANT-BASED EXTRACTS AS SUSTAINABLE TREATMENTS AGAINST RUST DISEASES IN WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract. Wheat (*Triticum aestivum* L.) is a vital crop that feeds approximately 30% of the global population. Its production is increasingly challenged by rust diseases, including stem rust (*Puccinia graminis* f. sp. *tritici*), leaf rust (*Puccinia triticina*), and stripe rust (*Puccinia striiformis* f. sp. *tritici*). Despite their prevalence, many farmers lack awareness of economical and non-toxic treatments. A susceptible wheat variety (Morocco) was treated with aqueous extracts of *Azadirachta indica*, *Nicotiana tabacum*, *Syzygium cumini*, and *Eucalyptus globulus*. Positive (Nativo) and negative (untreated) controls were included in the study. *A. indica* and *N. tabacum* effectively induced host resistance to leaf rust. *A. indica* at 50% concentration achieved the highest disease control (77.66%) compared to the positive control (73.66%) and negative control (0%). Disease severity was the highest in negative control (96%), followed by the *E. globulus* and *S. cumini* treatments (70.67% and 19.46%, respectively). No phytotoxicity was observed in the treated crops. Plant-based extracts can serve as natural, sustainable treatments to inhibit rust.

Keywords: *susceptible, Puccinia triticina, Puccinia graminis, Nicotiana tabacum, Syzygium cumini, Eucalyptus globulus, Azadirachta indica, Morocco, Nativo*

Abbreviations. AIE: *Azadirachta indica* A. Juss; NTE: *Nicotiana tabacum* L.; EJE: *Syzygium cumini* (L.) Skeels; EGE: *Eucalyptus globulus* Labill; CBF: Chemically treated fungicide (Local); UN: Untreated; TGW: Thousand Grain Weight; PDI: Percentage of disease incident; PDS: Percentage of disease severity

Introduction

Plants are relatively large contributors to a country's agricultural output. Pakistan is characterized with high quality soils in a vast diversity (Sher and Hussain, 2009). Wheat (*Triticum aestivum*) is a basic food source for many Asian countries, such as Pakistan, China, India, and Bangladesh. It has been estimated that global wheat production, on average, is around 750-770 million tons annually with a cultivated area of 220 million hectares whose daily calories are 15% (Balfourier et al., 2019). Local farmers and experts are under pressure due to the increasing need to produce food with rapidly increasing human population (Badar et al., 2023). Rust diseases of wheat are the most important diseases of that crop, and they have been a serious threat to wheat production worldwide. All three types of rust were found to deteriorate crop production; the severity of this disease depends on the stage at which it appears (Duplessis et al., 2021). The severity and infection type also depend on the wheat variety; it is the most serious concern for susceptible varieties of wheat. There are numerous factors responsible for the acceleration of the disease, such as growing conditions, climatic conditions, and methods of cultivation, the most important of which is the selection of the wheat cultivar (Hassan et al., 2022).

Wheat leaf rust is caused by *P. triticina*, which is a very destructive pathogen from South Africa, and it infects wheat under high relative humidity conditions with cool nights and the presence of dew (Nxumalo, 2018). Under these conducive conditions, the disease develops rapidly, causing yield loss due to decreased numbers of kernels per head and lower kernel weights in infected plants. *P. triticina* is now known to be the most significant pathogen in wheat production globally, causing significant yield losses across large geographical areas (Melvin et al., 2008; Figueroa et al., 2018). Rust pathogens are among the most destructive in several important crops, such as maize (*Zea mays* L.), coffee (*Coffea arabica* L.), and wheat (Cui et al., 2020). Wheat is highly susceptible to three different rusts: leaf (*P. triticina*), stem (*Puccinia graminis* Pers.), and stripe (*Puccinia striiformis* var. *striiformis* W. (Barreto and Evans, 1988; Robert, 1991; Cui et al., 2020), each of which can have a devastating effect and reduce yield (Markell et al., 2001).

P. triticina is the biotrophic parasite of wheat, it starts flushing with the suitable conditions required for its growth and spread such as temperature and moisture (Huerta-Espino et al., 2011; Figueroa et al., 2018). Attack by these pathogens became the ultimate cause for the reduction of crop yield and production, which is usually treated with synthetic fungicide solutions. The overuse of these commercial fungicides has numerous negative effects, such as causing unknown illnesses and allergies to people of specific regions having food treated with such chemicals. The most alarming factor induced by the application of these chemicals is that the crop becomes single-quality resistant (Hassan et al., 2022). *P. triticina* can infect a wide range of crops, such as Hordeum vulgare, Secale cereale, and Avena sativa, but *T. aestivum* is the most severely affected crop. Attack of this disease becomes severe with the availability of favorable conditions, such as low temperature (15 -25°C), high humidity, wind, and cultivation of susceptible varieties (Singh et al., 2002).

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There are some other useful methods to treat the pathogenic disease of crops in a safe way like the induction of host resistance and better cultivation strategies instead of common traditional methods. Yet, the most important countermeasure is the application of natural remedies (Temesgen, 2015; Kankwatsa et al., 2017). There are numerous plants with fungicidal compounds that can easily be isolated and can be applied as treatments, such as *A. indica*, *Moringa olifera,* and the bioagent *Trichoderma harzianum*. The application of these biological materials is an interesting and safe way to solve this problem (Shabana et al., 2017). Biocontrol of wheat rust and other diseases is an active control method that could be extensively utilized. It is an effective alternative to biocides that is seeing increased attention (Shahin et al., 2019).

Keeping in view the global food shortage and food crises, an experimental study was designed to introduce a safe alternative to chemical use for the induction of immunity in wheat against leaf rust disease caused by *P. triticina*. The focus of the present study was to introduce a safe and easy tool for farmers so that low scale production would be increased. A study was conducted to support local farmers in the countryside so that they could play a positive role in enhancing the production of food to combat food challenges.

Methodology

Experimental site and particulars

The present experiment was conducted in the greenhouse of the Pakistan Council of Scientific and Industrial Research Institute (PCSIR), Lahore, Punjab, Pakistan (31ºº28'50.48"N, 74º16'30.55"E, 692 feet elevation). The seeds of the wheat cultivar Morocco (a universal check for wheat rust) were provided by Punjab Seed Corporation, Ayub Agricultural Research Institute, Faisalabad, Pakistan. The experiment was conducted according to a randomized complete block design (RCBD) with plot dimensions of 4 m× 4 m keeping plant to plant distance 20-25 cm. There was a total of six treatments: four were plant extracts, one the positive control, and one the negative control. The positive control was treated with a locally available fungicide Nativo by Bayer {active ingredients Tebuconazole (50%) and Trifloxystrobin (25%)} commonly used by local farmers, while the untreated plot was considered as the negative control. All treatments, except the negative control, were applied to the crops through the foliar route at three different concentrations (10%, 20%, and 50%) one week before application of the artificial inoculation of *P. triticina* at the booting stage of the crop. The negative control was also treated again after the appearance of symptoms when the crop was at the heading stage.

Experimental plant material and extract preparation

The four plants, neem [*Azadirachta indica* A. Juss (GC.Herb.Bot.3980)], tobacco [*Nicotiana tabacum* L. (GC.Herb.Bot.3975)], jambolana [*Syzygium cumini* (L.) Skeels (GC.Herb.Bot.3976)], and eucalyptus [*Eucalyptus globulus* Labill. (GC.Herb.Bot.3977)] were selected for leaf extract purposes because of their antifungal activities. Fresh leaves were collected from well-established and healthy plants at the PCSIR and washed with distilled water. All the leaves were crushed into 80 mesh fine powder after shade drying at room temperature, followed by the addition of 100 mL sterilized distilled water to 10 g of each plant sample using a 250 mL conical flask and left for 8 h at 25 °C. Solutions

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were filtered through ashless filter paper, and its stock solution (1:1 mg/mL) was prepared and stored in a refrigerator at 4°C for further use (Iqbal et al., 2020).

Foliar application of plant extracts

Prepared experimental plant extracts were applied through the foliar route on all experimental plants three times, 24 h before and after artificial inoculation of the crop, while the third time the crop was sprayed on the appearance of symptoms.

Preparation of leaf rust disease causing inoculum

For artificial inoculation of *T. aestivum,* isolated and identified strains of *P. triticina* (RR-02) already reported in previous study conducted by Badar et al. (2023) were used. Strains were suspended in an isoparaffin oil solution. The concentration of *uredinia* spores was determined by loading 25 µL of inoculum on a hemocytometer. For convenience, every spore seen on the total grid was marked equal to 100 spores/mL. For homogenous spore suspension, 4.0 g spores were added to 1.0 L dist. H_2O and a few drops of Tween ® 20, followed by adjustment of the inoculum density to 4×10^6 spores using a hemocytometer (Nasr, 1977).

Artificial inoculation of leaf rust disease

All plants were artificially inoculated at Zadoks growth scale 39 and stage 14/15 according to the Joubert Scale (SA). The prepared suspension of *Pt* (*P. triticina*) strains was directly sprayed on leaves at Zadoks growth scale 39 (Zadoks et al., 1975). Autoclaved water was sprayed on the crop after artificial induction of the RR-02 strains, and transparent polyethylene bags were used to cover the plants to maintain 95-100% humidity at a temperature of approximately 15-18 °C (Navathe et al., 2020).

Assessment of disease infestation

Data regarding the percentage of disease incidence and efficacy of plant extracts were recorded after the first appearance of disease symptoms during April 2021. Data regarding disease severity was also assessed according to Peterson et al. (1948). The efficacy (%) of the tested plant extracts for disease control was determined according to the method adopted by Rewal and Jhooty (1985):

$$\text{Efficacy (\%)} = \text{Severity (\%) of the control - Severity (\%) of the treatment/Severity (\%) of the control} \times 100 \quad \text{(Eq.1)}$$

Assessment of yield and its components

Data regarding yield attributes, such as number of tillers, number of grains per spike, and thousand grain weight (TGW), were recorded at maturity. The number of tillers per unit area and the number of grains per spike were counted randomly in each treatment. One thousand grains were counted manually, and their weight was measured. Data were recorded in triplicate and the average was calculated. The increase over the control (%) in the yield components due to the application of each of the tested treatments was determined according to an equation proposed by Calpouszos et al. (1976), as follows:

$$\text{Increase over the control (\%)} = (Y_h / Y_d\text{-}1) \times 100 \quad \text{(Eq.2)}$$

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whereas,
Y_h = Yield of healthy (experimental) plants, and Y_d = Yield of diseased plants (negative control).

Statistical analysis

The data regarding disease incidence and efficacy of plant extracts against leaf rust disease were assessed using the Agricultural Statistics Software Package WASP - Web Agri Stat Package (https://ccari.icar.gov.in/waspnew.html). Obtained results were further verified statistically using Statistic 8.1 for the analysis of variance (ANOVA) with a least significant difference of 5% (Steel et al., 1997). Graphs were prepared using GraphPad Prism.

Results

Efficacy of Plant extracts against leaf rust

Statistically analyzed results of the study showed that disease control (%) was maximum by AIE extract at all concentrations (76.33, 76.66, and 77.66%) followed by NTE at its maximum concentration (75.25%). There was no disease control in the negative control (UN) group compared to that in the positive control (CBF) group (73.66%). There was no such disease control in EJE at all concentrations, in contrast to the other experimental plots and the positive control (*Figure 1*).

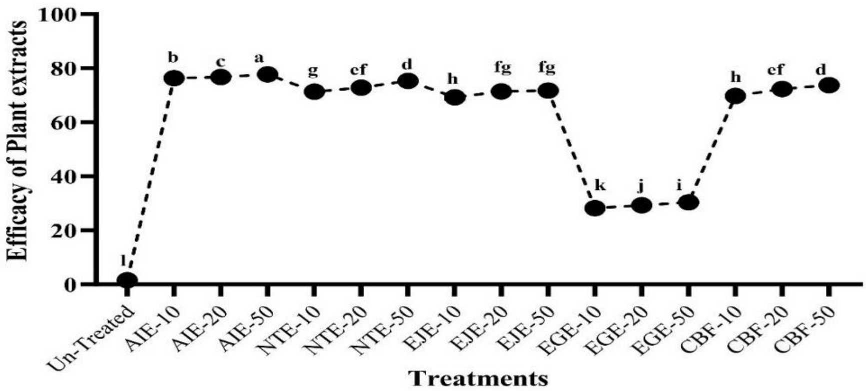

Figure 1. *Efficacy of plant extracts against the attack of leaf rust disease of wheat caused by P.triticinia (Pt). The abbreviations used in the study are as follows: AIE (Azadirachta indica A. Juss), NTE (Nicotiana tabacum L.), EJE (Syzygium cumini (L.) Skeels), EGE (Eucalyptus globulus Labill), CBF (Chemically treated fungicide), and UN (Untreated). All plant extracts were tested at three concentrations: 10%, 20%, and 50%. The efficacy of these treatments was analyzed using Two-way ANOVA, with results represented alphabetically (a–l). Letter 'a' denotes highly significant results, whereas letters b, c, d,e,f and g indicate lower significance compared to the AIE-treated crop (at 50 %). Letters, i, j, k, and l represent non-significant findings*

Percentage of disease incidents

The percentage of disease incidence was highest among untreated plots (96%), followed by EGE (70 %). Plants treated with AIE, NTE, and EJE were resistant, as the percentage of disease severity was less than 20% (*Figure 2*).

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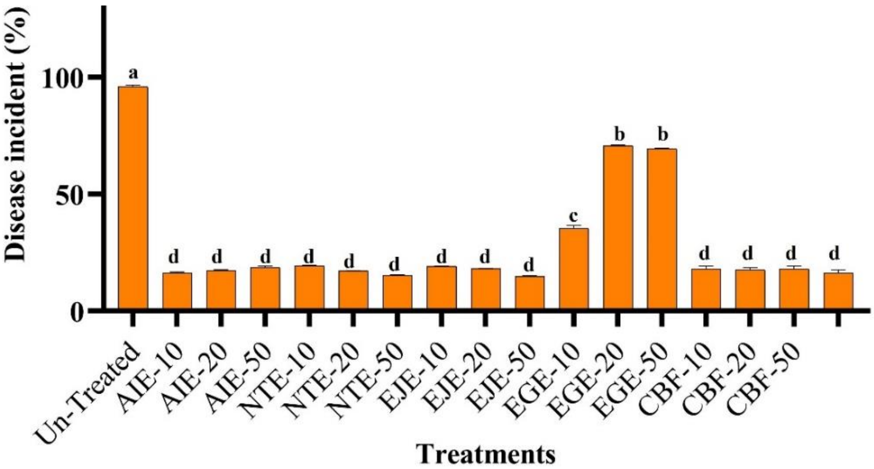

***Figure 2.** Percentage of disease incident (PDI) after the application of plant extracts. Figure illustrates the PDI observed after treating crops with various plant extracts at concentrations of 10%, 20%, and 50%. Statistical analysis was performed using Two-way ANOVA, and results are denoted by letters a–d to indicate significance levels:a: High PDI, b: Moderate PDI, c: Low PDI and d: Negligible PDI*

Growth attributes

The highest number of tillers was observed in the crop treated with AIE at 20% (357.33), while the lowest number of tillers was observed in the untreated crop (302.33). Data collected from the control plot (positive control) clearly demonstrated the impact of chemical fungicide on the number of tillers in comparison to the experimental plots and negative control (*Figure 3*). The number of tillers was higher in positive control (317.33) than in the negative control (302.33). Similar results were observed in the case of grains/spike, with plots having a greater number of infected tillers (un-treated) yielding the lowest grains/spike (36.16), followed by chemically treated plots (36) at all concentrations. The highest grains/spike were observed in AIE and NTE at 50% concentration (40.85 and 40.22) followed by EJE at 50% concentration (40.18) (*Figure 4*). A clear difference in thousand-grain weight (TGW) was observed among all treatments (*Figure 5*). The highest TGW was observed in AIE-treated plots at all concentrations (44.95, 45.45, and 47.77 g). The lowest TGW was observed in CBF when it was administered at 50% concentration (27.74 g) and in the untreated plot (30.72 g). TGW was also high in NTE and EJE at all concentrations, in contrast to un-treated plots (41.90, 43.61, 44.03 and 40.61, 41.14, 32.44 g). A higher percentage increase over the control for all tested growth attributes was observed in AIE- and NTE-based extracts.

Phytotoxicity

There were no signs or symptoms of phytotoxicity in any of the experimental crops. Observations revealed healthy plant growth and development in all the treatment groups. The absence of leaf discoloration, stunted growth, or other visible abnormalities further confirmed the lack of phytotoxic effects. This suggests that the applied treatments or experimental conditions did not negatively affect the physiological processes of the crops under study. The observed results indicate that experimental treatments were well tolerated by the crops, potentially allowing for their safe application in agricultural practices. The absence of phytotoxicity is particularly encouraging, as it suggests that the

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treatments may be utilized without compromising crop health or yield. Further long-term studies should be conducted to assess any potential cumulative effects and to evaluate the impact of treatments on crop productivity and quality.

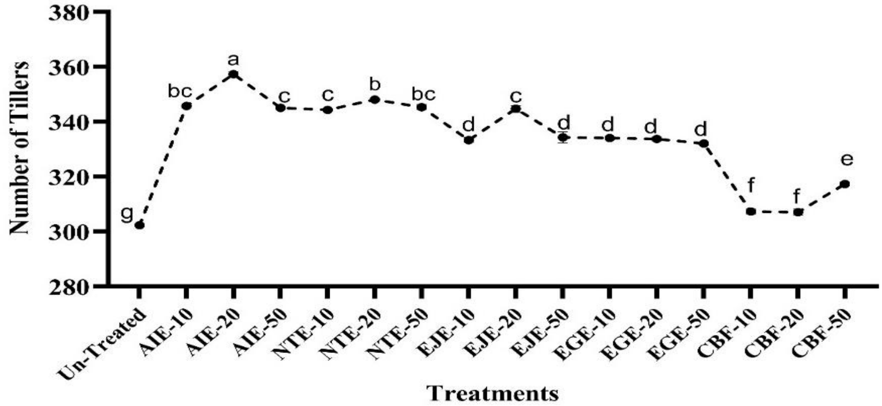

Figure 3. *Effect of Plant Extracts on number of Tillers m^{-2}. In this study, plant extracts from Azadirachta indica A. Juss (AIE), Nicotiana tabacum L. (NTE), Syzygium cumini (L.) Skeels (EJE), and Eucalyptus globulus Labill. (EGE) were evaluated alongside a chemically treated fungicide (CBF) and an untreated control (UN). Each extract was applied at concentrations of 10%, 20%, and 50%. The efficacy of these treatments was assessed using Two-way ANOVA, with results denoted by letters a - g. A result marked with 'a' indicates a highly significant effect, while 'b', 'c', and 'd' denote progressively lower levels of significance compared to the AIE-treated crops (at the conc of 20%). Letters 'e', 'f', and 'g' represent non-significant outcomes. This lettering system helps identify which treatments differ significantly from others, facilitating a clear understanding of the relative effectiveness of each plant extract*

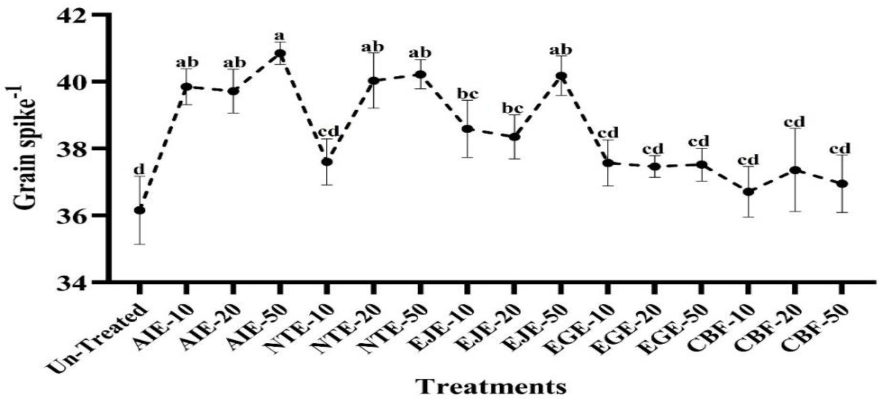

Figure 4. *Effect of Plant Extracts on number of grains/spikes. Where AIE: Azadirachta indica A. Juss, NTE: Nicotiana tabacum L., EJE: Syzygium cumini (L.) Skeels, EGE: Eucalyptus globulus Labill., CBF: Chemically treated fungicide, UN: Untreated. All the experimental plant extracts were applied at three different concentrations 10, 20 and 50%. Collected data for the efficacy of plant extracts were assessed through Two-way ANOVA, and the results represented alphabetically a-d. Letters 'a' indicate that the recorded results were highly significant, whereas letters 'ab' indicate that the level of significance was moderate in contrast to the AIE-treated crop (at 50%). The letters 'd, cd, and bc' represents non-significant results*

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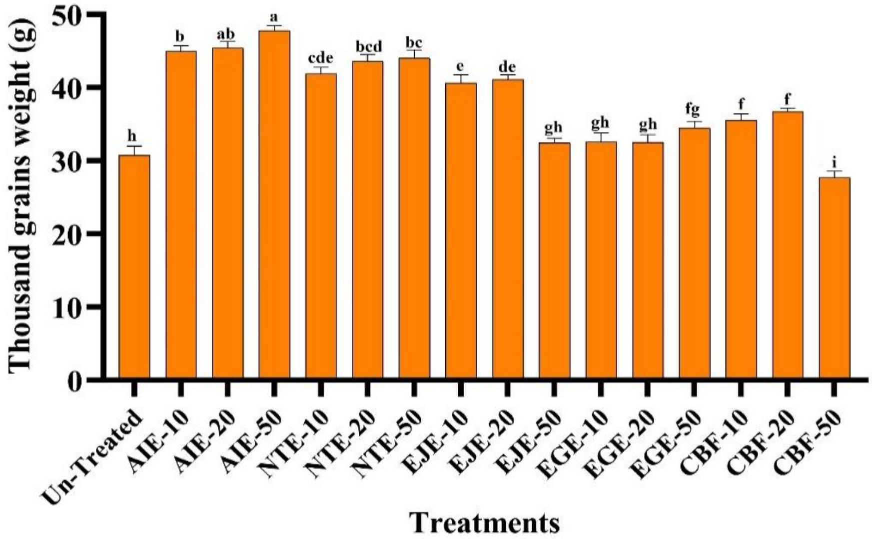

Figure 5. *Effect of Plant Extracts on Thousand grain weight (TGW) g. In this study, plant extracts from Azadirachta indica A. Juss (AIE), Nicotiana tabacum L. (NTE), Syzygium cumini (L.) Skeels (EJE), and Eucalyptus globulus Labill. (EGE) were evaluated alongside a chemical fungicide (CBF) and an untreated control (UN). Each extract was applied at concentrations of 10%, 20%, and 50%. Efficacy data were analyzed using Two-way ANOVA, with results denoted by letters a–i. Treatments marked with 'a', 'b', or 'ab' exhibited high significance, while those labeled 'cde, 'bcd', 'bc' moderate singificance while 'de', 'gh', or 'f' showed lower significance compared to others. And the letters 'i' or 'h' indicated non significant results*

Discussion

The results of the present field trial were in favor of the objective of the study, to overcome the yield losses of wheat crops through safe routes due to pathogenic diseases to fight with increasing issues of world hunger. There are numerous plants with the potential to fight these deadly pathogens in food crops. Wheat is the most common problem for wheat production around the world, which is very crucial to deal with. The use of chemical-based solutions is recommended to solve the yield- and growth-related problems of wheat crops in case of rust attack, which is not safe and economic (Rashad et al., 2012; Moni et al., 2016). Several studies have been carried out to highlight the use of chemical fungicides for controlling wheat disease, but due to health-related concerns, the negative and toxic impact of these solutions cannot be neglected, and they are also not economic on large and small scales (El-Sharkawy et al., 2018). There are different solutions to control the attack of rust on wheat, such as the use of commercial fungicides, cultivation of modern varieties, induction of resistance through genetic modifications, and better farming practices (Bariana et al., 2001). However, all these solutions have certain limitations, such as toxic effects on crops, which can lead to severe human diseases, and loss of traditional/old wheat varieties due to the cultivation of elite cultivars. Therefore, the most desired step towards better agriculture is the introduction of eco-friendly tools in agriculture, which can help to attain not only better production but also protection. Plants are a gift of nature due to their potential ingredients, which could easily solve this problem. Being easily available with properties to be used easily, plants are becoming the medium for choice to induce resistance among susceptible crops. Studies conducted by experts have highlighted the use of several microorganisms, such as

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bacteria, algae, and fungi, against these diseases for the development of host resistance (Shabana et al., 2017). It has been reported that plants are blessed to have metabolites that can play a better role in the induction of host resistance among crops when applied as pesticides and fungicides (Han et al., 2018). Plants are used as a natural solution to fight such diseases without imparting toxic or deteriorating effects on crops (El-Gamal et al., 2022). Not all plants are recommended for use as agricultural solutions against these pathogens because of their toxic effects; therefore, the use of any plant for solving agricultural problems is not recommended (El Khetabi et al., 2022).

Literature of published studies clearly represents an outstanding figure of the effectiveness of plant extracts against fungal pathogens; plants are marked to have a reasonable margin for the induction of immunity. The use of plant extracts against plant pests is an excellent medium of choice because they can increase the production of enzymes (POX and POD) that can aggregate phenolics and antioxidants, which are natural agents of plant defense mechanisms (Pathak et al., 2019). The outcomes of the present study are in line with those of a study conducted by Shabana et al. (2017), suggesting that plant extracts of *A. indica* A. Juss were 100% effective in controlling wheat rust disease caused by *P. triticina*. The findings of this study are 100 % in support of the present study because the route of application of plant extracts against wheat rust was foliar (Shabana et al., 2017). Moreover, it has been reported by numerous experts that plant extracts contain many active agents with wider abilities against phytopathogens. *Azadirchin, Artemesium, Caratenes, Emodin* and *Eucalyptolin* had been considered to cause inhibitory effects against pathogens. Phenolic compounds such as eugenol, carvacrol, and thymol are the most common plant components and are potent inhibitors of plant diseases caused by pathogens (Al Jumaili et al., 2018). Neem is a member of *Meliaceae*, which has more than 35 physiologically active constituents, such as combine and azadirachtin (Sujarwo et al., 2016). Leaf extracts of neem have shown outstanding control of wheat rust disease, with an increase in growth and yield (Afzal et al., 2023).

Conclusion

Based on the results of the present field trial, it can be concluded that plant extracts effectively induce immune responses in wheat crops, providing significant protection against rust diseases. These treatments are economical, easy to prepare, and safe for the environment, offering enhanced disease control and improved crop productivity without toxic effects. The use of plant-based extracts, such as *Azadirachta indica* and *Nicotiana tabacum*, demonstrated remarkable efficacy in reducing disease severity and enhancing natural resistance in treated crops. This approach not only minimizes reliance on synthetic chemicals but also promotes sustainable agricultural practices, making it a viable and eco-friendly solution for managing wheat rust diseases.

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Author's contribution. Conceptualization, investigation, data collection, and writing—original draft, R.B, A.A, M.M, and A.A. Software, script evaluation, and editing, I.N, I.A, M.S.A, H.R, R.I. and M.L. Writing—review and editing, R.B, A.A, M.M, and A.A, I.N, I.A, M.S.A, H.R, R.I. and M.L. Data curation and formal analysis, M.S.A. Visualization and validation, M.S.A., R.I. Methodology and references, R.I. Supervision, R.B. Funding Acquisition, M.L., and R.I. All authors reviewed the manuscript and agreed for final publication.

Data availability. All the data related to this work can be sourced from the corresponding authors.

Competing interests. The authors showed no relevant financial or non-financial interests to disclose.

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