

Suppression of phytopathogenic fungi using garlic, horsetail, and yarrow plant extracts

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ABSTRACT

Organic production is a system of sustainable agriculture based on a high respect for environmental principles through the rational use of natural resources, renewable energy sources, conservation of natural diversity, and environmental protection. Plant cultivation in organic production implies the application of preparations on a natural basis, which is most often used as a preventive measure without the application of agrochemicals. Therefore, the use of "domestic" natural pesticides is increasingly present. In this study, the antimicrobial effect of garlic (*Allium sativum* L.), horsetail (*Equisetum arvense* L.), and yarrow (*Achillea millefolium* L.) extract on the growth of phytopathogenic fungi of the genus *Fusarium*, *Alternaria*, and *Aspergillus* was tested. The potato dextrose agar was inoculated with the mycelia of fungi. The filter paper discs impregnated with 15 µL of plant extracts were placed onto agar. In the control sample, distilled water was used for the impregnation of paper discs. All experiments were performed in triplicate. Inhibition zone measurements were performed on the third day. The results showed that each of the mentioned plant extracts inhibited the growth of the phytopathogenic fungi. The lowest inhibition zone was observed using the garlic extract, while the highest inhibition zone was detected on the disc that was impregnated with yarrow extract. These results confirm the use of plant extracts in the suppression of phytopathogenic fungi.

Keywords: antifungal activity, incubation, plant extracts, phytopathogenic fungi, suppression

INTRODUCTION

The term „pesticides“ includes various compounds: insecticides, fungicides, herbicides, rodenticides, nematicides, etc. (Aktar et al., 2009). These substances are widely used in agriculture for crop protection from pests, diseases, and weeds (Nicolopoulou-Stamati et al., 2016). Pesticides have also been used to improve non-agricultural fields and green areas (Hoffman et al., 2000). In the last several decades, the widespread use of pesticides has been associated with the deterioration of environmental quality (Goulson, 2014; Zheng et al., 2016), thus the application of some pesticides in agriculture has

been prohibited and abandoned (Donley, 2019). The environmental issues caused by pesticides also include human health problems (Nicolopoulou-Stamati et al., 2016) because of the bioaccumulation of pesticides in the human body (Pirsaheb et al., 2015). Negative health effects include dermatological, neurological, carcinogenic, and reproductive problems related to pesticide exposure (Roeleveld and Bretveld, 2008; Salameh et al., 2006). Therefore, Nile et al. (2019) suggested that plant products can be used as an efficient alternative approach to pesticides.

Long-term research on plant extracts led to the production of powerful, low- cost, and environmentally-friendly pesticides (Miresmailli and Isman, 2014), which have the potential to solve pest and disease problems in plant production (Souto et al., 2021). The same authors have suggested that many of them are approved in biological control regulations.

In this research, plant extracts of garlic (*Allium sativum* L.), horsetail (*Equisetum arvense* L.), and yarrow (*Achillea millefolium* L.) were used to suppress growth of phytopathogenic fungi. The use of garlic extract against bacteria and fungi was confirmed in many studies (Krebs et al., 2006; Latha et al., 2009). The antifungal potential of horsetail was described in the research by Đurić et al. (2019), while yarrow extract can be used in the biological control due to bioinsecticidal (Daraban et al., 2022) and repellent (Magierowitz et al., 2020) properties. Thus, the objective of this paper was to determine the impact of the plants' extracts on the growth of phytopathogenic fungi of the genus *Fusarium*, *Alternaria*, and *Aspergillus*.

MATERIAL AND METHODS

The garlic extract was prepared by mixing garlic cloves with sterilized water up to a concentration of 0.07% (w/w) (C1). Before antifungal tests, this mixture was diluted in sterilized water (ratio 1:2) (C2). Horsetail extract was prepared by mixing horsetail shoots with sterilized water up to a concentration of 0.08% (w/w) (C1). This mixture was also diluted in sterilized water in a ratio of 1:2 (C2). Yarrow extract was prepared in a concentration of 0.015% (mixture of 15 g yarrow plant biomass and 1 L of sterilized water). The obtained extract was diluted in distilled water in a ratio of 1:2 (C2). All extracts were kept at +4 °C.

The antifungal property of the plant extracts was determined using the Kirby- Bauer test. Pure cultures of phytopathogenic fungi (*Aspergillus* sp., *Fusarium* sp., and *Alternaria* sp.) were grown on Potato dextrose agar (PDA, Himedia, India). The fungal isolates are the property of the Department of Microbiology at the Faculty of Agriculture and Food Science in Sarajevo (Bosnia and Herzegovina) Collection of microorganisms. The four

discs of fungal isolate (Ø 5 mm) were placed on a plate approximately 5 mm from the edge of the Petri dishes. Sterile filter paper discs (Ø 5 mm) containing 15 µl of the plant extract were placed in the centre of the Petri dish. In control, distilled water was applied to the disc. Incubation was performed at 22 °C for 6 days (Binder, Latvia). All tests were done in quadruplicates. Measurement of the inhibition zone was performed on the third day. The value of the inhibition zone was expressed in millimeters (mm), obtained results were statistically processed using the software package SPSS 20. To determine the statistically significant differences between the obtained values, the Independent Sample T-test ($P = 0.05$) was performed.

RESULTS AND DISCUSSION

Numerous authors report that plant extracts are promising alternatives to agrochemicals in contemporary agriculture (Kalidindi et al., 2015; Perello et al., 2013). The results obtained in this study point out the tested extracts can have antifungal potential ($P < 0.05$) against *Fusarium* sp, *Alternaria* sp, and *Aspergillus* sp. *in vitro* conditions. The level of inhibition depends on the origin and concentration of the plant extracts and the incubation time (Table 1).

According to Table 1, the inhibition zones affecting *Fusarium* sp. were higher ($P < 0.05$) in treatments with plant extracts compared with the control. Application of C1 extracts of yarrow and horsetail resulted in higher inhibition zones compared with C2 extracts. The highest inhibition zone was detected using yarrow plant extract (9.0 mm in C2 extract and 14.0 mm in C1 extract, which is statistically different) (Figure 1). Horsetail extract also showed a high inhibition zone (8.0, and 11.75 mm in C2 and C1 extracts, respectively, which is statistically significant). Garlic extract showed the lowest inhibition zone of *Fusarium* sp. and diameter values were similar using both extracts (7.75 and 8.0 mm).

The yarrow plant extract applied in C1 concentration was the most efficient extract against *Alternaria* sp. (Table 2) compared with C1 garlic and horsetail extracts. On the other hand, the C2 yarrow extract showed the lowest inhibition zone compared with the other two C2 extracts.

Table 1. Inhibition of *Fusarium* sp. growth by plant extracts

<i>Fusarium</i> sp.	Diameter of the inhibition zone (mm)						Control
	Yarrow extract		Garlic extract		Horsetail extract		
	Extract concentration (%)						
Replication	C2	C1	C2	C1	C2	C1	-
I	8.0	15.0	8.0	10.0	10.0	13.0	2.0
II	9.0	13.0	7.0	6.0	9.0	10.0	3.0
III	10.0	13.0	8.0	7.0	7.0	14.0	3.0
IV	9.0	15.0	8.0	9.0	6.0	10.0	2.0
Average	9.0 ^{aA} ± 0.82	14.0 ^{bA} ± 1.15	7.75 ^{aA} ± 0.50	8.0 ^{aA} ± 1.83	8.0 ^{aA} ± 1.83	11.75 ^{bA} ± 2.06	2.5 ^B ± 0.58

C1 - extract of the initial concentration; C2 - extract dissolved in water in a ratio of 1:2

a, b - values of the same extracts at different concentrations marked with different letters, have a statistically significant different effect, T-test ($P < 0.05$)

A, B - values of the different extracts at different concentrations compared with the control sample marked with different letters, have a statistically significant different effect, T-test ($P < 0.05$)

In line with that, all the tested extracts at all the tested concentrations showed higher ($P < 0.05$) activity than the control.

The effects of plant extracts on *Aspergillus* sp. growth

**Figure 1.** Inhibition of *Fusarium* sp. by C1 extract

are presented in Table 3. According to the results, garlic and horsetail extracts showed higher inhibition zones compared with yarrow extract and the control ($P < 0.05$). In all treatments, yarrow extract showed lower efficiency in the suppression of fungal growth. On the other hand, the inhibition zone of C2 horsetail and C1 garlic extract was higher compared to other treatments.

The results of this research revealed that the yarrow, garlic, and horsetail aqueous extracts were efficient ($P < 0.05$) at both concentrations of C2 and C1 in the growth suppression of phytopathogenic fungi. Generally, in most cases, the C1 concentration shows higher ($P < 0.05$) activity compared with the concentration of C2.

Salhi et al. (2017) found that various concentrations of plant extracts were effective against several *Fusarium* species. Wilson et al. (1997) pointed out that plant extract solutions play a significant role in the inhibition zone diameter of *Botrytis cinerea*. Karličić et al. (2021) reported pine bark extract as a biocontrol product against the *Botryosphaeriaceae*. In these studies, an increase in plant extract concentration resulted in higher antifungal activities, which is confirmed in our research.

Yarrow plant extract expressed remarkable antifungal

Table 2. Inhibition of *Alternaria* sp. growth by plant extracts

<i>Alternaria</i> sp.	Diameter of the inhibition zone (mm)						Control
	Yarrow extract		Garlic extract		Horsetail extract		
	Extract concentration (%)						
Replication	C2	C1	C2	C1	C2	C1	-
I	15.0	15.0	12.0	12.0	17.0	11.0	4.0
II	10.0	15.0	13.0	11.0	15.0	13.0	3.0
III	10.0	14.0	13.0	12.0	9.0	10.0	3.0
IV	12.0	14.0	12.0	9.0	10.0	12.0	4.0
Average	11.75 ^{aA} ± 2.36	14.50 ^{bA} ± 0.58	12.50 ^{aA} ± 0.58	11.0 ^{aA} ± 1.41	12.75 ^{aA} ± 3.86	11.5 ^{bA} ± 1.29	3.50 ^B ± 0.58

C1 - extract of the initial concentration; C2 - extract dissolved in water in a ratio of 1:2

^{a, b} - values of the same extracts at different concentrations marked with different letters, have a statistically significant different effect, T-test ($P < 0.05$)

A, B - values of the different extracts at different concentrations compared with the control sample marked with different letters, have a statistically significant different effect, T-test ($P < 0.05$)

Table 3. Inhibition of *Aspergillus* sp. growth by plant extracts

<i>Aspergillus</i> sp.	Diameter of the inhibition zone (mm)						Control
	Yarrow extract		Garlic extract		Horsetail extract		
	Extract concentration (%)						
Replication	C2	C1	C2	C1	C2	C1	-
I	10.0	15.0	13.0	19.0	14.0	20.0	2.0
II	11.0	20.0	10.0	20.0	15.0	17.0	1.0
III	10.0	16.0	14.0	20.0	16.0	18.0	2.0
IV	10.0	16.0	13.0	17.0	13.0	17.0	1.0
Average	10.25 ^{aA} ± 0.50	16.75 ^{bA} ± 2.22	12.50 ^{aA} ± 1.73	19.0 ^{bA} ± 1.41	14.50 ^{aA} ± 1.29	18.0 ^{bA} ± 1.41	1.5 ^B ± 0.58

C1 - extract of the initial concentration; C2 - extract dissolved in water in a ratio of 1:2

a, b - values of the same extracts at different concentrations marked with different letters, have a statistically significant different effect, T-test ($P < 0.05$)

A, B - values of the different extracts at different concentrations compared with the control sample marked with different letters, have a statistically significant different effect, T-test ($P < 0.05$)

activity, especially against *Aspergillus niger* (El-Kalamouni et al., 2017), which is contrary to our results. However, Khudher (2013) found the lowest inhibition percent of *Aspergillus ustus* using yarrow extract compared with sage and senna extracts. In addition, a moderate impact of yarrow extract on the inhibition of *Alternaria* (Itako

et al., 2008) and *Fusarium* (Steglińska et al., 2022) was observed, which was in contrast with our results. Our results are partially confirmed by Azaz et al. (2008) who found strong inhibition of *Alternaria* growth using some *Achillea* extracts.

Strong inhibition of *Aspergillus* growth using garlic

extract was confirmed by Yoshida et al. (1987). Although Santos et al. (2020) found that garlic extract completely controlled the growth of *Fusarium*, Sousa and Gervasia (2018) suggest a reduction of *Fusarium* occurrence to more than 80%, which follows our findings. Steglińska et al. (2022) reported the moderate effect of horsetail extract on *Alternaria* growth. Mir et al. (2021) found similar values of the inhibition zone against *Aspergillus* and *Fusarium* using an extract based on *Equisetum*.

CONCLUSION

The results confirmed that the application of the three plant extracts (garlic, yarrow, and horsetail) has inhibition effects on in-vitro fungal growth. The inhibition rate was more pronounced using 100% extracts compared with 50% extracts. The highest inhibition of *Fusarium* and *Alternaria* growth was observed using the yarrow plant extract, while horsetail and garlic extracts were more efficient in the case of *Aspergillus* sp. These findings suggest that yarrow, garlic, and horsetail extracts may have a potential application in the inhibition of fungal growth. Further research will be done regarding the chemical composition of these extracts and the potential use of these extracts as a compound of biopesticides.

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