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Full Length Article

Efficacy of plant extracts in controlling wheat leaf rust disease caused by *Puccinia triticina*

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ABSTRACT

The efficacy of eight plant extracts (garlic, clove, garden quinine, Brazilian pepper, anthi mandhari, 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.

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 agro-ecosystem. Eco-friendly control measures including plant

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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 (*Azadirchta indica*, A. juss) and garlic (*Allium sativum*); and essential oils such as nettle (*Urtica* spp.), rue (*Ruta graveolens*, Linn), thyme (*Thymus vulgaris*, Linn), wats 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, cost effective, nonhazardous 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.

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	*Allium sativum*	Bulbs	[26]
Clove	*Syzygium gromaticum*	Buds	[27]
Garden quinine	*Clerodendrum inerme*	Leaves	[28]
Brazilian pepper	*Schinus terebinthifolius*	Leaves	[29]
Anthi mandhari	*Mirabilis jalapa*	Roots	[30]
Black cumin	*Nigella sativa*	Seeds	[31]
White cedar	*Thuja accidentalis*	Leaves	[32]
Neem	*Azadirchta indica*	Seeds	[33]

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.

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]:

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

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