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# Effectiveness of plant extracts for repressing stem rust disease severity of wheat caused by *Puccinia graminis* f.sp. *tritici* Pers under field conditions

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#### **Abstract**

**Background:** Stem rust caused by *Puccinia graminis* f.sp. *tritici* Pers is one of the most devastating fungal foliar diseases, and causes substantial yield losses to wheat crops cultivated under field conditions. This study aimed to evaluate the efficacy of plant extract-based foliar sprays for wheat with carnation, ginger and cinnamon on the severity of stem rust infection of three wheat cultivars cultivated under field conditions for two consecutive winter seasons of (2020/21 and 2021/22).

**Results:** In both seasons our data showed that all treatments were associated with some degree of disease suppression recorded as a range of 2.3–8.6% compared to 15.3–24.5% in untreated control treatment. The plant extract treatment was more effective than Amistar, a commercial fungicide. They recorded disease severity as: scores ranged between 2.3 and 3.2%; 3.0–4.5%, and 3.0–6% for wheat *cvs*. Giza 1, Misr 2, and Sids 14, in respective order. Meanwhile, scores of 4.1, 7.5, and 8.6% were recorded at fungicidal treatment. Spaying wheat plants with carnation extract showed the lowest disease prevalence followed by ginger and cinnamon extract, respectively. Increased in grain yield was associated with reduced disease severity.

**Conclusions:** The present investigation demonstrated the efficacy using plant extracts against foliar diseases caused by *Puccinia* and it was suggested that they may be an alternative to traditional chemical treatment.

Keywords: Wheat stem rust, Puccinia graminis, Plant extracts, Foliar spray, Fungicide alternative

## **Background**

Wheat (*Triticum aestivum* L.), is vulnerable to infection by stem rust disease caused by *Puccinia graminis* f.sp. *tritici* Pers. (syn. *P. recondita* Rob. Ex Desm. f.sp. *tritici* Eriks. & Henn.) D.M. Henderson. Wheat grain is a common foodstuff, and its flour is used to produce bread, pasta, biscuits and other food products. Wheat agricultural waste and straw is also used for animal feed as is hay, grain, and wheat bran. Stem rust one of the main

diseases affecting large-scale wheat production. In Egypt, the estimated yield loss due to this disease can reach 50% in Egypt (Draz et al. 2015), while in other regions stem rust typically causes losses determined between 15 and 20% all-over the different producing regions (Figueroa et al. 2018). In the United States, stem rust disease is the most important wheat disease, and it is known to cause yield loss by affecting both quantity and quality of the plant (Leonard and Szabo 2005).

Diseases are commonly controlled by using known resistant varieties (Draz et al. 2015) and chemical fungicides (Barro et. al. 2017). However, the fungus known

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to cause stem rust fungus forms new pathogenic strains from year-to-year, and many of these strains have the potential to overcome plant resistance. In addition, the harmful effects of fungicide application on the environment are more widely understood than they were in the past. Therefore, new stem rust control methods designed to minimize the use of fungicide, are being developed. One such method involves the use of plant extracts, that contain natural substances with antifungal properties. Such extracts and associated organic materials had attracted considerable attention because they are ecofriendly control measures that both have a direct effect on phytopathogens and an indirect effect in boosting plant defense mechanisms (Gholamnezhad 2019), including the production of secondary metabolites, such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Cowan 1999). These compounds show high activity against pathogens and collectively serve as a key mechanism of plant defense against pathogenic microorganisms (Das et al. 2010).

The aim of the present investigation was to evaluate the activities of candidate plant extracts as foliar applications for suppressing the wheat stem rust disease under natural field conditions for the two consecutive seasons of (2020/21 and 2021/22).

# **Methods**

The effects of three candidate plant extracts on wheat stem rust was evaluated over two consecutive growing seasons on three wheat cultivars; (Giza 168, Misr 2, and Sids 14) that cultivated under field conditions. Field trails were performed at Kafr-Eldawar, in the El-Beheira Governorate, Egypt during 2020/21 and 2021/22 winter cultivation seasons.

#### Plant extracts preparation

Three plant extracts (Table 1) were tested for their effects on stem rust disease severity as a foliar wheat spray. Plant material was obtained from the Medicinal and Aromatic Plants Research Department, of the National Research Centre Giza (Egypt). Extract material was first washed with distilled water, then shade dried. Next, dried material was ground into powder, and the plant extracts were then prepared according to the method described by El-Mougy and Alhabeb (2009).

In brief, 250 g of powder of each plant was homogenized in ethanol (96%) and distilled water (100:400, v:v) for 10 min. These emulsions were then left in dark glass bottles for 72 h. to complete tissue maceration. Thin cheesecloth sheets were used to filter the obtained extracts. These were heated in a 60 °C water bath for 30 min to evaporate the remaining ethanol. The final extracts were stored in a refrigerator at 5 °C until needed. The commercial fungicide Amistar (*a.i.* Azoxystrobin 250 g/l) Syngenta https://www.syngenta.co.ke/product/crop-protection/fungicides/amistar-250-sc was used at the concentration of 0.5 ml/l.

During the 2020/21 cultivation season, an experimental field was comprised divided into plots each containing five  $1 \times 10$  m rows. These five plots were used as replicates for randomized block design. Growing wheat plants were subjected to spray treatment with plant extracts and the fungicide positive control was applied twice during the growing season. The first spray was performed one month after seedling appearance above the soil surface. The second was performed one month later.

Foliar spray of wheat cultivars was individually applied. For each of the plant extracts, the foliar spray consisted of 3 l of a 2% solution (by volume). Amistar was applied at a concentration of 0.5 ml/l. A negative control treatment received a spray of the same volume of distilled water. The same procedures were followed at the same experimental field for the second growing season during 2021/22.

During both cultivation seasons, it was scored stem rust disease occurrence and severity from 15 days after the application of the first spray. In both 2021 and 2022, encoring was repeated every 21 days throughout the growing season until the end of April (i.e., harvest time). These procedures were followed for all experimental and the control treatments. The calculation of

**Table 1** Plants classification and their main active components

Common name	Scientific name	Family	Major active component	Plant organ containing active component and its citation
Carnation	Dianthus caryophyllus L	Caryophyllaceae	Eugenol, benzyl benzoate and salicate	Flowers https://sphinxsai.com/2016/ph_vol9_ no4/1/(113-117)V9N4PT.pdf
Cinnamon	Cinnamomum burmannil (Nees & T. Nees) Blume	Lauraceae	cinnamic aldehyde	Cortical tissues DOI: https://doi.org/10.13140/ RG.2.1.2248.1523
Ginger	Zingiber officinale Roscoe	Zingiberaceae	bioactive phenolic and terpene compounds (gingerols, shogaols and paradols)	Roots DOI: https://doi.org/10.3390/foods 8060185

the grain yield was determined by computing the average thousand-seed weight of ten samples chosen at random from each experimental and control treatment. These weights were then computed as relative yields by ton. Disease severity and yield were calculated for both experiments as percentages.

# **Determination of disease severity**

Disease severity was estimated throughout the vegetative growth period. One hundred plants were randomly chosen and marked to determine the stem rust severity, as per the procedure described by Gomes et al. (2016). The average disease severity was computed at the end of the growing season (harvest). A five-point scale was used for score disease severity scores were recorded according to percentage of the stem plant area covered with rust urediniospores as follows:

No symptoms (score = 0); 1%-25% (score = 1), 26%-50% (score = 2), 51%-75% (score = 3) and more than 75% (score = 4).

The following formula was then used to calculating the percentage of disease severity:

#### Data analyses

CoStat version 6.3.3 (CoHort Software) was used to analyze the results. The data from each growing season was statistically analyzed separately using one-way analyses of variance. Means were then compared by Duncan multiple range tests at P < 0.05.

#### Results

It was examined the efficacy of plant extract sprays on stem rust disease severity in wheat cultivated under field conditions over two cultivation seasons. Data (Table 2) and (Fig. 1) showed that the wheat cultivar "Giza 168" was the most resistant to stem rust disease, followed by "Misr 2" and "Sids14", respectively.

In addition, it was found that all plant extract treatments were capable of suppressing stem rust disease severity, albeit to different degrees. In both growing seasons carnation extract treatment showed the greatest disease suppression effect, followed by ginger and cinnamon extracts, respectively. The fungicidal treatment was the least effective stem rust treatment, although it was significantly better than the control treatment.

Disease severity % = 
$$\frac{\text{Total number of diseased plants}}{\text{Total number of tested plants } x \text{ maximum disease rate}} \times 100$$

**Table 2** The use of plant extract for suppressing wheat stem rust disease under field conditions for the winter cultivation seasons 2020/21 and 2021/22

Wheat cultivar	Treatment	Concentrations	Disease severity % Cultivation season	
			2020/21	2021/22
Giza 168	Cinnamon extract	2%	3.2 ± 0.4 h	4.6 ± 1.2 h
	Carnation extract	2%	$2.3 \pm 0.5 hi$	$3.0 \pm 0.9i$
	Ginger extract	2%	$2.6 \pm 0.8 hi$	$3.1 \pm 0.7i$
	Amistar	0.5 ml/l	$4.1 \pm 0.5 \mathrm{g}$	$6.0 \pm 0.5 \mathrm{g}$
	Control	Distilled water	15.3 ± 2.1c	$18.5 \pm 1.9c$
Misr 2	Cinnamon extract	2%	$4.5 \pm 1.3 \mathrm{g}$	$6.1 \pm 0.8 \mathrm{g}$
	Carnation extract	2%	$3.0 \pm 1.1 \text{ h}$	$3.3 \pm 0.7i$
	Ginger extract	2%	$3.0 \pm 0.8  \text{h}$	5.3 ± 1.0gh
	Amistar	0.5 ml/l	$7.5 \pm 0.8e$	9.0 ± 0.7e
	Control	Distilled water	$20.6 \pm 1.8b$	$23.6 \pm 2.3b$
Sids 14	Cinnamon extract	2%	$6.0 \pm 2.0 ef$	8.3 ± 1.7ef
	Carnation extract	2%	$3.0 \pm 0.7  \text{h}$	$4.6 \pm 1.7  \text{h}$
	Ginger extract	2%	$5.0 \pm 0.6  \mathrm{fg}$	$6.5 \pm 1.3 \mathrm{g}$
	Amistar	0.5 ml/l	$8.6 \pm 0.6d$	$10.5 \pm 1.3d$
	Control	Distilled water	$24.5 \pm 2.3a$	$27.0 \pm 0.6a$
LSD 5%			1.11	1.21

Mean  $\pm$  standard deviations within each column followed by the same letters are not significantly different at P < 0.05

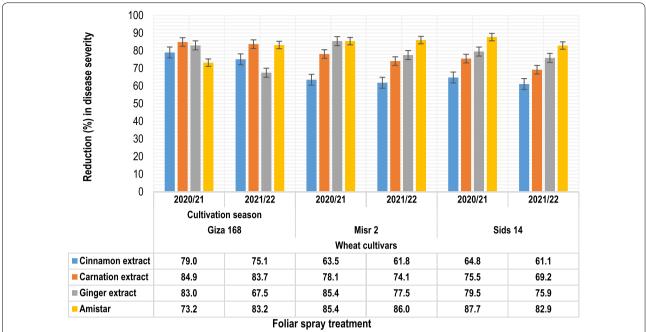


Fig. 1 Reduction in stem rust disease severity in response to plant extract spray treatment under field conditions during the cultivation seasons of 2020–2021 and 2021–2022

Data in Fig. 1 illustrated a high decrease in disease severity reached 84.9 & 83.7; 78.1 & 74.1; 75.5 & 69.2%, at carnation extract treatment for wheat cultivars; Giza 168, Misr 2 and Sids 14 at the two growing seasons 2020/21 and 2021/22, in relevant respective order. Reduction in disease severity at the treatments of ginger and cinnamon extracts attained 83.0 & 67.5; 85.4 & 77.5; and 79.5 & 75.95% for the first cultivated followed by 79.0 & 75.1; 63.5 & 61.8; and 64.8 & 61.1% throughout the second cultivated seasons for Giza 168, Misr 2 and Sids 14 wheat *cvs.*, in relevant special order.

On the other hand, the lowest effect on disease severity at Amistar fungicide treatment was 4.1 & 6.0; 7.5 & 9.0; and 8.6 & 10.5% (Table 1) with calculated reduction of 73.2 & 83.2; 85.4 & 86.0; and 87.7 & 82.9% (Fig. 1) for the wheat cultivars; Giza 168, Misr 2 and Sids 14, respectively, at the two successive growing seasons.

Wheat grain yield was estimated at harvest by selecting 10 samples out of 1000 kernels of each treatment and control condition. The resulting data presented in Table 3 and Fig. 2 showed that at the two growing seasons (2020/21 & 2021/22) the produced yield of all applied treatments was higher than that of control treatment which was (5.75–5.50); (5.50–5.50), (5.25–5.25) ton/hectare for wheat cultivars Giza 168; Misr 2; Sid 14, respectively. At all wheat cultivars, it was found that the highest produced yield was recorded at treatments of carnation followed by the ginger, cinnamon extracts then

fungicide treatment, in descending order. In the two cultivation seasons, the carnation extract treatment resulted in estimated yields of (8.5–8.00); (8.00–7.75); and (7.50– 7.00) ton/hectare for wheat cvs. Giza 168; Misr 2; Sid 14, respectively (Table 3), with increases over the control attained by (47.8–45.4); (45.4–40.9); and (42.8–33.3) %, respectively (Fig. 2). At ginger extract treatment, the calculated yield of Giza 168 (7.75-6.75); Misr 2 (7.50-7.00); and Sids 14 (7.00–6.75) ton/hectare (Table 3) with increases over control estimated by (34.7-22.7); (36.3-27.2); and (33.3-28.5) % (Fig. 2), respectively, for both cultivation seasons. Also, data in Table 3 showed that at cinnamon extract treatment the recorded produced yield for the two cultivation seasons was (7.25-7.25); (7.00-6.50); (6.75–6.25) ton/hectare as well as (7.00–6.75); (6.75-6.50) and (6.25-6.00) ton/hectare at fungicidal treatment for wheat cultivars Giza 168; Misr 2; Sid 14, in relevant respective order. Meanwhile, illustrated data in Fig. 2 showed percentages of yield increase over control treatment for wheat cultivars at the two cultivation seasons. At cinnamon extract treatment the produced yield increased by (26.0-31.8); (27.2-18.1); and (28.5-19.0) %, in the meantime, at Amistar treatment, the recorded increase was by (21.7-22.7); 22.7-18.1) and (19.0-14.2) %, in relevant respective order to wheat cultivars Giza 168; Misr 2; Sid 14 at the two growing seasons 2020/21 & 2021/22.

**Table 3** Average yield production (ton/hectare) in response to different plant extract treatments on three wheat cultivars grown under field conditions during 2020–2021 and 2021–2022

Wheat cultivar	Treatment	Concentration	Grain yield (Ton/hectare)	
			Cultivation seasons	
			2020/21	2021/22
Giza 168	Cinnamon extract	2%	7.25 ± 0.3bc	$7.25 \pm 0.6 \text{ cd}$
	Carnation extract	2%	$8.50 \pm 0.3e$	$8.00 \pm 0.3f$
	Ginger extract	2%	$7.75 \pm 0.6$ bc	$6.75 \pm 0.6c$
	Amistar	0.5 ml/l	$7.00 \pm 0.2b$	$6.75 \pm 0.5c$
	Control	Distilled water	$5.75 \pm 0.3a$	$5.50 \pm 0.2a$
Misr 2	Cinnamon extract	2%	$7.00 \pm 0.8b$	$6.50 \pm 0.5b$
	Carnation extract	2%	$8.00 \pm 0.6  \text{cd}$	$7.75 \pm 0.2e$
	Ginger extract	2%	$7.50 \pm 0.8 bc$	$7.00 \pm 0.3 \text{ cd}$
	Amistar	0.5 ml/l	$6.75 \pm 0.4b$	$6.50 \pm 0.5b$
	Control	Distilled water	$5.50 \pm 0.2a$	$5.50 \pm 0.2a$
Sids 14	Cinnamon extract	2%	$6.75 \pm 0.4b$	$6.25 \pm 0.5b$
	Carnation extract	2%	$7.50 \pm 0.5 bc$	$7.00 \pm 0.4 \text{ cd}$
	Ginger extract	2%	$7.00 \pm 0.6b$	$6.75 \pm 0.4c$
	Amistar	0.5 ml/l	$6.25 \pm 0.3b$	$6.00 \pm 0.5b$
	Control	Distilled water	$5.25 \pm 0.3 a$	$5.25 \pm 0.3a$
	LSD 5%		0.48	0.50

 $Mean \pm standard\ deviation\ within\ each\ column\ followed\ by\ the\ same\ letter\ are\ not\ significantly\ different\ at\ P<0.05$ 

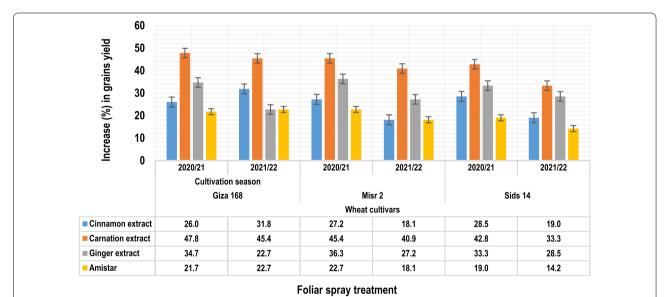


Fig. 2 Increase in grain yield by plant extract treatment of three wheat cultivars grown under field conditions during the cultivation seasons of 2020–2021 and 2021–2022

In addition, it was found that the fungicidal treatment showed the smallest increase in yield over the control treatment, with calculated yield increases of 21.7–22.7%; 22.7–18.1%; and 19.0–14.2% for the wheat *cvs*. Giza 168; Misr 2; and Sid 14, respectively, at the two cultivation

seasons. The yield produced by plants treated with the fungicidal treatment was 7.00–6.75; 6.75–6.50; and 6.25–6.00 ton/hectare for wheat *cvs*. Giza 168; Misr 2; and Sid 14 during the 2020/21 and 2021/22 growing seasons, respectively. Finally, it was observed the average grain

yield of the control condition to be 5.75–5.50; 5.50–5.50; and 5.25–5.25 ton/hectare, respectively for the same three cultivars.

#### Discussion

The goal of the present study was to develop alternative methods of controlling stem rust and thereby decrease dependence on chemical pesticides. Relevant to this concern, it was observed that plants can synthesize secondary metabolites which possessed antimicrobial effects and thus played important roles in natural plant defense mechanisms (Cown 1999; Das et al. 2010). However, few plants contained the components that showed toxicity against phytopathogens (Gujar et al. 2012). Gujar et al. (2012) noted that some volatile oils have been recommended as antimicrobial substances against phytopathogenic microorganisms, since these oils contain essential aromatic constituents capable of inhibiting microbial contamination and decrease spoilage of food commodities.

In this investigation it was found that treatment by carnation, ginger, and cinnamon extracts was associated with a significant reduction in stem rust disease and yield increase for all grown wheat cultivars. Moreover, it was found that treatment of carnation extract was the most effective, followed by ginger and cinnamon extracts, respectively. In contrast, the commercial fungicidal treatment showed fewer efficacies against stem rust disease. These results confirmed the previous reports that showed the effectiveness of plant extracts against plant foliar diseases, including Abd El-Malik and Abbas (2017) who sprayed susceptible wheat cultivars, Sids-1 and Gemmeiza-7 with plant extracts of neem (Azadirachta indica A. Juss), eucalyptus (Eucalyptus obliguia L" Her), cactus, garlic (Allium sativum L.) and pomegranate (Punica granatum L.) to combat wheat leaf rust caused by P. triticina. They found that all of these extracts significantly decreased rust severity. They concluded that the plant extract treatments may probably induced resistance in susceptible cultivars and /or had their own antifungal activity against the biotrophic pathogen. Furthermore, ELkhawaga et al. (2018) demonstrated the effectiveness of water and methanol extracts of henna (Lawsonia inermis L.), lantana (Lantana camara L.), acalypha (Acalypha sp. L.), chinaberry (Melia azedarach L.), and pomegranate (Punica granatum L.) as foliar applications on wheat plant against leaf rust. In this study, the authors found once again that all plant extract treatments reduced leaf rust disease severity and enhanced grain yield. These results agree with the findings of Draz et al. (2019), who attempted to replicate this experiment by applying the same plant extracts as foliar sprays on the susceptible wheat cultivar Gemmiza 7 for two cultivation seasons to test whether these sprays would protect wheat plants against leaf rust caused by P. triticina Eriks. These authors found that all the applied plant extracts significantly reduced the coefficient of disease infection (ACI) and increased yield. The authors also noted that lantana extract and fungicide diconazole significantly differed from the other treatments. Finally, they concluded that their results were associated with increases in total phenolic and oxidative enzyme (POX and PPO) content, and speculated that these compounds were responsible for inducing wheat resistance to leaf rust. Plant extracts have also been shown to contain compounds possessing activity against a broad spectrum of phytopathogens by stimulating the defense mechanism of infected plants (Srivastava et al. 2011). Numerous reports have demonstrated the efficacy of antifungal compounds present in botanical extracts against plant (Morsy et al. 2011). For example, both biotic and abiotic compounds may induce anti-phytopathogen immunity by acting as secondary messengers capable of promoting the immune protection of the host plant (Geetha and Shetty 2002) either by rising the activity of POX enzymes, by producing new isoforms of peroxidase (POD), by aggregating phenolic compounds (Hassan et al. 2007), or by repressing the production of antioxidant enzymes and catalases; that lead to oxidative stress (Radwan et al. 2008). In addition, abiotic elicitors also promote resistance via directly impacting the evolution and permanence of the pathogens or via indirect means, such as influencing plant metabolism and consequently the food supply of the pathogen (Khan et al. 2003). Under field conditions in Egypt, Shabana et al. (2017) reported that wheat seedlings treated with neem (Azadirachta indica A. Juss), clove (Syzygium aromaticum (L.) Merr & L.M. Perry) and garden quinine (Clerodendrum inerme L. Gaertn) extracts completely (i.e., 100%) prevented the development, of rust disease four days after inoculation, this favorably compared with treated by the fungicide Sumi-8 after artificial infestation with P. triticina, the pathogen known to cause leaf rust. Shabana et al. (2017) also found that neem extract was the most effective treatment, as its application was associated with a significant reduction in leaf rust infection when applied as a foliar spray to naturally infected mature wheat plants. Moreover, the consensus of many reports (Ayoub and Niazi 2001; Elsharkawy and El-Sawy 2015) was plant extracts contain active biological control agents against a wide spectrum of phytopathogenic microorganisms. Joseph et al. (2008) stated that the presence of antifungal components in botanical plant extracts, i.e., Azadirchin, Artemesium, Caratenes, Emodin and Eucalyptolin might be the cause of the inhibitory effects against pathogens. Finally, Fokkema (1993) stated that disease suppression by plant extracts might attributed to indirect mechanisms that was not yet clearly understood, but may involve induced resistance of host plants.

#### **Conclusions**

Generally, it might be suggested that utilization of plant extracts as foliar spray could be beneficial for controlling the stem rust disease of wheat. On the basis of the obtained results of the present study, as well as the previous reports, using plant extracts for controlling such plant diseases are considered successfully a promise for organic, fungicides alternatives and ecofriendly management of wheat foliar diseases.

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#### **Author contributions**

The idea and experimental design were prepared by all authors. NSE, NGE and MSAK performed the experiment, collected the field results and wrote the paper. MMA, NSE and NGE analyzed the recorded data and helped in the writing manuscript. MMA and NSE supervised the results analysis and corrected the manuscript written draft. All authors have read and approved the manuscript.

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### Availability of data and materials

All created and/or analyzed data during the present study are attainable in the manuscript, and the corresponding author has no interception to the availability of data and materials.

#### **Declarations**

#### Ethics approval and consent to participate

The study was conducted on natural occurrence of wheat foliar diseases and usage of beneficial fungi and bacteria that are available in the environment and the ethical approval is not demanded.

#### Consent for publication

Not Applicable.

# Competing interests

All authors declare that they have no conflict of interest.

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