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Original article

Grasp of wheat leaf rust through plant leaves extract and bioagent as an eco-friendly measure

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ABSTRACT

The efficiency in the treatment of leaf rust of wheat was examined for plant leaf extracts of Neem and Moringa at varied concentrations of 50, 100, and 150 ml correspondingly. All treatments decreased fungal growth in vitro by greater than 90%. The germination of spores was decreased by 91.99% in the presence of neem leaf extract at 150 ml concentration. The percentage of pustules/leaf was reduced by foliar spray of the same treatments on seedlings of the wheat plant. The wheat plants show the greatest response against the pathogen of leaf rust by plant extract second foliar application on the 4th day of infection. Spray application of 150 ml, 100 ml of neem leaf extracts, and 150 ml of Moringa leaf extracts at wheat seedlings, rust development completely prevented four days after leaf rust inoculation The application of treatments of all extract on wheat plants at the mature stage significantly reduced the disease (ACI, average infection coefficient) and increase the efficacy of plant extracts application as compared to control but Neem 150 ml treatment was most effective in all. The chlorophyll and phenol content in wheat plants represented a higher increase. In the case of the first or second-spray application, while the grain yield components of 1000 kernel weight had improved, the second-spray application was more efficient in that respect. To manage leaf rust disease in Bahawalpur, it may thus be inferred that plant extracts are beneficial for a safer biological control compared to synthetic fungicides.

1. Introduction

Leaf rust caused by the pathogen *Puccinia* has more potential to threaten the yield of wheat by up to 10% as compared to the other two types of rust stem and yellow rust. The severity of the disease depends upon the developmental stage of the plant and its susceptibility of the plant (Duplessis, et al., 2021). The variability of wheat growing conditions favorable climatic patterns, and genomic polyploidy nature it becomes the most financial and nutritional crop (Hassan, et al., 2022). The FAO report on the annual global supply and demand for wheat is ample for wheat production (FAO). The population development rate is increasing, which is estimated at 9 billion by 2025. To meet the demand for staple food the production of wheat is an important interest for its significance turn out (Schnurbusch, 2019).

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The favourable condition for rust to infect the plant at high relative humidity with a temperature range of 15–22 °C with optimum temperature for spore's development is 20 °C in only 6–8 h for the development of disease. Leaf rust infections can cause yield losses beyond 50 percent at even earlier stages. The damage to rust in leaves sometimes is less spectacular than that produced by strip (yellow), but as leaf rust frequently occurs the total yearly losses worldwide are likely more than the others. (Huerta-Espino et al., 2011).

Rust challenges the yield of the wheat crop so chemicals are used in terms to prevent and treat the disease incidence, but the chemical nature of the synthetic fungicide has a worse effect on the health and metabolic life of humans and animals. It also makes wheat single-quality varieties resistant to the pathogen (Hassan, et al., 2022). Some measures are proving very helpful to overcome the losses caused by Leaf rust followed by researchers as well as field workers including cultivars susceptibility, variety genetic resistance, climatic interaction, microorganism disease transmission capacity, and field management process (Temesgen, 2015). Some plants have pathogens-toxic substances that are isolated from plants and administered to diseased plants.

The compounds are referred to as botanical or botanical pesticides. Plant extracts including neem (*Azadirachta indica*, A. juss), moringa (*Moringa olifera*), and bioagent *Trichoderma herzianum* are commonly utilized in botanical substances. The biologist is interested to control the leaf disease severity by non-chemical method therefore researcher's main concern is to elevate the effect of synthetic fungicide causing environmental and biological pollution by bioagents and eco-friendly plant extracts. A plant-like Neem *Azadirachta indica*, Moringa, has an inhibitory effect on leaf rust (Shabana et al., 2016). The bioagent used as an antagonistic organism like Trichoderma species have been used as biological control agents for over 70 years, but just now have strains been widely available. Many strains of Trichoderma have been found to have potential use under biological control, especially *T. harzianum*, *T. viride*, and *T. virens* (Monte and Liobell, 2003).

These biocontrol agents are known to be natural having no side effects on human life but having a clear and effective result on the targeted organism. The bioagent is safe to use against diseases like wheat leaf rust due to its eco-friendly nature, cheapest, and ease to access. The research study is designed to assess the vulnerability of commercial wheat varieties against leaf rust caused by *Puccinia triticiana* and its biocontrol by the natural bioagents Trichoderma and plant leaf extracts. It may be a concern for future to work on the active substance of the used biocontrol agents to cope up the economic loss of wheat.

2. Material and method

Plant extracts of two plant species, including Neem leaves (*Azadirachta indica*), Moringa (*Moringa oleifera*) and Bioagent, (*Trichoderma harzianum*), were tested in the field conditions of regional Agriculture Research Institutes to determine their effectiveness as an inducer of materials to resist the infection of leaf rust. Fresh leaves of Neem and Moringa were taken from the field. Plant samples were cleaned to clear dust with tap water. Three different concentrations of 50 ml, 100 ml, and 150 ml of plant leave extract were made. For this purpose, take 50 g, 100 g, and 150 g of fresh leaves in pre-chilled mortar and pistil in chilled distilled water. All the plant leaf extracts were filtered with the help of a muslin cloth. The concentrated filtrates were diluted with distilled water to make a final stock solution of 50 ml, 100 ml, and 150 ml, respectively. For *Trichoderma herzianum* application the culture of *Trichoderma herzianum* was obtained from the Plant Pathology Department, Regional Agricultural Research Institute, Bahawalpur. The spore suspension of *Trichoderma harzianum* is prepared by adding 10 g of *Trichoderma herzianum* in 1 L of water with 50 ml of Limonoil. The Limonoil was used for its adhesive and wetting property that make the spore suspension more effective (Seng, et al., 2014). The stock solution is then stored at 4 °C.

For the experimental trial Morocco (Susceptible check) was sown in a single row of 2 m length with 30 cm row to row spacing to determine the efficacy of treatment in different concentrations. The inoculation of uredospore of *Puccinia triticiana* to wheat plant at two stages mainly at the seedling and booting stage (Shabana et al., 2017). The dusting method was used in the volume of 20 g talcum powder and 1 g of *Puccinia triticiana* spores at high temperature and humidity. Before dew began, dusting was performed at sundown. Inoculation was performed at the 7–8th stage of development. Distilled water was sprayed on the untreated controls The disease evaluation was recorded after observation based on the average coefficient of infection (ACI). The foliar spray was applied to the disease check-in in Morocco.

2.1. Foliar spraying treatment on wheat seedlings

Foliar treatment of plant extract of Neem and Moringa and *Trichoderm harzianum* a microbial plant biostimulant was tested on the wheat plant at the seedling stage. The inoculation was done at the seedling stage according to (Tervet and Cassel, 1951).

The foliar spray was done in Morocco due to its highly susceptible nature. For each treatment of extract, 1st spray was applied one day before inoculation. The second spray was applied after four days of inoculation. The residual effect of treatment was observed after 15 days of urediniospores inoculation. The control is only treated with water and an irrigation schedule. The disease severity, type, and many pustules were observed and noted according to Cobb's scale method after every interval of treatment.

2.2. Foliar spraying of Trichoderma herzianum and plant extracts under field conditions

The foliar spray of bioagent *Trichoderma herzianum* and plant extract of Neem and Moringa leaf extract in different concentrations were applied to the mature plant of wheat. The efficacy of all treatments on plants under field conditions was carried out in the experimental farm of Plant Pathology, RARI, Bahawalpur. The first spray was applied after the emergence of uredospores on the wheat plant. The second spray was done at 7 days interval after inoculation of the first disease incidence. A split-plot design was implemented for two sprays with three replicates. 1st and 2nd spray were applied on the main plot while subplot. The untreated control was represented by a subplot. Disease evaluations were carried out based on Johnston and Browder infection types of leaf rust (1966) where host response showed symptoms with infection type (Table 1). Disease severity (DS) was multiplied by constant values of infection type to get the average coefficient of infection (ACI). R = 0.2, MR = 0.4, MS = 0.8, and S = 1.0 were chosen as constant values for infection types. This method was used to classify the different kinds of infections (Johnston and Browder, 1964).

$$\mathrm{ACI} = DS \times IT$$

where DS is the disease severity and IT is the infection type to find the average coefficient of infection (Kumari et al., 2013). To find the disease severity the cobb's scale method is used.

After disease assessment, the efficacy of treatments applied would be checked by using the formula adopted by Rewal and Jhooty (1985). To evaluate the efficacy of plant leaves extract and bioagent *Trichoderma herzianum* on the mature stage of the wheat plant.

Table 1
Host response and infection type of leaf rust.

Infection type	Symbol	Host response	Symptoms
Highly Resistant	R	No uredia present	No symptom present
Resistant	HR	Some spores with necrosis	Necrotic surround the uredia
Moderately Resistant	MR	Medium sizer uredia with chlorosis and necrotic flecks	Yellow to brown flecks around the spores
Moderately Resistant moderately susceptible	MRMS	Small and medium-sized uredia with chlorosis	Powdery brown spores associated with chlorosis
Moderately Susceptible	MS	Medium to large uredia with no necrosis but some possible chlorosis	Spores on the leaf are brown
Susceptible	S	Large uredia with no chlorosis	The spores are very virulent enough to cause economic loss

$$\text{Efficacy} = \frac{C - T}{C} \times 100$$

where to find efficacy, C is the percentage of infection in control and T is the percentage of infection in the treatment.

2.3. Plant growth attributes

To estimate the plant height, spike length and root length the treated and untreated plant was measured from collar to the plant where the stem grows to the topmost level of the plant with the help of measuring tape. The 1000 kernel weight of wheat is determined at the harvesting stage for assessing the yield component as an indicator of yield value.

3. Biochemical analysis

3.1. Chlorophyll content

Fresh plant material of 0.25 g was chopped into 10 ml of 95% ethanol and grind in a pistil and mortar. Centrifuge the mixture at 3000 rpm for 10 min. Separate the supernatant by adding 95% ethanol to make a solution of 25 ml in a volumetric flask. Take absorbance at 663 nm, 645 nm, and 653 nm for chlorophyll *a* and *b* and total chlorophyll content respectively by spectrophotometer (Mehak, et al., 2021).

The following calculation will be done to calculate the total chlorophyll concentration.

$$\text{Chlorophyll } a = 12.7A_{663} - 2.69A_{645}$$
$$\text{Chlorophyll } b = 22.9A_{645} - 4.68A_{663}$$

whereas A_{663} is the absorbance at the wavelength of 663 nm. While A_{645} is the absorbance at the wavelength of 645 nm.

$$\text{Total chlorophyll} = \text{Chlorophyll } a + \text{Chlorophyll } b.$$

3.2. Phenolic content

0.25 g of plant leaf material was taken by adding 95% ethanol and grinding in pistil and mortar to make the solution of 25 ml in a test tube. Add 5 ml of folin ciocalteau reagent that is diluted 10 times with distilled water (Bungtongdee, et al., 2019). Add 5 ml of 7.5% sodium bicarbonate was added into the test tube. Gallic acid was used as a reagent grade. It was dissolved in 1 ml distilled water to make the stock solution. The stock solution is performed to make differently concentrated solutions Incubate the test tube at 25 °C for 20 min to complete the reaction. Then the absorbance was measured at 760 nm by using a spectrophotometer.

Fomula for gallic acid conc. from calibration/ standard curve

$$\frac{x = y - 0.03096}{0.0061} \therefore y = absorbance$$

Formula to calculate total phenol content.

$$C = \frac{C_1 \times V}{m}$$

where C_1 is the concentration of Gallic acid, V is the volume of extract and m is the weight of the plant.

3.3. Statistical analysis

Statistical analysis of the research parameters was evaluated with statistics by analysing the ANOVA test (Analysis of variance). Pairwise comparison of the parameters followed by least significant difference (LSD) test at $P \leq 0.05$. the graphical representation of data was presented by Microsoft Excel software.

4. Result and discussion

4.1. Effect of foliar spraying on wheat seedlings

The efficacy of plant leaf extracts and bioagents on the seedling stage shows that 150 ml conc. of Neem at the interval of four days of the application shows more effectiveness than 15 days of application which is 91.563% (Table 2). After Neem, *Trichoderma herzianum* was the most effective treatment show 84.55% efficacy in the plant response against leaf rust. Among all the concentrations of the biocontrol agent, the response of the plant at 4 four days intervals was more valuable as compared to the spray which was applied after 22 days interval. The Moringa with 50 ml concentration showed the least effective response when compared with other treatments and time intervals.

4.2. Effect of foliar spraying on mature plants under field conditions

The foliar spray effect of Neem, Moringa, and *Trichoderma herzianum* at the different concentrations on the mature plant under field conditions was checked. The average co-efficient of infection (ACI) shows that all treatment applied has a significant relation with the reduction of disease while at 2nd spray Neem 150 ml conc. *Trichoderma herzianum*, Neem 100 ml conc. and Moringa 150 ml concentration show 3.820%, 5.623%, 12.49% and 15.80% ACI respectively.

The efficacy of plant leaf extract and bioagent *Trichoderma herzianum* was checked to reduce the disease severity of wheat. When comparing it with ACI the high efficacy of Neem 150 ml concentration 82.020% reduces the ACI of leaf rust by 3.59% (Table 3) this shows that at high conc. of neem, the disease reduces with mean efficacy of 80%.

All the treatments have shown an effective role in efficacy as well as ACI and they also improve the 1000 kernel weight by 8.05%, 8.78%, 10.68, and 12.81% over the control. The yield parameters of 1000 kernel grain improve significantly with both sprays of the different intervals (Table 3), but the most effective results were obtained from the 2nd spray. Under field conditions, the second application of spray with Neem 150 ml concentration shows more effective control followed by *Trichoderma herzianum* and Neem 100 ml concentration. While Moringa with all concentrations has the least effective increase.

Table 2
Efficacy of plant extracts and bioagent *Trichoderma herzianum* on wheat seedling.

Treatment	One day interval	4 days after the interval	22 days after the interval	Mean Efficacy after the interval
Neem 50	54.980	64.230	71.227	63.479 F
Neem 100	75.500	77.780	80.300	77.860 C
Neem 150	87.650	91.540	95.500	91.563 A
Moringa 50		61.540	68.540	60.027G
Moringa 100	60.980	66.600	73.650	67.077 E
Moringa 150	70.000	73.440	78.300	73.913D
Trichoderma herzianum	80.000	84.550	88.000	84.183 B
Control	0.0000	0.0000	0.0000	0.0000 H
Mean	59.889 C	64.960 B	69.440 A	

Table 3
Effect of foliar spraying on efficacy, ACI and kernel weight of mature plants under field conditions.

Treatment	Efficacy 1st spray	Efficacy 2nd spray	ACI 1st spray	ACI 2nd spray	kernel weight 1st spray	kernel weight 2nd spray	Difference	Increase
Neem 50	39	44.24	48.8	44.608	33.32	34.65	1.33	3.99
Neem 100	66.55	71	13.38	11.6	37.773	41.09	3.317	8.78
Neem 150	79.78	82.02	4.044	3.596	38.32	43.23	4.91	12.81
Moringa 50	24	26.1	76	73.9	32.01	35.43	3.42	10.68
Moringa 100	43	50	57	50	34.95	37	2.05	5.86
Moringa 150	59	62	16.4	15.2	36	38.77	2.77	7.69
Trichoderma herzianum	71.32	77.5	5.736	4.5	37	39.98	2.98	8.05
Control	0	0	87	87	31.54	31.78	0.24	0.760938

LSD at 0.05 = 1.5941.
LSD for treatment = 0.88327.
1000-grain weight (Test weight).

4.3. Biochemical analysis

4.3.1. Chlorophyll content

The chlorophyll content shows that all the treatment shows an active response in increasing the total chlorophyll of the treated plant as compared to the control.

Trichoderma herzianum has significant interaction with the biochemical product of plants by increasing the photosynthetic activity of the plant. The photosynthesis activity increased by 0.45, 0.49, and 0.52 at 150 ml conc. of Moringa, 150 ml Neem, and *Trichoderma herzianum* respectively (Table 4). On the other hand, the Neem at 50 ml concentration shows minimum chlorophyll content as compared with the control (Fig. 1).

4.3.2. Phenolic content

The total phenolic content at 0.25 g dry weight of wheat leaf. There is a variation in results showing at different conc. Of plant extract and plant bio stimulant *Trichoderma herzianum.* All the bio-agents show positive results while Neem 50 ml concentration shows a minimum increase or less than the control. The increase of total phenolic content by Moringa 150 ml concentration, Neem 150 ml concentration, and *Trichoderma herzianum* by 3.56 mg, 7.3 mg, and 8.1 mg, respectively (Table 5, Fig. 2).

Table 4
Efficacy of plant extracts and bioagent Trichoderma herzianum on chlorophyll content.

Neem:	Sample dry weight	Total chlorophyll content
(Conc. 50 ml)	0.25 g	0.31
(Conc. 100 ml)	0.25 g	0.4
(Conc. 150 ml)	0.25 g	0.49
Moringa:		
(Conc. 50 ml)	0.25 g	0.43
(Conc. 100 ml)	0.25 g	0.46
(Conc. 150 ml)	0.25 g	0.45
Trichoderma Harzianum	0.25 g	0.52
Control	0.25 g	0.3

5. Discussion

To control rust disease the scientist, urge many managements of chemical, genetic, farming practices, and biological control (Harish et al., 2008). For eco-friendly measures, the earth reverses its system to a natural system. The application of plant products and bioremediation agents for plant protection against disease has recently become more important due to their widely accessible nature, bioagents, microorganism as a biostimulant, antibacterial activity, simple biodegradability, phytotoxicity, and generating host resistance. Numerous researchers have previously shown that certain plants have antifungal action against several plant diseases like leaf rust. Many bacterial and fungal strains are useful to control the disease. The fundamental processes of disease control by plant parts extracts are not apparent, although the participation of inducing resistance is considered (Shabana et al., 2017).

Several publications (El-Sharkawy et al., 2018) reveal that extracts of plants are efficient biocontrol agents against a variety of diseases in plants. Plants are capable of synthesis of secondary aromatic metabolites such as quinones, phenols, phenol acids, tannins, flavones, flavonoids and coumarins (Han et al., 2018). The phenolic structural components, such as eugenol, carvacrol and thymol, have been very potent against infections.

These groupings exhibit antibacterial action and act as strategies for plant protection against harmful microorganisms (Al-Jumaili, et al., 2018). Meliaceae plants, in particular neem, have at least 35 physiologically active components, the most important of which are combine and azadirachtin (Sujarwo et al., 2016).

The Neem plant extract shows a compatible response against leaf rust with about 98.99% efficacy when applied at a mature stage of wheat under field conditions in one and two sprays at the 4-day interval. While in seed soaking with Neem extract and at the seedling stage foliar application reduces leaf rust infection (ACI) and increases the efficacy of plant extract in controlling the disease. The use of Neem plant leaf extracts is also effective in increasing the 1000 kernel weight, spike length and plant height (Shabana et al., 2017).

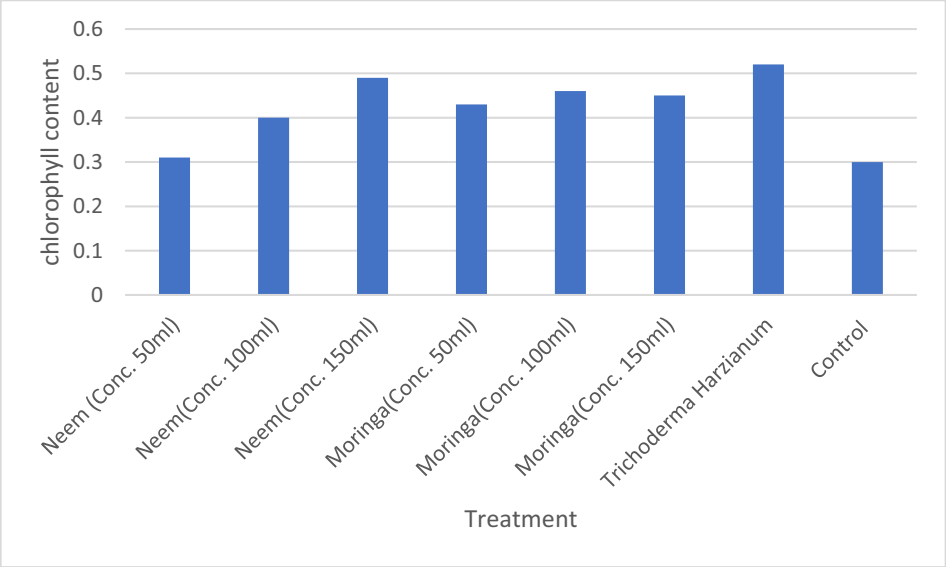

Fig. 1. Efficacy of plant extracts and bioagent *Trichoderma herzianum* on chlorophyll.

Table 5
Efficacy of plant extracts and bioagent Trichoderma herzianum on phenolic content.

Neem	Sample dry weight	Total phenolic content
(Conc.50 ml)	0.25 g	0.81 mg
(Conc. 100 ml)	0.25 g	1.37 mg
(Conc. 150 ml)	0.25 g	7.3 mg
Moringa		
(Conc.50 ml)	0.25 g	1.08 mg
(Conc. 100 ml)	0.25 g	1.22 mg
(Conc. 150 ml)	0.25 g	3.56 mg
Trichoderma harzianum	0.25 g	8.1 mg
Control	0.25 g	0.92 mg

Trichoderma herzianum is a beneficial microorganism used as an antagonist to many microorganisms' diseases like Leaf rust. It is also used to enhance the fertility of the soil, as a biopesticide, and as a biostimulant. These bioagents are non-polluting, low-cost, and non-hazardous, and may be made with readily available processes and materials. A second response in the improvement of the host defensive mechanism can be the type of action of abiotic inducers to prevent plant disease.

The bioagents *Trichoderma harzianum* was used to address the suppression of disease severity for three types of disease including stem rust, powdery mildew, and leaf blotch in different sowing dates trial (El-Mougy et al., 2020). The bioagent showed reduction in disease as compared to the control group. Infield experiment the *Trichoderma herzianum* increase root development and growth by the absorption of more nutrient from the soil. As compared to the control group the treatment with *Trichoderma herzianum* showed efficacy in 1000 kernel grain weight, spike length, a number of rootlets and tiller number also increase.

The rise in wheat production was comparable to the studies of Sallam Nashwa et al. (2008), who discovered that the preparation of Trichoderma spp. treatments increased the vegetative yield of bean plants when contrasted to infected controls.

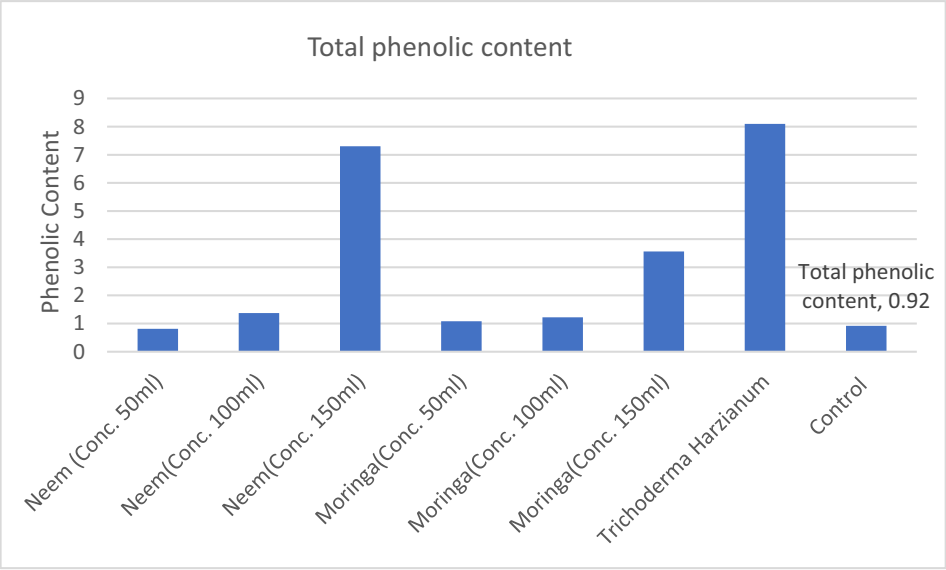

Fig. 2. Efficacy of plant extracts and bioagent *Trichoderma herzianum* on phenolic content.

6. Conclusion

The organic control of rust using plant extracts is an advanced alternative rust management technique that is current, advanced, and risk-free. Two plant leaf extracts at different concentrations and bioagent *Trichoderma herzianum* were tested to check leaf rust disease of the wheat (Neem, Moringa with 150 ml, 100 ml and 50 ml concentration). In the sowing stage, Neem extract reported a spore germination inhibition of 98.99% and a mature wheat plant of 82.90%. Spray on Wheat seedlings treatment with the plant extracts reduced the number of pustules/leaves and increased the effectiveness of foliar spraying. Neem extract was the most efficient treatment in this respect.

When administered one or four days after inoculation, it offered 82.90% control of the illness. After 22 days of inoculation, pustules started to emerge on control, but no pustules on neem 150 ml concentration, followed by *Trichoderma herzianum*, and moringa 150 ml concentration extracts. A foliar spray was the most efficient in decreasing leaf rust (ACI) infection and the neem extract at the mature plant stage. The applied treatment also shows a significant increase in photosynthesis, chlorophyll and grain production. Resistance to leaf rust in Pakistan's wheat cultivars has been shown in current results.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Al-Jumaili, A., Kumar, A., Bazaka, K., Jacob, M.V., 2018. Plant secondary metabolite-derived polymers: a potential approach to develop antimicrobial films. Polymers 10 (5), 515.

Bungtongdee, N., Sopalun, K., Laosripaiboon, W., Iamtham, S., 2019. The chemical composition, antifungal, antioxidant and antimutagenicity properties of bioactive compounds from fungal endophytes associated with Thai orchids. J. Phytopathol. 167 (1), 56–64.

Mehak, G., Akram, N.A., Ashraf, M., Kaushik, P., El-Sheikh, M.A., Ahmad, P., 2021. Methionine-induced regulation of growth, secondary metabolites and oxidative defense system in sunflower (*Helianthus annuus* L.) plants subjected to water deficit stress. PLoS One 16 (12), e0259585.

Hassan, A., Akram, M.U., Hussain, M.A., Bashir, M.A., Mostafa, Y.S., Alamri, S.A.M., Hashem, M., 2022. Screening of different wheat genotypes against leaf rust and role of environmental factors affecting disease development. J. King Saud Univ.-Sci. 34 (4), 101991.

Duplessis, S., Lorrain, C., Petre, B., Figueroa, M., Dodds, P.N., Aime, M.C., 2021. Host Adaptation and Virulence in Heteroecious Rust Fungi. Annu. Rev. Phytopathol. 59 (1), 403–422.

El-Mougy, N.S., Khalil, M.S., El-Gamal, N.G., Abdel-Kader, M.M., 2020. Impact of grain treatments with bioagents for suppressing foliar diseases severity of three wheat cultivars under field conditions. Arch. Phytopathol. Plant Protect., 1–17

El-Sharkawy, H.H., Rashad, Y.M., Ibrahim, S.A., 2018. Biocontrol of stem rust disease of wheat using arbuscular mycorrhizal fungi and Trichoderma spp. Physiol. Mol. Plant Pathol. 103, 84–91.

FAO, 2009. Agribusiness handbook: Wheat flour. Rome. (Available at http://www.fao.org/docrep/012/al376e/al376e.pdf).

Han, J.W., Ruiz-Garcia, L., Qian, J.P., Yang, X.T., 2018. Food packaging: A comprehensive review and future trends. Compr. Rev. Food Sci. Food Saf. 17 (4), 860–877.

Harish, S., Saravanakumar, D., Radjacommare, R., Ebenezar, E.G., Seetharaman, K., 2008. Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. BioControl 53 (3), 555–567.

Huerta-Espino, J., Singh, R.P., Germán, S., McCallum, B.D., Park, R.F., Chen, W.Q., Bhardwaj, S.C., Goyeau, H., 2011. Global status of wheat leaf rust caused by *Puccinia triticina*. Euphytica 179 (1), 143–160.

Johnston, C.O., Browder, L.E., 1964. Seventh revision of the international register of physiologic races of *Puccinia recondita* f. sp. Tritici.

Monte, E., Liobell, A., 2003. Trichoderma in organic agriculture. In: Proceeding V World Avocado Congress (pp. 725–733).

Rewal, H.S., Jhooty, J.S., 1985. Differential response of wheat varieties to systemic fungitoxicants applied to control *Ustilago tritici* (Pers.) Rostr.

Sallam Nashwa, M.A., Abo-Elyousr, K.A.M., Hassan, M.A., 2008. Evaluation of Trichoderma species as biocontrol agent for damping-off and wilt diseases of Phaseolus vulgaris L. and efficacy of suggested formula. Egypt J. Phytopathol. 36 (1–2), 81–93.

Schnurbusch, T., 2019. Wheat and barley biology: Towards new frontiers.

Seng, J., Herrera, G., Vaughan, C.S., McCoy, M.B., 2014. Use of Trichoderma fungi in spray solutions to reduce Moniliophthora roreri infection of Theobroma cacao fruits in Northeastern Costa Rica. Rev. Biol. Trop. 62 (3), 900–907.

Shabana, Y.M., Abdalla, M.E., Shahin, A.A., El-Sawy, M.M., Draz, I.S., Youssif, A.W., 2017. Efficacy of plant extracts in controlling wheat leaf rust disease caused by Puccinia triticina. Egypt. J. Basic Appl. Sci. 4 (1), 67–73.

Sujarwo, W., Keim, A.P., Caneva, G., Toniolo, C., Nicoletti, M., 2016. Ethnobotanical uses of neem (*Azadirachta indica* A. Juss.; Meliaceae) leaves in Bali (Indonesia) and the Indian subcontinent in relation with historical background and phytochemical properties. J. Ethnopharmacol. 189, 186–193.

Temesgen, B.G., 2015. Identification, genetic studies and molecular characterisation of resistance to rust pathogens in wheat.

Tervet, I., Cassel, R.C., 1951. The use of cyclone separation in race identification of cereal rusts. Phytopathology 41, 282–285.