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Efficacy of plant extracts in controlling wheat leaf rust disease caused by *Puccinia triticina*Yasser M. Shabana^{a,*}, Mohamed E. Abdalla^a, Atef A. Shahin^b, Mohammed M. El-Sawy^b, Ibrahim S. Draz^b, Ahmed W. Youssif^b^a Plant Pathology Department, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt^b Agricultural Research Centre, Plant Pathology Research Institute, Giza 12619, Egypt

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ABSTRACT

The efficacy of eight plant extracts (garlic, clove, garden quinine, Brazilian pepper, anthi mandhaari, black cumin, white cedar and neem) in controlling leaf rust disease of wheat was investigated *in vitro* and *in vivo*. *In vitro*, all treatments inhibited spore germination by more than 93%. Neem extract recorded 98.99% inhibition of spore germination with no significant difference from the fungicide Sumi-8 (100%). Under greenhouse conditions, seed soaking application in neem extract (at concentration of 2 ml/L) resulted in 36.82% reduction in the number of pustules/leaf compared with the untreated control. Foliar spraying of plant extracts on wheat seedlings decreased the number of pustules/leaf. Foliar spraying of plant extracts four days after inoculation led to the highest resistance response of wheat plants against leaf rust pathogen. Spray application of wheat seedlings with neem, clove and garden quinine extracts, four days after inoculation with leaf rust pathogen completely prevented rust development (100% disease control) and was comparable with the fungicide Sumi-8. Foliar spray application of wheat plants at mature stage with all plant extracts has significantly reduced the leaf rust infection (average coefficient of infection, ACI) compared with the untreated control and neem was the most effective treatment. This was reflected on grain yield components, whereas the 1000-kernel weight and the test weight were improved whether under one- or two-spray applications, with two-spray application being more effective in this regard. Thus, it could be concluded that plant extracts may be useful to control leaf rust disease in Egypt as a safe alternative option to chemical fungicides.

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1. Introduction

Leaf rust disease of wheat, caused by *Puccinia triticina* Eriks. (syn. *P. recondita* Rob. Ex Desm. f.sp. *tritici* Eriks. and Henn.) has always been one of the major constraints in wheat production. It causes severe yield losses that could reach 50% in Egypt [1]. Injudicious use of synthetic fungicides for controlling plant diseases has ultimate negative effects on human and animal health and agroecosystem. Eco-friendly control measures including plant extracts and organic materials, which act directly on the plant pathogens or indirectly by inducing resistance in plants [2], have gained considerable attention as alternative means to synthetic fungicides.

Efforts have been made to control plant diseases using plant extracts [3–15]. They gave evidences that the plant extracts are effective bioagents against a wide range of plant pathogens viz., fungal, bacterial and viral pathogens. Plant seed oils had been also

used to control plant pathogens [16–19]. Plant extracts of many higher plants like neem have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials [20].

Some plants contain components that are toxic to pathogens when extracted from plant and applied on infected crops. These components are called botanical pesticides or botanicals. Commonly used botanicals include plant extracts such as neem (*Azadirachta indica*, A. juss) and garlic (*Allium sativum*); and essential oils such as nettle (*Urtica* spp.), rue (*Ruta graveolens*, Linn), thyme (*Thymus vulgaris*, Linn), and tea tree (*Melaleuca alternifolia*) [21]. Plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins [22]. The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the plant pathogens. These groups of compounds show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms [23]. The underlying mechanisms are not clearly understood, but involvement of induced resistance is considered [24]. These bioagents are nonpolluting,

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cost effective, non-hazardous and can be prepared with available materials in the field.

The ultimate aim of this research is to develop safe alternative control strategies to reduce dependency on synthetic fungicides. The present study investigated the role of some plant extracts in controlling wheat leaf rust disease under *in vitro* and *in vivo* conditions.

2. Materials and methods

The experiments of the study were carried out in the laboratory, greenhouse and field at the Wheat Disease Research Department, Sakha Agricultural Research Station, Plant Pathology Research Institute, Agricultural Research Center of Egypt, during the period from 2012 to 2014.

2.1. Preparation of *P. triticina* inoculum

The causal fungus of leaf rust, *P. triticina* was isolated from infected wheat leaves collected from the commercial wheat fields and the Egyptian Wheat Rust Trap Nurseries during winter growing seasons of 2012/13 and 2013/14.

Since the causal fungus of leaf rust is an obligate parasite, rust isolates were maintained on living plants. For multiplication of *P. triticina* uredospores, the collected samples were transferred onto seedlings of the highly susceptible wheat variety “Morocco” to obtain enough inoculum for further investigations.

The inoculation of wheat plants was carried out as described by Ref. [25], whereas seedling leaves were rubbed gently between moistened fingers with tap water and sprayed with water in the incubation chambers, then inoculated by sprinkling or brushing the collected uredospores over the plant leaves, and then re-sprayed gently with water. The inoculated plants were incubated in a dark dew chamber at 18 °C for overnight then moved to the greenhouse and maintained at 18–25 °C. After incubation (12 to 15 days post-inoculation), uredospores were collected.

2.2. Preparation of plant extracts

Plant parts used in the present study were obtained from the Department of Medicinal Plants and Aromatic Research, Horticultural Research Institute, Agricultural Research Center (ARC), National Research Centre (NRC) and Faculty of Pharmacy, Cairo University, Egypt.

The extraction process was conducted at the Unit of Oil Extraction, Department of Medicinal Plants and Aromatic Research, Horticultural Research Institute, ARC. Plant extracts were prepared by grinding the used part (Table 1) of plants individually with sterilized distilled water in a blender. The eight tested plant extracts and their extraction methods are presented in Table 1.

Table 1

List of the plant extracts used for controlling leaf rust of wheat, common name, scientific name, part used, and reference for extraction method.

Plant name	Scientific name	Part used	Reference
Garlic	<i>Allium sativum</i>	Bulbs	[26]
Clove	<i>Syzygium gromaticum</i>	Buds	[27]
Garden quinine	<i>Clerodendrum inerme</i>	Leaves	[28]
Brazilian pepper	<i>Schinus terebinthifolius</i>	Leaves	[29]
Anthi mandhaari	<i>Mirabilis jalapa</i>	Roots	[30]
Black cummin	<i>Nigella sativa</i>	Seeds	[31]
White cedar	<i>Thuja occidentalis</i>	Leaves	[32]
Neem	<i>Azadirchta indica</i>	Seeds	[33]

2.3. In vitro assay

2.3.1. Effect of plant extracts on spore germination of *P. triticina*

Eight plant extracts listed in Table 1 were tested for their inhibitory effect on spore germination of *P. triticina*. For each treatment, a concentration of 0.2% (v/v) was used *in vitro* and tested using cavity slides with three replications, which were incubated at 15–18 °C. Ten microscopic fields were examined 8–10 h after treatment. A negative control treatment was maintained using distilled water. Sumi-8 fungicide at 0.35 ml/L was used as a positive control (check treatment). The percent of spore germination was calculated by the following formula adopted by Ref. [14]:

$$PG = A/B \times 100$$

where: PG = Percent of spore germination, A = Number of spores germinated and B = Number of spores observed.

Inhibition percent of spore germination was calculated using the following formula [34,35]:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

where: C = germination percent of spores in the negative control, T = germination percent of spores in the treatment.

2.4. Greenhouse assay

2.4.1. Seed soaking treatment

Plant extracts listed in Table 1 were evaluated as seed-soaking treatment for their efficacy in suppressing leaf rust disease under greenhouse conditions. For each treatment, seeds of the highly susceptible wheat variety Morocco were individually soaked in the tested plant extracts at concentration of 0.2% (v/v) for 24 h. Meanwhile, Sumi-8 fungicide at 0.35 ml/L was used as standard treatment (positive control). Negative control treatment was made by soaking seeds in sterilized distilled water. After soaking, seeds were sown in pots (15 cm in diameter) with three replicates for each treatment. After 7 days, seedlings in each treatment were sprayed with water containing a few drops of Tween-20, then inoculated with uredospores of *P. triticina* using rubbing technique according to Ref. [25]. The plants were then incubated in a dark dew chamber at 100% relative humidity for 24 h, then moved to the benches in the greenhouse and maintained at 18–25 °C under a photoperiod of 16 h light (7600 Lux) and 8 h dark [36]. After 15 days from inoculation, disease severity was recorded in terms of infection types and number of pustules/leaf. Infection types, from 0 to 4, were recorded as described by Ref. [37], where 0, 0, 1 and 2 are resistant, and 3 and 4 are susceptible while X is mesothetic (Table 2). The number of pustules/leaf was counted as described by Ref. [38].

Table 2

Infection types of wheat leaf rust used in disease assessment adopted from [37].

Infection type	Host response	Symptoms	
Resistant	0	Immune	No uredia or other macroscopic sign of infection
	0	Nearly immune	No uredia, but hypersensitive necrotic or chlorotic flecks present
	1	Very resistant	Small uredia surrounded by necrosis
Susceptible	2	Moderately resistant	Small to medium uredia surrounded by chlorosis or necrosis
	3	Moderately susceptible	Medium-sized uredia that may be associated with chlorosis
Mesothetic	4	Very susceptible	Large uredia without chlorosis or necrosis
	X	Heterogeneous	Random distribution of variable-sized uredia on a single leaf

Efficacy of treatments was calculated by the following equation:

$$\text{Efficacy (\%)} = \frac{\text{No. of pustules in the negative control} - \text{No. of pustules in the treatment} \times 100}{\text{No. of pustules in the negative control}}$$

2.4.2. Foliar spraying treatment on wheat seedlings

The plant extracts were tested for their efficacy in controlling leaf rust using foliar spray application on wheat seedlings in the greenhouse. For each treatment, grains of the highly susceptible wheat variety Morocco were sown in pots (15 cm diameter) with three replicates (10–15 grains/pot). Sumi-8 fungicide was used as standard treatment (positive control). Seedlings sprayed with water supplemented with four drops of Tween 20 were served as negative control (untreated control). All extracts were separately sprayed one day before inoculation with uredospores and one and four days after inoculation with uredospores. Inoculation and incubation were performed as described by Ref. [25]. After 15 days from inoculation, disease severity was rated in terms of infection types and number of pustules/leaf was recorded.

2.5. Efficacy of plant extracts under field conditions

The plant extracts were evaluated as foliar spray applications on mature wheat plants to determine their efficacy for controlling leaf rust disease and their effect on yield components. This experiment was carried out at the Experimental Farm of Sakha Agricultural Research Station during two winter seasons (2012/13 and 2013/14).

Split plot design with three replicates was adopted in this respect. The main plots were represented by one- and two-spray applications for each treatment. The first spray application was applied soon after the appearance of disease symptoms and the second spray application was carried out 15 days after the 1st spray application. Sub-plots were represented by the tested plant extracts.

Grains of the susceptible wheat varieties, “Gemmiza-7” (2012/13 season) and “Sids-1” (2013/14 season), were sown in experimental units (plots), each containing three rows with 3-m long and 30-cm apart at a rate of 8 g of grains/row. All plots were surrounded by a spreader area of one meter in width planted with the highly susceptible wheat variety “Morocco.” All cultural practices recommended in the commercial fields, i.e., fertilization, irrigation and other management practices, were applied. At boot stage, the spreader plants were inoculated according to the method of Ref. [39]. The spreader plants were moistened by a fine spray with water then dusted with uredospores powder mixture (one volume of fresh uredospores: 20 volume of talcum powder). Dusting was carried out at sunset to be favored with high relative humidity at night. Fungicide “Sumi-8” (35 ml/100 L water) was used as check treatment (positive control). Plants sprayed with just water served as untreated control treatment (negative control).

Disease assessment based on average coefficient of infection (ACI) according to Ref. [40] was used by multiplying disease severity (DS) by constant values of infection type (IT). The constant values for infection types were used, where $R = 0.2$, $MR = 0.4$, $MS = 0.8$ and $S = 1.0$. Infection types were scored according to Ref. [37]. Disease severity (DS) was estimated as percentage coverage of leaves with rust pustules using Modified Cobb's scale [41].

At harvest, 1000 kernel weight and test weight (1000 ml) parameters were estimated as indicators of yield components. Yield reduction (%) was estimated using the following equation adopted by Ref. [42]:

$$\text{Yield reduction (\%)} = 1 - y_d/y_h \times 100$$

where: y_d = yield of untreated plants (negative control), y_h = yield of treated plant.

The efficacy of treatment was determined according to the following equation adopted by Ref. [43]:

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

where: C = infection (%) in the negative control, T = infection (%) in the treatment.

The statistical analysis was done for each experiment individually using Duncan's new multiple range test according to the method adopted by Ref. [44].

2.6. Statistical analysis

Data were statistically analyzed using the Statistical Analysis System package (SAS software, Cary, NC, USA). Data were evaluated by one way ANOVA (analysis of variance) test. Pairwise comparison was carried out with least significant difference (LSD) test at $P \leq 0.05$.

3. Results

3.1. Effect of the plant extracts on spore germination of *P. triticina*

In vitro, treating of *P. triticina* uredospores with all plant extracts significantly inhibited spore germination by more than 93% (Table 3). The most effective plant extracts in this regard were neem (98.99% inhibition), clove (98.86%), anthi mandhaari (98.72%), and black cumin (98.61%) (Table 3). These were followed by white cedar (97.76% inhibition) and then Brazilian pepper (97.12%), while garden quinine and garlic extracts were the least effective inhibitors for uredospore germination. Sumi-8 fungicide has totally inhibited uredospore germination (100% inhibition) (Table 3).

3.2. Efficacy of plant extracts under greenhouse condition

3.2.1. Seed soaking treatment

Results showed that seed treatment with Sumi-8 fungicide led to 100% protection against leaf rust infection (Table 4). Among the plant extracts tested, white cedar extract was the most

Table 3

Effect of plant extracts on spore germination of *Puccinia triticina* compared with the fungicide Sumi-8.

Treatment	Germination (%)	Inhibition (%)
Garlic	5.02 b ^a	93.71
Clove	0.91 e	98.86
Garden quinine	5.26 b	93.41
Brazilian pepper	2.30 c	97.12
Anthi mandhaari	1.02 e	98.72
Black cumin	1.11 e	98.61
White cedar	1.79 d	97.76
Neem	0.80 e	98.99
Sumi-8 (fungicide)	0.00 f	100.00
Control (untreated)	79.78 a	00.00

LSD: at 5% = 0.35, at 1% = 0.48

^a Values followed by the same letter are not significantly different according to LSD test at $P = 5\%$.

Table 4
Effect of seed soaking with plant extracts on leaf rust severity of wheat at seedling stage.

Treatment	Type of infection ^a	No. of pustules/leaf	Efficacy (%)
Garlic	4	55.70 f ^b	15.60
Clove	4	49.30 d	25.30
Garden quinine	4	54.00 ef	18.18
Brazilian pepper	4	50.00 d	24.24
Anthi mandhaari	4	51.30 d	22.27
Black cumin	4	53.30 e	19.24
White cedar	4	39.33 b	40.41
Neem	4	41.70 c	36.82
Sumi-8 (fungicide)	0	0.01 a	100.00
Control (untreated)	4	66.00 g	00.00

^a 4 = Susceptible, 0 = Resistant.

^b Values followed by the same letter are not significantly different according to LSD test at $P = 5\%$.

effective one, recording 40.41% efficacy followed by neem (36.82%), clove (25.30%), Brazilian pepper (24.20%) and *Anthi mandhaari* (22.27%), while garlic extract was the least effective one (15.60%) (Table 4). These results may indicate that such plant extracts are inducing resistance in wheat seedlings against leaf rust infection as seed soaking treatment.

3.2.2. Foliar spraying on wheat seedlings

Data in Table 5 indicated that all the tested plant extracts reduced the number of pustules/leaf as foliar spraying on wheat seedlings compared with the untreated control. Foliar spray application four days after inoculation was the best timing treatment showing plant resistance response (R) compared with the application one day before or after inoculation. Among all plant extracts tested, neem extract was the most effective treatment and recorded 86.30% efficacy when applied one day before inoculation and 100% efficacy when applied one or four days after inoculation (Table 5). Its effect was equivalent to that of Sumi-8 fungicide (Table 5). After 22 days from inoculation, the tested plant extracts except garlic extract gave evidence to have positive residual/continued protective effect even better than that of the fungicide Sumi-8 (Table 5).

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

The effect of foliar spraying of plant extracts on leaf rust infection (average coefficient of infection, ACI) and wheat yield components (1000 kernel weight and test weight) was evaluated under field conditions during two seasons, 2012/13 and 2013/14.

At the first season (2012/13), where wheat cultivar “Gemmiza 7” was used, data in Table 6 revealed that the application of either one or two sprays of all plant extracts significantly reduced leaf rust infection, with neem extract being the most effective. It was more effective than the fungicide for one spray application, while equivalent to it for the two-spray application with regard to ACI, efficacy, and the 1000-kernel weight. In general, two-spray application was more effective than one-spray application (Table 6).

All the used plant extracts improved the 1000-kernel weight with two-spray application being more effective than one spray. With two-spray application, extracts of clove, Brazilian pepper, neem, and garlic were the best treatments, improving 1000 kernel weight by 19.13%, 19.11%, 19.06%, and 19.06% increase over the control, respectively. Their effect was significantly equivalent to that of the fungicide Sumi-8 (20.23% increase in 1000-kernel weight) (Table 6).

With regard to test weight parameter, data showed that two-spray application was better than one spray. Also, the used plant

Table 5
Effect of spraying the wheat seedlings with eight plant extracts on leaf rust and their residual effect.

Treatment	Time of application			Residual effect after 22 days for the last group (4 days after inoculation)								
	One day before inoculation			Four days after inoculation								
	Infection type	No. of pustules/leaf	Efficacy (%)	Infection type	No. of pustules/leaf	Efficacy (%)	Infection type	No. of pustules/leaf	Efficacy (%)			
Garlic	4 (S)	65.30 d ^a	16.60	3 (S)	17.00 e	78.29	3 (S)	13.30 c	83.00	3 (S)	13.30 d	83.00
Clove	3 (S)	12.70 b	83.78	0; (R)	0.01 a	100.00	0; (R)	0.01 a	100.00	0; (R)	0.0 a	100.00
Garden quinine	3 (S)	20.00 c	74.46	0; (R)	0.01 a	100.00	0; (R)	0.01 a	100.00	0; (R)	0.0 a	100.00
Brazilian pepper	3 (S)	13.70 b	82.50	4 (S)	11.70 d	85.06	2 (R)	3.00 b	96.17	2 (R)	3.0 b	96.17
Anthi mandhaari	4 (S)	18.30 c	76.63	0; (R)	3.00 b	96.17	1 (R)	2.00 b	97.45	1 (R)	2.0 ab	97.45
Black cumin	4 (S)	21.70 c	72.29	3 (S)	9.30 cd	88.12	2 (R)	1.00 ab	98.72	2 (R)	1.0 ab	98.72
White cedar	4 (S)	20.70 c	73.56	3 (S)	6.70 c	91.44	2 (R)	0.01 a	100.00	2 (R)	0.0 a	100.00
Neem	4 (S)	10.70 b	86.33	0; (R)	0.01 a	100.00	1 (R)	0.01 a	100.00	1 (R)	0.0 a	100.00
Sumi-8 (fungicide)	0 (R)	0.01 a	100.00	0 (R)	0.01 a	100.00	0 (R)	0.01 a	100.00	4 (S)	7.0 c	91.06
Control (untreated)	4 (S)	78.30 e	00.00	4 (S)	78.30 f	00.00	4 (S)	78.30 d	00.00	4 (S)	78.30 e	00.00
LSD at 5%		3.94			2.67			1.84			2.062	
LSD at 1%		5.36			3.63			2.94			2.813	

^a Values followed by the same letter(s) are not significantly different according to LSD test at $P = 5\%$.

Table 6
Effect of foliar spraying of plant extracts on leaf rust infection (average coefficient of infection; ACI) and yield components of wheat (cv. Gemmiza 7) under field conditions during 2012/13 growing season.

Treatment	ACI		Efficacy (%)		1000-Kernel weight (g)				Test weight (g)			
	One spray	Two sprays	One spray	Two sprays	One spray	Two sprays	Difference	Increase (%)	One spray	Two sprays	Difference	Increase (%)
Garlic	30.00 c ^a	20.00 c	66.67	77.78	49.66 a	50.60 ab	0.94**	19.06	692.00 e	702.50 c	10.50**	3.27
Clove	30.00 c	20.00 c	66.67	77.78	49.83 a	50.63 ab	0.80*	19.13	697.87 b	704.57 b	6.70**	3.57
Garden quinine	31.67 c	23.67 b	64.81	73.70	45.96 c	47.98 d	2.02**	12.89	694.67 c	696.20 e	1.53**	2.34
Brazilian pepper	38.33 b	21.67 bc	57.41	75.92	49.52 a	50.62 ab	1.10**	19.11	689.23 f	690.77 g	1.53**	1.54
Anthi mandhaari	30.00 c	10.00 d	66.67	88.89	43.46 e	48.61 cd	5.15**	14.38	693.00 de	694.30 f	1.30**	2.06
Black cumin	27.33 d	10.00 d	69.63	88.89	44.29 d	49.92 b	5.63**	17.46	692.63 de	694.57 f	1.93**	2.10
White cedar	20.00 e	10.00 d	77.78	88.89	43.80 de	48.89 c	5.09**	15.04	693.50 d	698.67 d	5.17**	2.70
Neem	11.67 f	4.33 e	87.03	95.19	43.86 de	50.60 ab	6.22**	12.08	700.10 a	706.53 a	6.43**	3.86
Sumi-8 (fungicide)	30.00 c	5.00 e	66.67	94.44	47.42 b	51.10 a	3.68**	20.33	684.83 g	695.20 ef	10.37**	2.19
Control (untreated)	90.00 a	90.00 a	00.00	00.00	42.50 f	42.50 e	0.00 ^{ns}	00.00	680.27 h	680.27 h	0.00 ^{ns}	0.00
LSD at 5%		2.710					0.641				0.900	
LSD at 1%		3.683					0.875				1.323	

^a Values followed by the same letter(s) are not significantly different according to LSD test at $P = 5\%$ (*) and $P = 1\%$ (**).

Table 7
Effect of foliar spraying of plant extracts on leaf rust infection (average coefficient of infection; ACI) and yield components of wheat (cv. Sids 1) under field conditions during 2013/14 growing season.^a

Treatment	ACI		Efficacy (%)		1000-Kernel weight (g)				Test weight (g)			
	One spray	Two sprays	One spray	Two sprays	One spray	Two sprays	Difference	Increase (%)	One spray	Two sprays	Difference	Increase (%)
Garlic	33.33 b	23.33 b	58.34	70.84	42.23 c	44.61 b	2.83**	13.02	704.00 a	708.73 b	4.73**	4.13
Clove	30.00 c	20.00 b	62.50	75.00	40.52 f	41.68 e	1.16**	5.60	695.07 bc	699.00 d	3.93**	2.70
Garden quinine	30.00 c	20.00 b	62.50	75.00	41.57 de	42.62 d	1.05**	7.98	703.47 a	711.10 a	7.63**	4.48
Brazilian pepper	34.33 b	23.00 b	57.09	71.25	43.70 a	45.69 a	1.98**	15.73	696.07 b	699.27 d	3.20**	2.74
Anthi mandhaari	20.00 d	9.33 d	75.00	88.34	41.46 e	42.62 d	1.16**	7.98	693.53 cd	698.80 d	5.27**	2.67
Black cumin	30.00 c	10.00 cd	62.50	87.50	42.96 b	43.79 c	0.83**	10.94	694.57 bc	699.07 d	4.50**	2.71
White cedar	21.67 d	5.00 e	72.91	93.75	43.14 ab	44.92 b	1.78**	13.81	693.97 cd	702.97 c	9.00**	3.28
Neem	8.33 e	5.00 e	89.59	93.75	41.63 de	42.90 d	1.27**	8.69	694.13 cd	699.70 d	5.57**	2.81
Sumi-8 (fungicide)	30.00 c	5.00 e	62.50	93.75	42.08 cd	45.07 b	2.99**	14.19	692.83 d	703.43 c	10.60**	3.35
Control (untreated)	80.00 a	80.00 a	00.00	00.00	39.47 g	39.47 f	0.00 ^{ns}	00.00	680.77 e	680.63 e	-0.130 ^{ns}	0.00
LSD at 5%			3.506				0.567				1.389	
LSD at 1%			4.766				0.774				1.895	

^a Values followed by the same letter(s) are not significantly different according to LSD test at $P = 5\%$ (*) and $P = 1\%$ (**).

extracts increased the test weight compared with the untreated control. The best treatment was neem extract followed by clove and garlic extracts, which were all better than the fungicide Sumi-8 (Table 6).

In the second season (2013/14), where wheat cultivar “Sids 1” was used, data presented in Table 7 revealed that the application of either one or two sprays of all used plant extracts showed high efficacy against leaf rust, reducing the ACI in comparison with the untreated control. The treatment of two-spray application seemed to be more effective than one spray. For one- or two-spray application, the most effective treatment was neem (89.59 and 93.75% efficacy, respectively), which was more effective than the fungicide Sumi-8 (for one-spray application) and had equal efficacy with the fungicide for the two sprays (Table 7).

All plant extracts significantly improved yield components by increasing the 1000-kernel weight and the test weight either under one- or two-spray application, but two-spray application was more effective. Under two-spray application, Brazilian pepper was the most effective extract, improving the 1000-kernel weight by 15.73% over the untreated control. It was followed by white cedar (13.81%) and garlic (13.02%), which were equivalent to the fungicide Sumi-8 (14.19%) (Table 7). With regard to the test weight, garden quinine was the most effective extract, increasing the test weight by 4.48% over the untreated control followed by garlic (4.13%) and both were significantly more effective than the chemical fungicide (3.35%); then came the white cedar extract, increasing the test weight by 3.28%. The latter was equivalent to the fungicide Sumi-8 (Table 7).

4. Discussion

Biological control of wheat rusts by using plant extracts is a modern, advanced and risk-free alternative method of rust management [45]. Several plant extracts are known to play an important role in the management of plant diseases [7,9,12,14,15]. They act directly or indirectly against plant pathogens, either to inhibit fungal growth and multiplication or by inducing resistance in crop plants. In this study, *in vitro*-tested plant extracts (neem, clove, antha mandhaari, black cumin, white cedar, Brazilian pepper, garlic and garden quinine) inhibited spore germination of wheat leaf rust *P. triticina* by 93% or more. Neem extract caused 98.99% inhibition to spore germination, which was not significantly different from the fungicide Sumi-8 treatment (100% inhibition).

Seed soaking or foliar spray treatments of wheat seedlings with the plant extracts reduced the number of pustules/leaf, with foliar spraying being more effective. In this regard, neem extract was the most effective treatment. It gave 100% control of the disease when applied one or four days after inoculation. After 22 days from inoculation, pustules started to appear on plants treated with the fungicide (Sumi-8), while no pustules developed on plants treated with neem, white cedar, garden quinine, or clove extracts. Applying a foliar spray at the mature plant stage showed high efficacy in reducing the leaf rust infection (ACI) and neem extract was the most effective treatment. Two-spray application seemed to be more effective than one spray.

All plant extracts improved the 1000-kernel weight and test weight under one- or two-spray application but two-spray application was more effective.

It is evident from several reports [5,7–15] that plant extracts are effective biocontrol agents against a wide range of plant pathogens. Plants have the ability to synthesize aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins [22]. The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly

active against pathogens. These groups of compounds show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms [23]. Plants of Meliaceae family, especially neem, contain at least 35 biologically active principles of which nimbin and azadirachtin [46] are the most active insecticidal ingredients and are present predominantly in the seeds, leaves and other parts of the neem tree. The active ingredients of neem constitute mostly of triterpenoides, e.g. nimbin, nimbidine, azadirachtin etc. [47]. The inhibitory effect of the plant extracts might be attributed to the presence of antifungal components, i.e., Azadirchin in *Azadirachta indica*, Artemesium in *Artemisia annua*, Caratenes in *Ocimum sanctum*, Emodin in *Rheum emodi* and Eucalyptolin in *Eucalyptus globulus* [9].

The underlying mechanisms of disease suppression by plant extracts are not clearly understood, but involvement of induced resistance is considered [24]. The phenomenon of inducing resistance in plants by biotic and abiotic compounds, such as some microorganisms, natural active ingredients (allicin, fulvic acid and eugenol), salicylic acid, phosphates, and plant oils, potentially offers an alternate, more environmentally approach to crops protection against infection with many diseases [15,48,49]. These bioagents are nonpolluting, cost effective, non-hazardous and can be prepared with available materials in the field. The mode of action of abiotic inducers for controlling plant diseases may include acting as second messengers in enhancing the host defense mechanism [50], activating resistance by increasing the activity of peroxidase (PO), the synthesis of new POD isoforms, the accumulation of the phenolic compound [51], or through inhibition of some antioxidant enzymes and catalases, thereby leading to production of elevated amounts of H₂O₂ [52] and finally enhancing resistance by direct effects on multiplication development and survival of pathogens or indirect effects on plant metabolism with subsequent effects on the pathogen food supply [53].

Finally, it could be concluded that the used plant extracts having resistance mechanisms may be useful to control leaf rust disease of wheat. On the basis of the results obtained during the experiment and reports of success of plant extracts in controlling plant pathogenic fungi, the tested plant extracts hold promise for the organic and ecofriendly management of foliar diseases of wheat. The findings of these studies may become the foundation for the use of biocontrol agents as a safe and cost-effective control method against leaf rust of wheat.

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