

SHORT COMMUNICATION

Antifungal activity of aqueous extracts of spices against bean rust (*Uromyces appendiculatus*)

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ABSTRACT

This pot culture study done under controlled conditions, evaluated the antifungal activity of aqueous extracts [1, 2 and 3 % (w/v)] of 8 spices [basil (*Ocimum basilicum* L.), black cumin (*Nigella sativa* L.), black pepper (*Piper nigrum* L.), celery (*Apium graveolens* L.) fennel (*Foeniculum vulgare* Mill.), laurel (*Laurus nobilis* L.), parsley (*Petroselinum crispum* Mill.) and rosemary (*Rosmarinus officinalis* L.)] against bean rust. All spice extracts except parsley, when applied 2 h before pathogen inoculation significantly controlled (26.5 – 96.1 %) the rust development. Black cumin extract was most effective followed by laurel. Black cumin extracts at 2 and 3 % concentrations provided rust control similar to mancozeb fungicide. There was no synergistic effect, when black cumin and laurel extracts were combined. None of the tested spice extracts were phytotoxic to bean leaves.

Key words: Alternative control, botanical fungicides, organic farming, plant extracts.

INTRODUCTION

Bean rust, caused by *Uromyces appendiculatus* (Pers.) Unger, is major disease of bean (*Phaseolus vulgaris* L.) and reduces the yields from 18 to 100 % in dry and snap beans (19,25). Its control primarily depends on host resistance, but owing to high genetic diversity of pathogen and its ability for new race development, resistance is not long lasting (29). Hence, in most bean-growing areas of Europe and United States, fungicides are used for its control, however, resistance to fungicides is major reason of poor disease control (1,12,26). To overcome this problem, higher concentrations of fungicides are used, but it increases the risk of high-level toxic residues in the products (6).

Black cumin is grown in Mediterranean countries and has been used as food preservative and medicinal plant since ancient times (23). Its seeds contain essential oils, proteins, alkaloids and saponins and their biological activity has been shown due to thymoquinone, the major component of essential oil (2). Black cumin seed extracts or oil possess antimicrobial activity (20) and inhibitory effects against *Candida albicans* (C.P. Robin) Berkhout (15). Laurel has been used as herbal medicine owing to its antibacterial

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and antifungal effects (8). Various compounds (phenolics, terpenoids and alkaloids) are present in plants and they jointly or independently possess biological activities. These phytochemicals act in different ways against several disease complexes, hence, may be applied for plants disease control like agricultural chemicals. They are being considered as potential alternatives to synthetic fungicides, or as lead compounds for new classes of synthetic fungicides, such as podoblastin produced by *Podophyllum peltatum* L. (21).

The widespread use of pesticides increases the cost, handling hazards and is a threat to human health and environment (9,10,11). In recent years, a greater awareness of environmental and health hazards and the development of resistance to fungicides has increased the public demand to reduce the pesticides use in agriculture (17,27). Therefore, alternative control methods are needed. Among the various choices, plant extracts seem to be an alternative to currently used fungicides to control phytopathogenic fungi, as they are (i). rich source of bioactive chemicals (13), (ii). biodegradable in nature, (iii). non-pollutant and (iv). have no residual or phytotoxic properties. These natural products have the potential to replace the present fungicides (24,28). This study aimed to assess the antifungal activity of aqueous extracts of spices against bean rust in pot culture.

MATERIALS AND METHODS

The selected dry spice plant materials (Table 1) were purchased from Bursa, Turkey and their taxonomy was confirmed by Dr. G. Kaynak, Department of Biology, Uludağ University, Bursa, Turkey. Dry materials were grounded in Mill to obtain a fine powder. Powdered spice samples were separately added to 100 ml distilled water and stirred for 4 h at 25°C to obtain 1, 2 and 3 % (w/v) concentrations. The aqueous extracts were filtered twice through four layers of cheese cloth and used immediately for spraying plants.

Table 1. The spices extracts used

Common name	Botanical name	Family	Used part
Basil	<i>Ocimum basilicum</i> L.	Lamiaceae	Leaves
Black cumin	<i>Nigella sativa</i> L.	Ranunculaceae	Seeds
Black pepper	<i>Piper nigrum</i> L.	Piperaceae	Fruits
Celery	<i>Apium graveolens</i> L.	Apiaceae	Fruits
Fennel	<i>Foeniculum vulgare</i> Mill.	Apiaceae	Fruits
Laurel	<i>Laurus nobilis</i> L.	Lauraceae	Leaves
Parsley	<i>Petroselinum crispum</i> Mill.	Apiaceae	Leaves
Rosemary	<i>Rosmarinus officinalis</i> L.	Lamiaceae	Leaves

Fungal culture, spore propagation and growth of plants

Leaves infected with *Uromyces appendiculatus* (Pers.) Unger, were collected from bean fields in Bursa, Turkey. The urediniospores were gently removed with spatula from the surface of rust-infected leaves, dried in desiccator at room temperature for 12 h and then kept at 5°C until use. The spores were inoculated to bean (*Phaseolus vulgaris* L. cv. Gina) plants grown in climate-controlled room. Inoculated plants were covered with polyethylene bags to maximize the relative humidity to facilitate infection. Covered plants

were kept at 18°C for 24 h in dark and then at 20°C for 10 days without the polyethylene bags. The light intensity inside the climate room was (167 $\mu\text{E}/\text{m}^2/\text{s}$) with a 12-h supplemental photoperiod. Fresh spore were removed from the surface of pustules 12 days after inoculation and immediately used in *in vivo* experiments.

Pot experiments

Experiment 1: The treatments consisted of two factors: (i). 8 spices (basil, black cumin, black pepper, celery, fennel, laurel, parsley and rosemary) and (ii). Extracts concentrations 4 (0,1,2,3%). The efficacy of aqueous extracts of spices was tested in a climate-controlled room as described above. Two bean plants (cv. Gina) were grown per pot (7 cm dia), Gina cultivar was selected due its high susceptibility to *U. appendiculatus* (4). In the first set of experiments, each concentration of aqueous extract was sprayed (until run-off) on primary leaves in 12-day-old bean plants, 2 h before inoculation with *U. appendiculatus*. Plants were inoculated with 2×10^4 urediniospores/ml spore suspension of *U. appendiculatus*. The spore suspension (containing 0.01 % Tween 20) was sprayed with hand sprayer. A mancozeb (Dithane M-45, 80 WP, Cyanamid Turkey, label rate, 0.2 %) fungicide, was used to compare the efficacy of spice extracts. Control plants were sprayed with distilled water. The efficacy of spices was assessed 10 days after the inoculation. Disease development was evaluated by counting the number of pustules within randomly selected three different one- cm^2 areas per leaf. Data were converted to the control percentage as compared to controls.

Experiment 2: Black cumin and laurel showed higher efficacy, hence, these were tested alone or in mixture in second set of experiments. The treatments consisted of two factors : (i). 2 spices (Black cumin and laurel) and (ii). Extracts concentrations : 3 (0, 0.75,1.5%). The powdered black cumin and laurel samples were prepared in 100 ml distilled water in 1:1 ratio (0.75 g from each). The extract was stirred for 4 h at 25°C to obtain spice extract concentration of 1.5 %. The pot experiment and inoculation procedures were same as described above.

Both these experiments were conducted in triplicate and their treatments were replicated thrice and each replicate comprised of three pots per treatment. Analysis of variance was performed and means were evaluated by LSD test ($P \leq 0.05$).

RESULTS AND DISCUSSION

We assessed the antifungal activity of 8 spices extracts against bean rust (caused by *U. appendiculatus*). Among the aqueous extracts of 8 spices, black cumin and laurel showed higher disease control (96.1 and 79.3 %, respectively) (Fig. 1). The celery extract caused lowest inhibition of 26.5 %, while the parsley extracts did not control bean rust. The mancozeb fungicide completely inhibited the bean rust. Black cumin extracts at 2 and 3 % concentrations proved as effective as mancozeb (Fig. 1).

The results indicate that black cumin is most effective spice in inhibiting the bean rust and followed by laurel (Table 2).

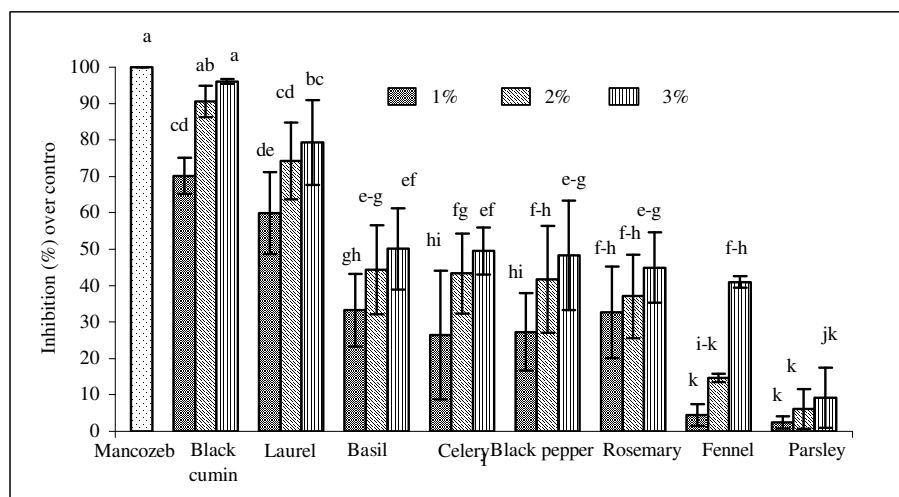


Figure 1. Effect of aqueous extracts of various spices on the control of bean rust under controlled conditions. Vertical bars show standard deviation. Values with different letters show significant difference as determined by LSD test.

Table 2. The efficacy of aqueous extracts of spices against bean rust under controlled conditions

Spice Extract	Rust control efficacy (%)
Mancozeb	100.0
Black cumin (<i>Nigella sativa</i> L.)	85.6
Laurel (<i>Laurus nobilis</i> L.)	71.2
Basil (<i>Ocimum basilicum</i> L.)	42.6
Celery (<i>Apium graveolens</i> L.)	39.7
Black pepper (<i>Piper nigrum</i> L.)	39.2
Rosemary (<i>Rosmarinus officinalis</i> L.)	38.2
Fennel (<i>Foeniculum vulgare</i> Mill.)	20.1
Parsley (<i>Petroselinum crispum</i> Mill.)	5.9

Data are mean of three concentrations (1,2,3 %) of each tested spice.

Owing to higher efficacy of black cumin and laurel extracts, these were selected for further studies. At 0.75 %, the efficacy of black cumin and laurel was similar (Fig. 2). The efficacy of combined application of both extracts was identical to black cumin and no synergistic effect was observed, (Fig. 2). The synthetic fungicide mancozeb used as reference fungicide was superior to spice treatments. None of the tested spice extracts were phytotoxic to bean leaves.

Aqueous extracts of *Reynoutria sachalinensis* (F. Schmidt) Nakai protected the cucumber (*Cucumis sativus* L.) plants from powdery mildew, *Sphaerotheca fuliginea* (Schl. Pollacci), infection by 90% and was comparable to myclobutanil and sulfur fungicides (7). Kim et al. (16) reported that extracts of *Achyranthes japonica* (Miq.) Nakai

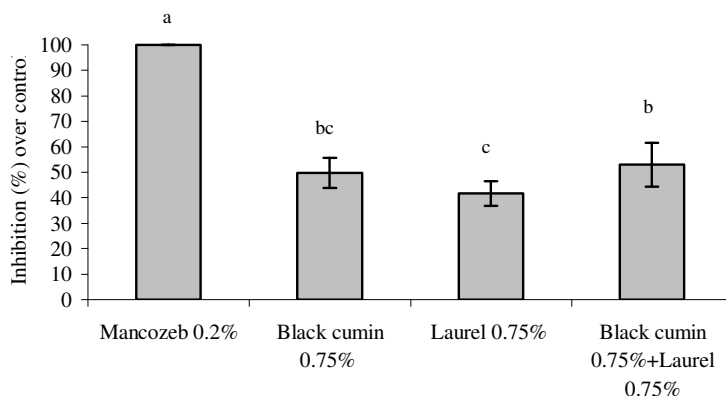


Figure 2. Effect of aqueous extracts of black cumin and laurel alone or in combination on the control of bean rust under controlled conditions. Vertical bars show standard deviation. Values with different letters show significant difference as determined by LSD test.

and *Rumex crispus* L. effectively controlled the cucumber powdery mildew. Plant-derived compounds effectively controlled the rice blast caused by *Pyricularia oryzae* Cav. (3). Kamalakannan *et al.* (14) reported that spraying with *Prosopis juliflora* (Sw.) DC., leaf extract significantly reduced the rice blast and also increased the yield. Kagale *et al.* (13) reported that leaf extract of *Datura metel* L., *Zizyphus jujuba* Mill., *Ipomea carnea* Jacq, *Azadirachta indica* A. Juss., and *Vitex negundo* L. showed strong inhibitory effect against *Rhizoctonia solani* Kuhn., and *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Dye, on rice (*Oryza sativa* L.) plants under *in vitro* and *in vivo* conditions. Leaf and seed extracts of *Pithecellobium dulce* (Roxb.) Benth showed fungicidal effects on sporulation and mycelial growth of *Botrytis cinerea* Pers., *Penicillium digitatum* (Pers.: Fr.) Sacc., and *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill, on strawberry (*Fragaria vesca* L.) fruit (5). The extracts of *Macleaya cordata* Willd. R. Br., *Polygonum cuspidatum* Sieb. et Zucc. and *Scutellaria baicalensis* Georgi demonstrated antifungal activity against stem blister canker (*Botryosphaeria dothidea* Moug.ex Fr.) on *Populus* genus (30). Aqueous extract of neem (*Azadirachta indica* A. Juss.) leaves controls leaf stripe pathogen (*Drechslera graminea* Rabenh. Shoemaker) on barley (*Hordeum vulgare* L.) (22). Li and Zhihui (18) reported that garlic (*Allium sativum* L.) extract controlled the *Phytophthora capsici* Leonian.

CONCLUSIONS

The aqueous extracts of black cumin and laurel effectively controlled the infections of *U. appendiculatus*, hence, they may be possible alternative to synthetic fungicides. These spice extracts may be used alone in organic agriculture or in rotation with synthetic fungicides in an Integrated Pest Management (IPM) programme in the conventional bean growing. Further studies are needed to evaluate the efficacy of black cumin and laurel extracts in the fields.

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