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Research Article

Understanding the influence of applying plant extracts and microorganism culture filtrates against barley leaf rust disease

B.R. MOHDLY[1], Fatmah AHMED SAFHI[2], M.A. ABOU-ZEID[3]*, A.A. ABDEL-FATTAH[1], Ameina S. ALMOSHADAK[4], Enas A. ALMANZALAWI[4], Tahani M. ALQAHTANI[4], Diaa ABD EL MONEIM[5], Rana A.M. ELESSAWY[1]

[1]*Barley Dis. Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt; eng_gazar@yahoo.com; amratef01065515637@gmail.com; rana.elessawy@ufl.edu*
[2]*Princess Nourah bint Abdulrahman University, College of Science, Department of Biology, P.O. Box 84428, Riyadh 11671, Saudi Arabia; faalsafhi@pnu.edu.sa*
[3]*Wheat Dis. Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt; m.abouzeid@arc.sci.eg (*corresponding author)*
[4]*King Abdulaziz University, Faculty of Science, Department of Biological Sciences, Jeddah 21589, Saudi Arabia; ealmanazalawy@kau.edu.sa; talkahtani@kau.edu.sa; aalmashdaq@kau.edu.sa*
[5]*Arish University, Faculty of Environmental Agricultural Sciences, Department of Plant Production (Genetic Branch), El-Arish 45511, Egypt; dabdelmoniem@aru.edu.eg*

Abstract

Rust fungi are devastating plant pathogens, and several *Puccinia* species have exerting substantial financial impacts on global barley cultivation. Fungicides are used on a large scale as an effective method for combating phytopathogenic fungi. The negative environmental impacts of fungicides are steadily escalating on a daily basis. Consequently, researchers are currently exploring alternative approaches to mitigate the use of fungicides, such as the utilization of plant extracts. This method has proven effective due to its incorporation of natural antifungal substances. Among the nine natural elicitors that were tested, the application of plant extracts on barley seedlings resulted in an increase in the incubation and latent periods of *Puccinia hordei*. These periods are integral components of partial and induced resistance, effectively mitigating the incidence of barley leaf rust disease by over 70% on mature plants. Similarly, the biochemical analyses demonstrated a notable augmentation in all the tested treatments' overall phenolics and oxidative enzyme activities (peroxidase and polyphenol oxidase). Random amplified polymorphic DNA (SCoT) test serves as a viable approach for assessing the impact of plant extracts and microorganisms on barley plants. The results obtained from this study indicate that the detection of DNA polymorphism through SCoT analysis holds a significant potential powerful tool to evaluate genetic changes compared with untreated plants although some of them tested displayed high similarities at the morphological reaction.

Keywords: *Hordeum vulgare*; leaf rust; *Puccinia hordei*; induced resistance; plant extracts; SCoT; polyphenol oxidase (PPO); peroxidase (POX); total phenolics

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Introduction

Barley (*Hordeum vulgare* L.) is the fourth most significant cereal crop plant worldwide (Eshghi and Akhundova, 2010). It follows wheat, rice, and maize (Raikwar, 2013) and contributes approximately 7% to the overall production of cereals worldwide. Barley is commonly employed as a feed source for poultry and cattle and subsequently finds application in the production of malt and beverages. Only 5% of the overall production is utilized for human consumption (Singh *et al*., 2016; Sapkota *et al*., 2023). It is grown in the winter season, particularly in the Egyptian rainy areas (North Coast), New Valley, North and South Sinai, Delta, and South Egypt. The crop is commonly referred to as a "poor man's crop" due to its low input requirement and better adaptability to drought, salinity, alkalinity, and marginal lands (Verma *et al*., 2012). This cereal is adapted to dry areas characterized by erratic rain and poor soil fertility, often described as low-input barley production systems (Gyawali *et al*., 2018). The production of barley faces a multitude of biotic and abiotic factors. Barley rust diseases are widely identified as a significant biotic limitation to the improvement of barley production. The prevailing rust disease affecting barley on a global scale is leaf rust, caused by the fungal pathogen Puccinia hordei. Currently, this rust disease of barley holds significant global importance, as it is widely prevalent in barley-growing regions across Europe, the USA, North Africa, New Zealand, Australia, certain parts of Asia, and the Andes region of South America (Dickson, 1956; Mathre, 1982; Sayed *et al.,* 2019) In susceptible cultivars, the infection caused by *P. hordei* can lead to a substantial reduction in barley yield, with potential losses of up to 30% (Griffey *et al*., 1994; Whelan *et al*., 1997; Bai *et al.,* 2022) which result in significant yield reduction and poor grain quality (Mathre, 1997). Leaf rust is a prevalent and highly destructive rust disease in Egypt, known for its tendency to escalate into epidemic proportions. Barley rust, a recurring phenomenon in Egypt's barley-growing regions, significantly reduces grain yield. This impact is particularly pronounced in the Northern areas of the Delta, where disease development is facilitated by favorable environmental conditions, such as high relative humidity (El-Orabey *et al*., 2017; Mehnaz, 2021). the utilization of barley cultivars resistant to diseases has proven to be an effective strategy in disease management and mitigating potential reductions in crop yield. The leaf rust fungus has the ability to generate novel races characterized by the potential to overcome the plant's inherent resistance (Murray *et al*., 2009; Bai *et al.,* 2022). Additionally, it is worth noting that the utilization of synthetic fungicides has been found to have adverse effects on the environment (Barro *et al*., 2017). For example, during and after the application of pesticides, a substantial amount of pesticides could end up in soil, ground- and surface water or air during and after the application of pesticides. Furthermore, there is a growing concern regarding chemicals due to their adverse effects on human health, other organisms specifically targeted, and their behavior and persistence within the natural environment (Jespers, 1994). Consequently, alternative methods are developed to reduce the use of fungicides (Dubey *et al*., 2008). Natural products have proved to be potential sources of environmentally safe antimicrobial agents, which could be beneficial to plant protection and plant disease control (Wang *et al*., 2004). Previous studies have demonstrated the efficacy of botanical extracts in managing barley leaf rust through the activation of a defense mechanism in the affected plants (Chakraborty and Chakraborty, 2010; Srivastava *et al*., 2011). There has been considerable interest in the investigation of the efficacy of botanical extracts in preventing plant diseases through their antifungal activities (Morsy *et al*., 2011; Bhuvaneshwari *et al*., 2015). The potential mechanism of action of abiotic inducers against plant pathogens involves acting as a secondary messenger to enhance the host defense mechanisms (Geetha and Shetty, 2002). This can be achieved by potentially increasing the activity of peroxidase (POX), polyphenol oxidase (PPO) isozymes, and phenolic compounds (Hassan *et al*., 2007). In addition, abiotic inducers have been found to augment resistance by exerting direct effects on the development and survival of the pathogens (Khan *et al*., 2003). Biological control agents such as *Trichoderma* spp. and *Bacillus* spp. can also be used for managing barley rusts (El-Sharkawy *et al*., 2015; Huang and Pang, 2017; El-Sharkawy *et al*., 2018). During the cell cycle, DNA undergoes conformational

and structural changes, the results confirmed the effects of using of plant extracts and microorganisms on DNA instability and the yield components. The present study aimed to evaluate the use of some biological control against i.e. eight plant extracts and two microorganisms to control leaf rust disease in barley Also, evaluation was conducted to estimate the effect of different treatments on the significant increase of the grain yield and its components; An ultimate goal of this study was to assess the diversity among by this control agent barley plants using SCoT analysis on DNA extracted from each treatment, to barley plants.

Materials and Methods

Plant materials and preparation of inocula

The experiment was conducted at Agricultural Research Stations located in Sakha and Giza, ARC, Egypt, over the course of two consecutive growing seasons (2021/2022 and 2022/2023). The highly susceptible barley cultivar to stem rust *Puccinia horde*, *i.e.,* 'Giza 123', was used to evaluate the capacities of some plant extracts (8), two microorganism culture filtrates, one green alga extract, and two fungicides to activate and induce host resistance, at seedling and adult stages. Eight-day-old seedlings eight days old were grown in 7 cm diameter clay pots at a rate of 10 seeds per pot under greenhouse conditions. The maximum temperature was 20 °C, falling to a minimum of 15 °C at night under 14 h natural daylight. The first formed leaves' upper and lower surfaces were sprayed with the elicitor solutions. After 48 h elicitor spraying, leaves of seedlings were inoculated by a suspension of *Puccinia hordei* uredospores (25 mg of spores /100 ml of water) according to Walters and Murray (1992) and Cao *et al*. (2014 a). The control plants were sprayed with distilled deionized water. Subsequently, the inoculated plants treated with natural products (Table 1) were kept inside and incubated in moist chambers for 24 h to provide the high relative humidity that allows the rust spores to germinate and cause infection. Then, the disease assessment was carried out. The seedling experiment was laid out in a randomized complete block design (RCBD) with three replicates for each treatment.

At the adult plant stage, the grains were sown in random plots (2×3 m^2) at the rate of 8 lines/plot. The agricultural practices were implemented in accordance with the recommended guidelines for growing barley. Additionally, the experiment was surrounded by a 1 m belt of a mixture of highly susceptible cultivars, *i.e*., 'Gus'.

Table 1. Extracts of eight plants, two microorganism culture filtrates, one green algae extract and two fungicides, used as elicitors to induce resistance in barley plants against *P. hordei*

No.	Treatment	Recommended dose	
		g /ml	mg /ml
Plant extract			
1	*Cinnamomum zeylanicum*	10	
2	*Curcuma longa*	10	
3	*Lepidium sativum*		20.0
4	*Rhamnus cathartica*	10	
5	*Ricinus communis*	10	
6	*Saussurea costus*	10	
7	*Opuntia ficus-indica*	10	
8	*Zingiber officinale*		20.0
Green algae extract			
9	*Laurencia obtusa*		20.0
Organism culture filtrate			
10	*Bacillus subtilis*		5.0
11	*Trichoderma viride*		5.0

Fungicide			
12	TILT		3.50
13	Opera	0.15	

Preparation of the natural product solutions

a) The plant water extracts of *Curcuma longa*, *Cinnamomum zeylanicum*, *Rhamnus cathartica*, *Saussurea costus*, *Lepidium sativum,* and *Zingiber officinale* were prepared according to the method described by Hussain *et al.* (2012). The used part of the aforementioned six plants was ground individually to semi-powder. In contrast, the used part of the two plants, *Ricinus communis* and *Opuntia ficus-indica,* and the sea green algae, *Laurencia obtuse,* were minced individually into a semi-suspension.

Ten g of the ground or minced samples were extracted by 100 ml of sterilized distilled water in a conical flask and kept for 8 h at room temperature. The extract was separated from the solid residue by filtering through filter paper, and the obtained extracts were pooled. A stock solution of each extract was kept in the refrigerator at – 4 °C until use.

Preparation of the microorganism culture filtrates

b) The *Trichoderma viride* and *Bacillus subtilis* isolates used in the current study were obtained from the Mycological Research and Plant Disease Survey Department, Giza, Egypt. Potato dextrose broth medium was utilized for growing *T. viride* isolate, and yeast extract - triptone broth medium was used for growing *B. subtilis*. The bioagent isolates were inoculated in 250 ml flasks containing 100 ml broth media and incubated at 26±2 °C for seven days with continuous shaking. Colonized media were filtered through a sterilized membrane (0.45 µm mesh) (Lifshitz *et al*., 1986). The upper and lower surfaces of the initial barley leaves were treated with the filtered cultural media 48 hours prior to inoculation with the pathogen. Control treatments for barley leaf rust disease were administered using fungicidal concentrations of 3.50 mg TILT /L and 0.15 mg Opera /L. The application of the spraying process, using a hand sprayer, was performed on the leaves until they were sufficiently wet, as described by (Cao *et al*., 2014 b).

Assessment of resistance components at seedling and adult stages

a) Incubation period (IP)

The time (days) between setting uredospores on the leaf surface (infection process) and the appearance of the first signs of the disease was recorded to calculate the incubation period for each treatment according to a study by Holliday (1989).

b) Latent period (LP)

The calculation of the latent period involved determining the number of days that elapsed from inoculation until the point at which 50% of uredinia became visible on plant leaves (Parlevliet, 1975; 1985).

c) Infection type (IT)

A scale of 0-4 was utilized to document the infection type (IT) within a time frame of 12 to 20 days following inoculation. The classification of infection types into categories 0, 1, and 2 is associated with a host reaction that exhibits resistance (Stakman *et al*., 1962; Vander Plank, 1968).

d) Final rust severity (FRS %)

Rust severity (%) was recorded weekly after the initial infection occurred, using the modified scale of Peterson *et al*. (1948). Adult plant reactions were scored as the percentage of rust severity (%) at the time when rust first had just appeared until the early dough stage (Large, 1954) when rust severity % reached its maximum and final level in the untreated control plants of the highly susceptible (Das *et al*., 1993).

The efficacy of a certain treatment was determined according to the following equation adopted by (Rewal and Jhooty, 1985):

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

where C = infection in the control and T = infection in the treatment

Biochemical assay

Biochemical changes in the barley leaves were estimated to determine their effects on some biochemical components, such as phenols and oxidative enzymes. The assay was carried out at the Laboratory of the Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Egypt.

a) Estimation of phenol content

Total phenol content was estimated according to the method described by Malick and Singh (1980); 0.5 g of fresh leaves was ground with 10 ml of 80% ethanol and stored in a dark bottle at 4 °C for 72 h. Extracts were combined and filtered for determination using a Unico UV-2100 Spectrophotometer. The total phenol was expressed in mg g^{-1} fresh weight.

b) Estimation of oxidative enzyme activity

Peroxidase (POX) activity was directly determined according to a typical procedure proposed by Hammerschmidt *et al.* (1982). Polyphenol oxidase (PPO) activity was determined according to the method described by Malick and Singh (1980). First, 0.5 g of leaf material was homogenized at 0-4 °C in 3 ml of 50 Mm TRIS buffer (PH 7.8), containing 1 mM EDTA-Na_2 and 7.5% polyvinyl pyrrolidone, the homogenates were centrifuged (12,000 rpm, 20 min, 4 °C). Then, the total soluble enzyme activities were measured spectrophotometrically in the supernatant. Measurements were carried out at 25 °C, using a spectrophotometer (model UV-160A, Shimadzu).

Molecular studies

Start codon-targeted (SCoT) polymorphism marker

SCoT-PCR Reactions

a) SCoT-PCR Reactions

A total of SCoT primers were used in the detection of polymorphism Table 2. The amplification reaction was carried out in 20 µl reaction volume containing 10 µl Master Mix (sigma), 2 µl primer (10 pcmol), 2 µl template DNA (10 ng), and 6 µl d H_2O, according to Ibrahim *et al*. (2019), Elshafei *et al*. (2021) and Hanaa *et al*. (2021).

b) DNA extraction and purification

Total DNA was extracted from approximately 100 mg of plant leaves for each treatment (*Laurencia obtusa*, *Saussurea costus*, *Opuntia ficus-indica*, *Bacillus subtilis*, *Trichoderma viride* and TILT) using DNeasy Plant Kit (QIAGEN, Germany). The extracted DNA concentration and quality were estimated by NanoDrop.

Table 2. Sequences of primers used in this study

Primer	Name Sequence
SCoT-1	5'-ACGACATGGCGACCACGC-3'
SCoT-2	5'-ACCATGGCTACCACCGGC-3'
SCoT-3	5'-ACGACATGGCGACCCACA-3'
SCoT-4	5'-ACCATGGCTACCACCGCA-3'
SCoT-5	5'-CAATGGCTACCACTAGCG-3'
SCoT-6	5'-CAATGGCTACCACTACAG-3'

c) Thermocyling profile PCR

PCR amplification was performed in a Perkin Elmer/Gene Amp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94 °C. Each cycle consisted of a denaturation step at 94 °C for 45 s, an annealing step at 50 °C for 50 s, and an elongation step at 72 °C for 1 min. The primer extension segment was extended to 7 min at 72 °C in the final cycle.

d) Detection of the PCR Products

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000) Williams *et al*. (1990).

e) Estimation of genomic template stability

Analysis of DNA variations was determined by scoring present (1) or absent (0) of SCoT bands for the bulked samples of each treatment (six treatments) in addition to the control. Polymorphism observed in the DNA profiles included the disappearance of normal bands and the appearance of new bands in treated samples compared with non-treated ones (set to 100%) (Atienzar *et al*., 1999). The GTS was calculated as follows:

GTS (%) = (1- a /n) x 100

Where a is the number of polymorphic bands detected in each treated sample equal to the sum of the disappearance of normal bands and appearance of new bands, and n is the number of total bands in the control.

Statistical analysis

All the obtained recorded data were statistically analyzed using Fisher's analysis of variance (ANOVA) and LSD test was applied at 5% and 1% probability levels to compare the differences of phenotypic mean expression of one hundred wheat genotypes and check variety Gomez and Gomez 1984.

Results

At the seedling stage

Data presented in Table 3 show the effect of pretreatments on barley leaf rust disease (*Puccinia hordei*) in the seedling stage on the susceptible variety, 'Giza 123'. The pretreatment of barley seedlings with plant extracts resulted in an elongation of both the incubation and latent periods of *Puccinia hordei*, which are constituents of the partial resistance induced. The results showed that the three extracts of *Saussurea costus*, *Ricinus communis*, and *Laurencia obtuse* increased the incubation period by 16.67, 13.89, and 11.11 days, respectively. Similar approximate results were obtained using *T. viride* culture filtrate (22.22 days) and B. subtilis culture filtrate (11.11 days). However, the most significant increase in the incubation period was observed with fungicidal treatments of TILT and Opera, which resulted in increases of 44.44 and 33.33 days,

respectively. In contrast, the three plant extracts (*Curcuma longa, Rhamnus cathartica,* and *Lepidium sativum*) did not significantly affect the incubation period, with only a 2.78-day increase compared to the control. *Saussurea costus* extract and *T. viride* culture filtrate resulted in the most prolonged latent periods (17.00 and 13.33 days), respectively, followed by the extracts of *Ricinus communis*, *Zingiber officinal*, *Opuntia ficus-indica* and *B. subtilis* culture filtrate (16.00, 16.00, 16.25 and 16.50 days, respectively for each with (6.67, 6.67, 8.33 and 10.00) increase, respectively. The highest increase was in (LP) of each of TILT and Opera (20.00 and 19.00 days) by percentages (33.33 and 26.67%), while *Curcuma longa*, *Rhamnus cathartica* and *Lepidium sativum* exhibited adverse effects because they did not lead to a significant increase in the latent period compared with the untreated control (Table 3).

Table 3. Effect of using 11 elicitors on barley seedlings infection by leaf rust fungus, expressed as incubation period, latent period and infection type in the seasons 2021/2022 to 2022/2023

No.	Treatment	Incubation period		Latent period		Infection type
		Day	Increase%	Day	Increase%	
Plant extract						
1	*Cinnamomum zeylanicum*	9.25	2.78	15.00	0.00	3
2	*Curcuma longa*	9.50	5.56	15.75	5.00	2
3	*Lepidium sativum*	10.25	13.89	16.00	6.67	2
4	*Rhamnus cathartica*	9.25	2.78	15.00	0.00	3
5	*Ricinus communis*	10.50	16.67	17.00	13.33	2
6	*Saussurea costus*	9.25	2.78	15.00	0.00	3
7	*Opuntia ficus-indica*	9.50	5.56	16.00	6.67	2
8	*Zingiber officinale*	9.75	8.33	16.25	8.33	2
Green algae extract						
9	*Laurencia obtusa*	10.00	11.11	15.25	1.67	2
Organism culture filtrate						
10	*Bacillus subtilis*	10.00	11.11	16.50	10.00	2
11	*Trichoderma viride*	11.00	22.22	17.00	13.33	2
Fungicide						
12	TILT	13.00	44.44	20.00	33.33	1
13	Opera	12.00	33.33	19.00	26.67	1
14	Control	9.00		15.00		4
	L.S.D. (5%)	0.421		1.656		

The data was recorded as a reaction Infection type (IT) result of TILT and Opera application from susceptible (4) to resistant (1). In addition, *Cinnamomum zeylanicum*, *Laurencia obtusa*, *Ricinus communis*, *Saussurea costus*, *Zingiber officinale*, *Opuntia ficus-indica* extracts, and *B. subtilis* and *T. viride* culture filtrates changed reactions to moderately resistant (2) against *P. hordei* comparing with the untreated control which was recorded susceptible reaction (4).

At the adult stage

Data presented in Table 4 show the effect of water plant extracts on barley leaf rust disease severity in adult plants. The application of pre-infection spraying on 'Giza 123' barley plants using 11 elicitors was found to have a significant impact on reducing barley leaf rust infection. The effectiveness of this treatment was measured by the average final rust severity, expressed as a percentage. The plant extracts of *Saussurea costus* and *Opuntia ficus-indica* were particularly effective, reducing the rust severity to 13.33% and 13.58%, respectively. Nevertheless, the efficacy of microorganisms in terms of average disease severity was highly significant, as

indicated by the percentage. The disease severity values for the treatments with *Trichoderma viride* filtrate and *Bacillus subtilis* filtrate were 9.16% and 10.00%, respectively. These values were not significantly different from the fungicide treatment using TILT (5.00%) and Opera (5.83%). In comparison, the non-treated control had an average disease severity value of 88.33%.

Table 4. Effect of induced resistance by 11 elicitors on adult barley plants against leaf rust infection in Agricultural Research Stations, Sakha and Giza under field conditions during two the growing seasons 2021/2022 and 2022/2023

Treatment	Final rust severity (%)		Mean	Efficacy%		Mean
	Sakha	Giza		Sakha	Giza	
Plant extract						
Curcuma longa	53.33	42.66	47.99	40.74	50.78	45.66
C. zeylanicum	33.33	30.67	32	62.97	64.61	63.77
Ricinus communis	36.67	25.75	31.21	59.26	70.29	64.66
Rhamnus cathartica	53.33	50.25	51.79	40.74	42.02	41.36
Saussurea costus	16.67	10	13.33	81.48	88.46	84.9
Lepidium sativum	53.33	50.25	51.79	40.74	42.02	41.36
Zingiber officinale	33.33	30.75	32.04	62.97	64.52	63.72
Opuntia ficus-indica	16.67	10.5	13.58	81.48	87.89	84.62
Green algae extract						
Laurencia obtusa	23.33	18.33	20.83	74.08	78.85	76.41
Organism culture filtrate						
Bacillus subtilis	10	10	10	88.89	88.46	88.67
Trichoderma viride	8.33	10	9.16	90.74	88.46	89.62
Fungicide						
TILT	5	5	5	94.44	94.23	94.33
Opera	6.67	5	5.83	92.59	94.23	93.39
Control	90	86.67	88.33	-	-	-
L.S.D. (5%)	10.96	8.74	9.85	-	-	-

The efficacy of the treatments was found to be highly significant in controlling the infection in the tested plant species, namely *Laurencia obtusa*, *Opuntia ficus-indica*, and *Saussurea costus* extracts, as well as Bacillus subtilis and *Trichoderma viride* filtrates. The effectiveness of these treatments exceeded 75%. Additionally, the fungicide treatments, specifically Opera and TILT, demonstrated an efficacy rate of over 90% (Figure 1 and Table 4).

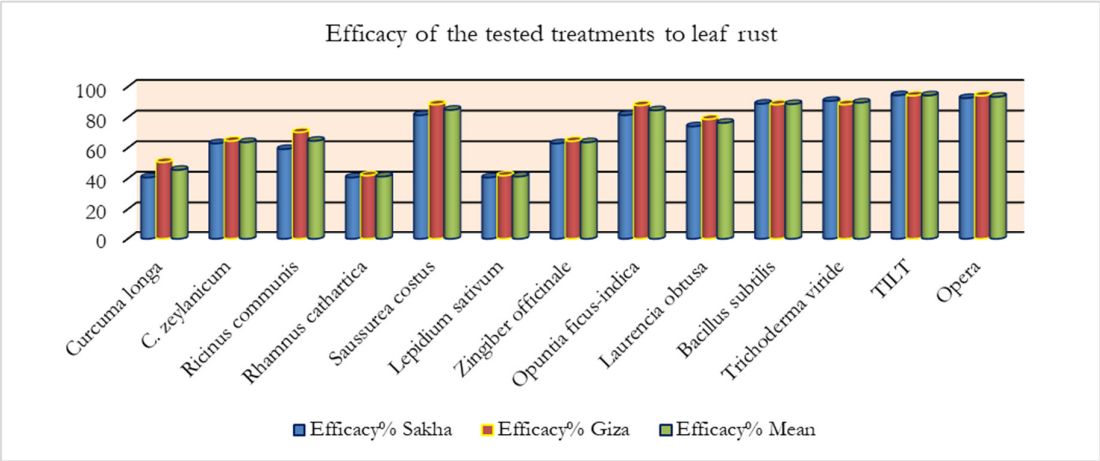

Figure 1. Efficacy of the test natural products from some plants, microorganisms and fungicides against infection of barley leaves by the leaf rust fungus (*Puccinia hordei*) under field conditions

The data presented in Table 4 demonstrate that the application of the tested botanical extracts resulted in a significant increase in total phenol contents compared to the control group that did not receive any treatment. The results presented in Table 4 demonstrate that the application of green algae extract exhibited the highest efficacy in enhancing the overall phenolic content of barley leaves infected with *Puccinia hordei*. It is evident from the data that the total phenol levels significantly increased following the application of the botanical extracts, compared to the control group that did not receive any treatment. The extracts of *Laurencia obtusa*, *Opuntia ficus-indica*, and *Saussurea costus* demonstrated the highest efficacy in enhancing the overall phenolic content of barley leaves infected with *Puccinia hordei*. Specifically, 24 hours after application, the total phenolic contents reached 64.50 mg/g, 61.75 mg/g, and 57.25 mg/g, respectively. In comparison, the fungicide TILT ranked fourth with a phenolic content of 33.75 mg/g, followed by the microorganisms Bacillus subtilis (15.25 mg/g) and *Trichoderma viride* (13.50 mg/g). However, the total phenolic contents in the non-treated control were 10.75 mg/g.

The biochemical analysis of barley leaves involved the treatment of three plant extracts, as depicted in Table 5. In general, it was observed that all of the plant extracts that were tested exhibited a significant increase in total enzyme activity. *Laurencia obtusa* extract treatment was the most effective in enhancing the activity of (POX) by a percentage of 1.0854 mg/g, followed by *Opuntia ficus-indica* extracts 0.9973 mg/g and *Saussurea costus* 0.9858 mg/g while the control 0.0265 mg/g, on the other hand, fungicide TILT had the lowest efficacy from the control 0.0234 mg/g.

Data in Table 5 illustrates that spraying barley plants with the tested plant extracts before *Puccinia hordei* infected increased polyphenol oxidase (PPO) activity. *Lepidium sativum* extract was the most effective (0.5843 mg/g), followed by each *Opuntia ficus-indica* and *Saussurea costus* extract (0.4735 and 0.3204 mg/g), respectively. The lowest effective treatment in this respect was by *Trichoderma viride* (0.3170) and *Bacillus subtilis* (0.2953) compared to the non-treated and infected control (0.3082 mg/g). In contrast, TILT (0.2875) demonstrated the lowest efficacy compared to the control (0.3082).

Table 5. Effect of some plant treatment on the activity of peroxidase, polyphenol oxidase enzymes and the total phenolic contents in barley leaves, infected with *Puccinia hordei*

No.	Treatment	Total phenol mg/g	Enzyme activity	
			Peroxidase	Peroxidase
1	*Laurencia obtusa*	64.50	1.0854	0.5843
2	*Saussurea costus*	57.25	0.9858	0.3204
3	*Opuntia ficus-indica*	61.75	0.9973	0.4735
4	*Bacillus subtilis*	15.25	0.0589	0.2953
5	*Trichoderma viride*	13.50	0.0986	0.3170
6	TILT	33.75	0.0234	0.2875
7	Control	10.75	0.0265	0.3082

Estimation of genome template stability using SCoT markers

A SCoT analysis was conducted on DNA samples obtained from various treatments, including *Laurencia obtusa, Saussurea costus, Opuntia ficus-indica, Bacillus subtilis, Trichoderma viride*, TILT, and control to evaluate the genetic effects. Seven oligonucleotide primers were utilized to discriminate controls from barley plants treated. These primers were able to generate reproducible SCoT bands with template DNA from treated barley plantlets and control. The SCoT analysis yielded 6-15 bands in control in the different used genotypes, and the total number of amplified bands from the six primers were identified in the control treatment 57 bands (Table 6 and Figure 2). Polymorphism was calculated as DNA fragments' presence and/or absence between the samples. Different polymorphic bands were detected for the different primers in each barley plant treated. In all cases, the detected values of GTS % increased to more than 79% in all genotypes, where the detected value of GTS % was 79% in treatments (1,5). The maximum value of GTS% in 2 treatments reached 89%, while it was 81% in each of the 3,4 genotypes. Previous studies have shown that changes in band patterns observed in DNA fingerprint analyses reflected DNA alterations due to spraying plants with the used treatments and were loss of normal bands and appearance of new bands compared with the normal control plants.

The findings presented in Table 7 demonstrate that the application of various elicitors resulted in a significant improvement of yield components in barley plants infected with *Puccinia hordei*. Specifically, the elicitors led to notable improvements in 1000-kernel weight, spike length, flag leaf area, and days to maturity compared to the non-treated control group. Notably, *ficus-indica* and *Lepidium sativum* exhibited extracts with the highest 1000-kernel weight (62.0 and 60.2 g) and spike length (10.9 and 10.5 cm^2) respectively.

Table 6. Genome template stability %, the total number of bands in control and polymorphic bands in for six treatments using six SCoT primers.

	Control	*Laurencia obtusa*		TILT		*Saussurea costus*		*Bacillus subtilis*		*Opuntia ficus-indica*		*Trichoderma viride*	
	n	p	d	p	d	p	d	p	d	p	d	p	d
SCoT-1	15	0	4	0	3	0	4	0	3	0	5	0	5
SCoT-2	10	0	3	0	0	1	0	0	3	0	2	2	0
SCoT-3	6	3	0	2	0	2	0	2	0	0	1	2	0
SCoT-4	9	0	1	0	0	0	1	0	1	0	1	0	0
SCoT-5	8	0	0	0	0	0	1	0	1	0	1	0	0
SCoT-6	9	1	0	0	1	1	1	0	1	1	1	1	0
Total	57	4	8	2	4	4	7	2	9	1	11	5	5
a			12		6		11		11		12		10
a/n			0.21		0.11		0.19		0.19		0.21		0.18
1-(a/n)			0.79		0.89		0.81		0.81		0.79		0.82
GTS%			79		89		81		81		79		82

p: disappearance of normal bands, d: appearance of new bands, a = (p+d): denotes polymorphic band.

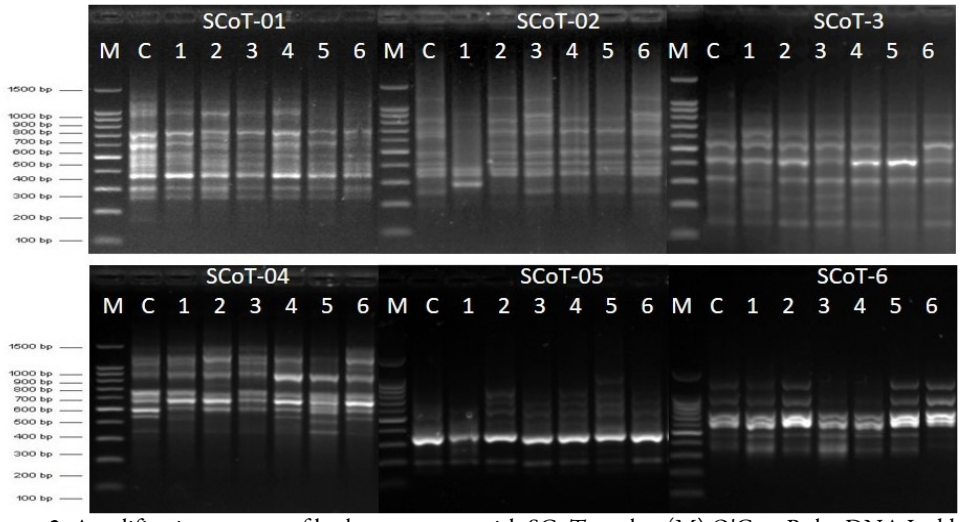

Figure 2. Amplification pattern of barley genotypes with SCoT marker (M) O'GeneRuler DNA Ladder Mix, ready to use, (c) control and (1-6) variations between treatment

Effect of the test elicitors on yield components

Data in Table 7 show that all the tested elicitors significantly increased yield components of barley plants infected with *Puccinia hordei* in terms of 1000-kernel weight, spike length, flag leaf area and days to maturity, compared to the non-treated control, *O. ficus-indica* and *Lepidium sativum* extracts gave the highest 1000-kernel weight (62.0 and 60.2 g), respectively and spike length (10.9 and 10.5 cm^2), respectively. The highest flag leaf area was also obtained by *O. ficus-indica* extract (10.5 cm^2), followed by *Laurencia obtusa* extract (10.0 cm^2), *S. costus* extract (9.5 cm^2) and each of *R. communis* extract and *T. viride* filtrate by percentage (9.2 cm^2). *L. obtusa* and *O. ficus-indica* extracts were the best treatments in increasing the days to maturity of the *P. hordei* infected barley plants by number 128.3 days, while, *R. cathartica* and *L. sativum* gave the lowest values for crop components. The findings of this study suggest that the application of plant extracts and microorganism culture filtrates has the potential to enhance the yield components of barley.

Table 7. Effect of the test elicitors on yield components of infected barley adult plants, in Agricultural Research Stations, Sakha and Giza under field conditions, during the seasons 2021/2022 and 2022/2023

Treatment	1000-grain weight (g)			Spike length $(cm)^2$			Flag leaf area $(cm)^2$			Time to maturity (days)		
	Sakh.	Giza	Mea.	Sakh.	Giza	Mea.	Sakh	Giza	Mea.	Sakh.	Giza	Mean
Curcuma longa	48.1	49.4	48.8	8.7	8.3	8.5	8.3	7.9	8.1	124.0	122.5	123.3
C. zeylanicum	53.2	52.5	52.9	8.7	8.9	8.9	8.9	8.8	8.9	127.2	126.3	126.8
Laurencia obtusa	60.8	59.5	60.2	10.7	10.3	10.5	10.2	9.8	10.0	128.7	127.8	128.3
Ricinus communis	58.8	59.1	59.0	9.5	9.4	9.5	9.5	8.9	9.2	127.2	126.7	126.9
Rhamnus cathartica	48.1	45.4	46.8	8.7	7.5	8.1	8.1	7.9	8.0	124.7	125.5	125.1
Saussurea costus	50.4	53.2	51.8	10.8	9.5	10.2	9.7	9.3	9.5	126.8	125.5	126.2
Lepidium sativum	45.1	47.4	46.3	8.3	8.5	8.4	8.7	7.1	7.9	124.3	125.2	124.8
Zingiber officinale	51.6	50.7	51.2	9.2	8.7	8.9	7.8	8.3	8.1	126.9	125.2	126.1
Opuntia ficus-indica	61.9	62.1	62.0	11.0	10.8	10.9	10.6	10.4	10.5	127.7	128.8	128.3
Bacillus subtilis	49.6	48.0	48.8	8.7	8.9	8.8	8.5	8.9	8.7	125.5	125.7	125.6
Tricoderma viride	51.9	49.3	50.6	9.4	9.3	9.4	9.3	9.0	9.2	125.7	126.5	126.1
TILT	50.1	49.4	49.8	8.9	8.5	8.7	7.9	7.8	7.9	125.3	124.0	124.7
Opera	48.9	47.3	48.1	8.7	8.1	8.4	7.8	7.0	7.4	124.3	125.2	124.8
Control	39.0	40.1	39.6	6.8	6.5	6.7	6.3	6.5	6.4	122.9	122.8	122.9
L.S.D. (5%)	0.165	0.183	0.173	0.123	0.131	0.127	1.03	0.98	1.05	1.37	1.04	1.21

Discussion

There is growing concern regarding the use of chemicals for plant protection due to their adverse effects on humans, as well as other target organisms, and their behavior and persistence in the environment (Jespers, 1994). Consequently, the scientific community, agro-industry, and pharmaceutical companies are compelled to explore natural compounds that can meet consumer demands (Harvey, 2008). Plant, fungal, and bacteria culture filtrates containing physiologically active biochemicals hold significant promise in the development of new agents that can significantly contribute to the well-being of human beings. In this context, systematic screening of secondary metabolites of folk herbs and fungi may result in discovering novel and effective antimicrobial compounds (Hussain *et al*., 2011). The findings showed that the prior application of the plant extracts of *Curcuma longa*, *Cinnamomum zeylanicum*, *Laurencia obtusa*, *Ricinus communis*, *Saussurea costus*, *Lepidium sativum*, *Zingiber officinale*, and *Opuntia ficus-indica*. In this complete resistance, they changed the infection type of the susceptible barley cultivar ('Giza 123') to a resistant type and a partial resistance reduction infection type (IT), lengthening the latent period and incubation period. Similar results were found by Barna *et al*. (1998), who reported a significant reduction in the leaf rust infection of wheat plants, using some plant extracts (garlic, clove, garden quinine, Brazilian pepper, rose pepper, black cumin, white cedar, and neem), sprayed pre-infection on wheat seedlings. In addition, foliar spraying of plant extracts (pomegranate, *eucalyptus*, cactus significantly decreased leaf rust severity of wheat (Abd El-Malik and Abbas, 2017; Thabet *et al*., 2023).

The prior application of the green algae extracts significantly decreased leaf rust. Genuine mosses constitute a large group of nonvascular higher plants comprising about 14,000 species. Over several hundred new compounds have been isolated from bryophytes, and their structures elucidated (Veljić *et al*., 2009). Despite a number of secondary metabolites identified from various mosses, the chemical profiles of most species are insufficiently known or even unknown. The identified secondary metabolites derived from mosses encompass terpenoids, flavonoids, bibenzyls, as well as fatty acid derivatives (Veljic *et al*., 2009). Chloroform extract of the sea green algae *Laurencia obtusa* collected from three sites along the Red Sea coast of Egypt was efficient as an antimicrobial against *Pseudomonas aeruginosae* and *Fusarium* spp. (Deyab, 2011). The microdilution method evaluated the moss *Rhodobryum ontariense* against *Aspergillus versicolor*, *Penicillium funiculosum,* and *P. ochrochloron*. The extract was active against all the fungi tested but to varying degrees (Pejin *et al*., 2012).

Induced resistance is environmentally friendly and confers to long-lasting protection against a broad spectrum of plant pathogens, diseases, bacteria, fungi, oomycetes, and nematodes (Durrant and Dong, 2004). Induced resistance is generally characterized by the increased synthesis of the chemical compounds in plants that can prevent pathogen's growth and development, due to the gradual activity in antioxidants enzymes, peroxidase (POX) and polyphenol oxidase (PPO), then in a biochemical increase (Phenol) or what knows by systemic acquired resistance. (Agrios, 2005).

The tested plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids and coumarins (Cowan, 1999). The components with phenolic structures, like carvacrol, eugenol, and thymol, are highly active against pathogens. Obtained findings are in agreement with Karavaev *et al*. (2002) who reported that the aqueous extracts from bird cherry tree *Padus avium*, aspen *Populus tremula*, and celandine *Chelidonium majus* effectively suppressed the *P. triticina* and antifungal activity was attributed to the high content of phenolic compounds in the leaves of these plants.

Spraying *Puccinia hordei*-infected barley plants with the tested plant extracts increased the activity of peroxidase (POX) and polyphenol oxidase (PPO). *Laurencia obtusa*, *Opuntia ficus-indica,* and *Saussurea costus* extract treatment was the most effective in enhancing the activity (POX) and (PPO). The underlying mechanisms of disease suppression by plant extracts are not clearly understood, but the involvement of induced resistance is considered (Fokkema, 1993). The plant extract treatments exhibited substantial increases in POX

and PPO, as corroborated by the findings of Kumar *et al*. (2017). Karavaev *et al*. (2002) recorded a high POX activity in the wheat leaves treated with the aqueous extracts from bird cherry tree, *Padus avium*, aspen *Populus tremula*, and celandine *Chelidonium majus* to resist rust leaf disease caused by *P. triticina*.

In the present study, the barley seedlings treated with *Trichoderma viride* and *Bacillus subtilis* culture filtrates also showed significantly reduced the disease incubation (IP) period latent period (LP) and Infection type (IT). The probable bio-control mechanisms exerted by *Trichoderma* filtrate may directly affect the pathogen *via* antibiosis, or indirectly through induction of the plant resistance (El-Sharkawy *et al., 2015).*

Adult plants also showed a significant decrease in percentage final rust severity (FRS %) and enhanced antioxidant enzyme activity or enzymes POX and PPO associated with induced resistance.

The obtained findings are in agreement with Kiani *et al*. (2021) who reported that the wheat seedlings treated with endophytic bacteria showed significantly reduced stripe rust disease severity and moderately resistant phenotype. These seedlings also showed enhanced activity of antioxidant enzymes or enzymes PAL, SOD, PO, and PPO. The three bacteria identified in this study have great potential and can be used against other cultivars and stripe rust races to explore their biocontrol potential.

The effects of the use of SCoT for the detection of variations in the activity of the DNA polymerase, be attributed to visible changes in the electrophoretic profiles of SCoT reaction products. The changes in amplified band fluorescence intensity, obvious disappearance of amplified bands, and appearance of new PC products occurred in SCoT profiles generated from the Cd-exposed organisms in comparison to the control (Doyle and Doyle, 1987; Atienzar *et al*., 2002; Atia *et al.,* 2021; Samah *et al*., 2021). The changes to the DNA of the treated plants consequently resulted in the appearance of DNA bands. The appearance of new products to oligonucleotide primers after the structural change or because some changes in DNA sequence have occurred due to mutations or large deletions or homologous recombination. In the current study to evaluate the genetic effects, SCoT analysis was performed on DNA extracted from each treatment, and seven oligonucleotide primers were utilized to discriminate controls from barley plants treated. The SCoT analysis yielded 6-15 bands in control in the different genotypes, and the total number of amplified bands from the six primers were identified in the control treatment 57 bands. Previous studies have shown that changes in band patterns observed in DNA fingerprint analyses reflected DNA alterations as a result of spraying plants with the used treatments and were loss of normal bands and appearance of new bands in comparison with the normal control plants. This result was consistent with Omar (2021) hypothesis that The SCoT Random amplified polymorphic DNA (SCoT) test is a feasible method to evaluate the effect of plant extracts and microorganisms on wheat plants the same result. In addition, other writers like (Atienzar *et al*., 1999; Enan, 2006). These findings suggest that DNA polymorphism detected by SCoT analysis could be a powerful tool to evaluate genetic changes as compared with untreated plants.

Conclusions

The findings obtained indicate that the application of the tested plant extracts can enhance the yield components of barley, specifically by increasing the weight of 1000 kernels and the length of the spike. These findings are consistent with the previous studies conducted by Shabana *et al.* (2017) and Shreen *et al*. (2019). They found that the 1000-kernel weight of wheat plants infected with *P. triticina* sprayed with Brazilian pepper extract improved by 15.73% compared to the untreated control, followed by white cedar (13.81%) and garlic (13.02%). Additionally, Abd El-Malik and Abbas (2017) reported that the foliar spraying of plant extracts (pomegranate, eucalyptus, and cactus) significantly increased wheat yield components, including 1000-kernel weight and spike weight.

Authors' Contributions

Conceptualization MBR, MAA, AAA and RAE Data curation; MBR, MAA, AAA, FAS and RAE Formal analysis; MBR, MAA, EAA and RAE Funding acquisition; FAS , MBR, MAA, AAA, TMA, DAA and RAE Investigation; MBR, TMA, ASA and RAE Methodology; MBR, MAA, AAA and RAE Project administration; MBR, MAA, AAA, TMA, DAA and RAE Resources; MBR, MAA, AAA, FAS and RAE Software; MBR, MAA, AAA and RAE Supervision; MBR, MAA, AAA and RAE Visualization; Writing – original draft; MBR, FAS , MAA, AAA and RAE. Writing - review MBR, MAA, AAA and RAE and editing MBR, MAA, AAA, TMA, DAA and RAE and All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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