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IN VITRO STUDY ON THE USE OF QUINOA (*Chenopodium quinoa* WILLD.) EXTRACTS FROM TO LIMIT THE DEVELOPMENT OF PHYTOPATHOGENIC FUNGI

Summary

The aim of the study was to search for alternatives to chemical fungicides currently used in plants protection against Botrytis cinerea, Rhizoctonia solani, Phoma exiqua var. exiqua, Sclerotinia sclerotiorum, Fusarium poae. Various concentrations of aqueous extracts of Chenopodium quinoa Willd leaves, stems and inflorescences were tested in the laboratory conditions. Antifungal activity of the extracts was evaluated based on surface growth and sporulation intensity of the test fungi. The applied concentrations of aqueous extracts (25.0, 10.0, 1.0 and 0.1 $mm^3 \cdot cm^{-3}$) significantly modified the examined parameters of particular fungi. The strongest fungistatic activity was noted for 25.0 $mm^3 \cdot cm^{-3}$ concentration of C. quinoa stems and inflorescences extracts with respect to B. cinerea. They limited this fungus linear growth by 42.9% and 53.3%, and sporulation by 53.6% and 67.85%, respectively. In turn, a very intense inhibition (53.5–88.3%) in S. sclerotiorum colony growth on the media with higher concentrations of all analyzed types of extracts was accompanied by stimulation of sporulation. The lowest concentration of the leaves extract in 43.1% inhibited the growth of mycelium and in 52.1% sporulation of P. exiqua var. exiqua. Unfortunately, most of the analyzed concentrations to a very little degree inhibited the surface growth of R. solani and F. poae hyphae.
Keywords: *Chenopodium quinoa, antifungal activity, fungi pathogenic to horseradish roots*

BADANIA *IN VITRO* NAD WYKORZYSTANIEM WYCIĄGÓW Z KOMOSY RYŻOWEJ (*Chenopodium quinoa* WILLD.) DO OGRANICZANIA ROZWOJU GRZYBÓW FITOPATOGENNYCH

Streszczenie

Celem pracy było znalezienie alternatywnych dla chemicznych środków grzybobójczych stosowanych obecnie w ochronie roślin przed Botrytis cinerea, Rhizoctonia solani, Phoma exiqua var. exiqua, Sclerotinia sclerotiorum, Fusarium poae. W warunkach laboratoryjnych testowano różne stężenia wodnych wyciągów z liści, łodyg i kwiatostanów Chenopodium quinoa Willd. Aktywność przeciwgrzybową wyciągów oceniano na podstawie rozrostu powierzchniowego i intensywności zarodnikowania grzybów testowych. Zastosowane stężenia wodnych wyciągów (25,0; 10,0; 1,0 i 0,1 $mm^3 \cdot cm^{-3}$) istotnie modyfikowały badane parametry poszczególnych grzybów. Najsilniejszym działaniem fungistatycznym odznaczały się 25,0 $mm^3 \cdot cm^{-3}$ stężenia wyciąwów z łodyg i kwiatostanów C. quinoa w odniesieniu do B. cinerea. Ograniczały one odpowiednio o 42,9 i 53,3% rozrost liniowy oraz sporulację w 53,6 i 67,85% tego grzyba. Z kolei na podłożach z wyższymi stężeniami wszystkich analizowanych rodzajów wyciagów obserwowano intensywne hamowanie (53,5-88,3%) rozrostu kolonii *S.sclerotiorum*, któremu towarzyszyła stymulacja zarodnikowania. *Najniższa koncentracja wyciągu z liści w 43,1% hamowała rozrost mycelium oraz w 52,1% sporulację P. exiqua var. exiqua. Niestety, większość analizowanych stężeń w bardzo małym stopniu hamowała powierzchniowy rozrost strzępek R. solani oraz F. poae.*
Słowa kluczowe: *Chenopodium quinoa, aktywność grzybobójcza, grzyby patogeniczne korzeni chrzanu*

1. Introduction

Infectious diseases of plants to a high degree reduce the amount and quality of crops. Globally, it is estimated that more than 80% of economically important diseases is caused by pathogenic fungi [32]. Most of them, closely related to the soil environment, constitute a threat to the plants starting from seed material placing in the soil. Among them, the special attention should be paid to polyphagous species such as *Sclerotinia sclerotiorum, Rhizoctonia solani, Phoma exiqua, Botrytis cinerea* and *Fusarium* spp. Although the pathogenicity of these fungi is clearly visible in the growing season, intense destruction of plant tissues occurs during the storage. These fungi can be transferred with the seed material. Internationally important pathogen *S. sclerotiorum* causes white mold and *Sclerotinia* stem rot in about 500 plant species [27]. It is known from the numerous reports that, especially in temperate climate, this fungus causes severe losses in the cultivation of economically important plant species, including: root vegetables, beans, tomato, pepper, potato, sunflower, soybeans, Brassicaceae, Malvaceae and numerous ornamental plants [5, 21, 26, 33, 37]. Similarly, a broad host range is also noted for worldwide distributed fungus *Rhizoctonia solani*. It attacks important cultivated plant species belonging to the families Solanaceae, Poaceae, Brassicaceae, Asteraceae and many others [9, 11, 18, 22]. *R. solani* causes a variety of disease symptoms, usually their type is related to the parts of the plant (seedling blight, root and storage organs rot, leaf spot, carcinoses). Polyphagous fungus *Phoma exiqua* var. *exiqua* reduces the ability of seeds germination, contributes to seedling blight, root rot and infects stems and leaves [1, 19, 39]. In Poland, this species is the most commonly isolated from soybeans, beans, potatoes, horseradish and herbs [15, 19, 39]. In turn, neurotrophic pathogenic *Botritis cinerea* is responsible for gray mold, and that dis-

ease belongs to the most important in Europe. This fungus infects more than 230 plant species, is the cause of post-harvest rot of mainly perishable vegetable raw materials, serious losses (over 50%) are noted for strawberries and grapevines [7, 23, 29]. Quick and efficient limitation of plant fungal diseases and protection of crops against storage rots is achieved by the use of synthetic fungicides. Reasonable, compatible with the label, use of chemical agents results in the desired effect in the form of improved yield, quality features, and even an increase in the nutritive value of plant material [8]. Unfortunately, wrong, too intensive use of agrochemicals is often observed in practice. It should be remembered that pesticides are also toxic to non-target organisms, often eliminating useful ones from the environment, and also cause pests resistance. Pesticide residues are found in soil, air, drinking water, and above all in fresh fruits and vegetables [3, 8, 10, 24]. Osman and Al-Rehiayam [24] reported that among the pesticides, fungicides are the most carcinogenic. The negative effect on the environment is partially minimized by successive elimination from the European market of pesticide active substances characterized by acute toxicity, long half-life and dangerous accumulation in the food chain [12]. An alternative to synthetic fungicides may be constituted by natural compounds present in plant extracts, which do not exhibit toxicity to the environment and humans. Numerous global research confirms high fungicidal activity of extracts from various species of herbal plants [10]. Fungicidal properties were demonstrated for flavonoids, phenolic compounds, unsaturated lactones, sulfur compounds, cyanogenic glycosides, glucosinolates and saponins contained in different parts of the plant [6, 10]. Quinoa (*Chenopodium quinoa* Willd.) is a particularly rich source of saponins and other bioactive compounds [2, 20, 25, 28, 31]. The seeds of this plant actively inhibit the growth of *Escherichia coli*, *Staphylococcus aureus* bacteria and *Botrytis cinerea* fungus [6, 20,25]. Assuming that particular parts of *C. quinoa* are differentiated in terms of bioactive compounds content, the differentiated effect on fungi should be expected. The aim of this study was to evaluate antifungal effects of aqueous extracts of quinoa leaves, stems and inflorescences on economically important pathogens: *S. sclerotiorum*, *R. solani*, *P. exiqua* var. *exiqua*, *B. cinerea* and *Fusarium poae*.

2. Material and methods

2.1. Examined fungi: the following fungi were used in the experiment: *Sclerotinia sclerotiorum* (Lib.) de Bary., *Rhizoctonia solani* Kühn., *Phoma exigua* var. *exiqua* Desm., *Botrytis cinerea* Pers. and *Fusarium poae* (Peck) Wollenw., and they were isolated from the roots of horseradish grown in private farm located in the province of Lodz.

To the experiment used a two-week culture of fungi grown in temp. 23°C on standard PDA medium with the participation of chloramphenicol.

2.2. Extracts preparation: *Chenopodium quinoa* was derived from experimental field belonging to the Institute of Plant Production and located in Prusy near Krakow. Aerial parts of the plant were collected in the flowering stage. Leaves, stems and inflorescences were separated in the laboratory, they were purified with tap water, surface sanitized by immersion for 30 seconds in 1% sodium hypochlorite solution, rinsed in sterile distilled water. The plant material was dried at 45°C in an electric dryer and then milled. The portions of 30 g of powdered plant material were transferred to a sterile 300 mL flasks and poured with 150 ml of sterile distilled water. The flask contents were tightly sealed with aluminum foil and placed for 24 hours in shaking incubator at 40°C. Then, the content of the flask was filtered through four layers of sterile gauze. The obtained filtrates were added in an appropriate amount to PDA medium.

2.3. Antifungal activity: a suitable quantity of particular aqueous extracts of quinoa leaves, stems and inflorescences in sterile conditions was added to a standard glucose – potato medium (PDA) at a temperature of about 45°C. Prepared media of the following *C. quinoa* extracts concentrations: 0.1; 1.0; 10,0 and 25.0 $mm^3 \cdot cm^{-3}$, were poured into Petri dishes of 90 mm diameter. Inoculum of the examined fungi was placed on the central part of solidified medium in the form of 5 mm agar disk overgrown with 10-day mycelium. The medium in Petri dishes without plant extracts was the control. The experiment was conducted in five replicates. The diameter of the colonies respective species of fungi measured until overgrowing surface of the Petri dish in any combination. Daily measurements of fungal colonies diameter were used to calculate the coefficient of growth [T] of examined fungi according to the formula of Kowalik and Krechniak [17]:

$$T = \frac{A}{D} + \frac{b_1}{d_1} + \cdots + \frac{b_x}{d_x}$$

T – linear growth coefficient; A – an average of colony diameter measurements [mm],
D – number of days since experiment beginning to the end;
b_1, b_x – increase in colony diameter since the last measurement [mm]; d_1, d_x – number of days since the last measurement.
An effect of *C. quinoa* extracts on the linear growth of fungi was evaluated on the basis of the difference between the diameter of fungus colony on Petri dishes with given types and concentrations of the extracts, and the diameter of colonies on the control plates. The results were presented as percentage factor of growth inhibition/stimulation according to the formula presented in the work of Gleń and Boligłowa [13]:

$$H = \frac{K-A}{K} \cdot 100\%$$

H – coefficient of fungus linear growth inhibition; K – colony diameter on the control Petri dish;
A – colony diameter on the plate with known concentration of plant extract.

After three weeks, the number of spores of the examined fungi was determined in each plate using a Thoma hemocytometer placed under a light microscope. For this purpose, each of the of the Petri dish cork borer having a diameter of 1 cm was excised of five pucks overgrown by mycelium. Then they were transferred to a test tube and poured over 10 ml of sterile distilled water. The tubes were shaken and then filtered through the triple a gauze, a drop of the spore suspension was placed in a Thoma hemocytometer. The coefficient of sporulation inhibition/stimulation was calculated for the obtained number of spores in the same way as in case of linear growth evaluation.

The results were subjected to two-factor analysis of variance, where the first factor was the kind of aqueous extract (leaves, stems, inflorescences), the second was its concentration. The significance was evaluated using Duncan's test with a significance level of $\alpha = 0.05$.

3. Results and discussion

In vitro study demonstrated that aqueous extracts of *Chenopodium quinoa* significantly modified the surface growth and sporulation process of the examined fungi (Table 1 and 2).

Table 1. Linear growth coefficient of the tested fungi depending on the examined factors [T]
Tab. 1. Współczynnik tempa wzrostu liniowego grzybów testowych w zależności od badanych czynników[T]

Fungi	Concentration [$mm^3 \cdot cm^{-3}$]	Aqueous extracts of:			Mean	$LSD_{0,05}$
		Inflorescences	Leaves	Stalks		
Botrytis cinerea	25	33,98	40,40	36,38	36,92	5,19
	10	40,10	36,00	36,69	37,59	
	1,0	42,68	34,40	40,85	39,31	
	0,1	49,20	50,08	49,64	49,65	
	Control	52,50			52,50	
	Mean	43,69	42,67	43,22		
	$LSD_{0,05}$	r.n. - n.s.				
	$LSD_{0,05}$ for interaction	r.n. – n.s.				
Fusarium poae	25	38,65	45,10	42,61	42,12	1,45
	10	43,50	47,60	44,65	45,25	
	1,0	43,90	44,50	48,67	45,69	
	0,1	47,92	44,20	52,45	48,19	
	Control	46,02			46,02	
	Mean	43,99	45,48	46,88		
	$LSD_{0,05}$	1,13				
	$LSD_{0,05}$ for interaction	2,57				
Phoma exiqua var. exiqua	25	34,50	32,47	30,52	32,49	2,22
	10	29,65	30,82	30,15	30,20	
	1,0	28,50	28,91	29,30	28,90	
	0,1	27,82	11,67	28,00	22,49	
	Control	29,80			29,80	
	Mean	26,73	26,73	29,55		
	$LSD_{0,05}$	1,94				
	$LSD_{0,05}$ for interaction	3,79				
Rhizoctonia solani	25	48,30	48,50	48,38	48,39	1,91
	10	46,40	47,04	46,40	46,61	
	1,0	47,10	45,41	46,32	46,27	
	0,1	46,30	36,50	42,31	41,70	
	Control	46,60			46.60	
	Mean	46,94	44,81	46,00		
	$LSD_{0,05}$	n.s.				
	$LSD_{0,05}$ for interaction	n.s.				
Sclerotinia clerotiorum	25	25,17	18,17	13,67	19,00	6,15
	10	28,83	20,83	18,83	22,83	
	1,0	29,17	21,33	21,67	24,05	
	0,1	35,33	37,33	41,50	38,05	
	Control	45,20			45,20	
	Mean	32,74	28,57	28,17		
	$LSD_{0,05}$	1,80				
	$LSD_{0,05}$ for interaction	7,90				

Source: own work / Źródło: opracowanie własne

The results indicate the differentiated reactions of the examined fungi species on both the type of an extract as well as its concentration. In general, the inhibitory effect of extracts was more frequently recorded for the linear growth of examined fungi colonies than sporulation process. Among the analyzed fungi, the strongest surface growth inhibition was noted for *S. sclerotiorum*. Significantly stronger reduction in the growth rate of this fungus was found on media containing higher concentrations of the extract (25, 10, 1 $mm^3 \cdot cm^{-3}$). Moreover, stems and leaves extracts, as compared to the inflorescences, more strongly inhibited the growth of *S. clerotiorum* hyphae, which is reflected in inhibition coefficients presented in Figure 1. Even up to 88.3% lower diameter compared to the control was found on the media containing 25 mm^3 extract from *C. quinoa* stems, however, the amount of ascospores was 28.5% higher. In case of fungi pathogenic for the plants, an increased sporulation is observed in adverse thallus growth conditions [14]. According to Hodges [16], this is a defensive reaction of the fungi ensuring the continuity of the species. Stronger fungistatic properties of quinoa stems extracts can be explained by higher content of antimicrobial compounds. The rationale for such conclusion are the study conducted by Singh et al. [30], who found that the older plant organs e.g. outer bark protecting the stems with dying cells, contains higher amounts of tannins and polyphenols soluble in wa-

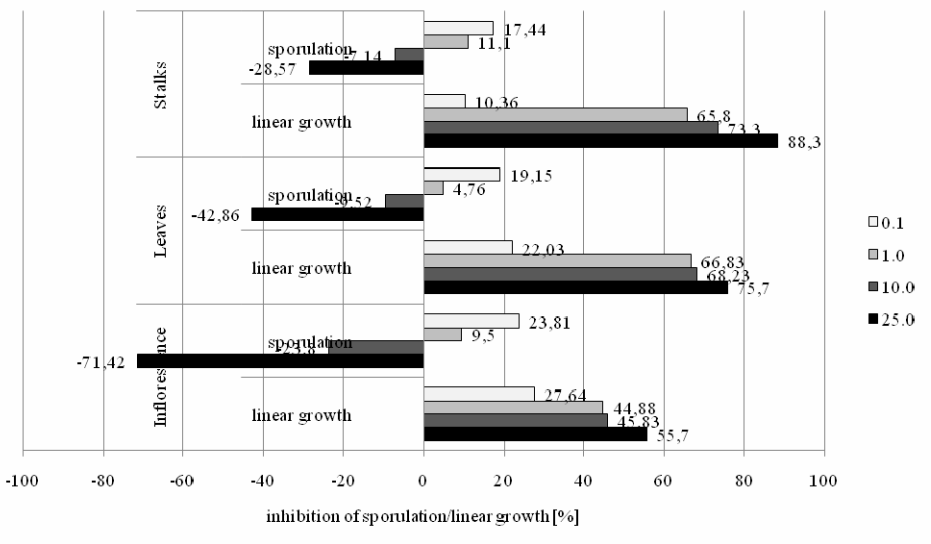
ter. These compounds have proven antimicrobial (*Streptococcus pneumonia, Enterobacter aerogenes, Klebsiella pneumonia*) and antifungal activity (*Candida albicans*). Their presence is also noted in *C. quinoa,* however this species contains saponins in all its parts [6, 34, 38]. Triterpenoid saponins, which constitute up 30% in the outer husks of quinoa seeds, limit the growth of *Candida albicans* and *Botrytis cinerea* fungi [6, 35, 36]. Quinoa seeds were not examined in this study, however, the extracts prepared from leaves, stems and inflorescences equally limited linear growth of *B. cinerea* (Table 1, Fig. 2). The factor which significantly affected this feature was the concentration. The highest inhibition in mycelium growth, on a level of 53.5% and 53.4%, was noted for the media containing 1.0 $mm^3 \cdot cm^{-3}$ of leaves extract, and 25.0 $mm^3 \cdot cm^{-3}$ of inflorescences extracts, respectively (Fig. 2). At the same time, the rate of *B. cinerea* growth in the presence of extracts from inflorescences and stems significantly decreased with an increasing concentration, and the reverse reaction was observed for the leaves. Rapid spread and infectivity of the fungus causing gray mold are conditioned by conidia [29]. The process of their production was significantly limited by higher concentrations (25.0 and 10.0 $mm^3 \cdot cm^{-3}$) of the extracts from inflorescences and stems, and lower (1.0 and 0.1 $mm^3 \cdot cm^{-3}$) from the leaves of *C. quinoa* (Table 2, Fig. 2). The strongest inhibition at the level of 67.85% was recorded on the medium with the highest concentration of inflorescences extract.

In relation to other species: *Phoma exiqua* var. *exiqua*, *Fusarium poae, Rhizoctonia solani,* the examined extracts exhibited significantly weaker activity. In case of *Phoma exiqua* var. *exiqua,* 52.1% colony growth inhibition, accompanied by 47.8% reduction in sporulation (Fig. 3) was found only for the lowest concentration of the leaves extract. Unlike in previously analyzed fungi, the quinoa extracts in lower concentrations demonstrated greater effect on the reduction of sporulation process and surface growth of mycelium. In turn, the highest concentration, especially inflorescences extract, generally the most stimulated the examined features of *P. exiqua* var. *exiqua*. In the light of the study conducted, *C. quinoa* leaves extract essentially showed no fungistatic activity with regard to *F. poae* (Table 1 and 2, Fig. 4). Generally, the highest concentration of inflorescences and stems extract inhibited the linear growth of this species mycelium by 18.22% and 8.47%, respectively, with concurrent stimulation (63% and 16.27%) of macro-conidia production (Fig. 4). In this study, non-sporulating *R. solani* fungus appeared to be the least responsive species to the aqueous extracts of quinoa. The kind of applied extract had no significant effect on the growth of *R. solani* colonies (Table 1). Very poor fungistatic activity was demonstrated for 0.1 $mm^3 \cdot cm^{-3}$ aqueous extract from quinoa leaves. An inhibition in the surface growth of *R. solani* colony in this case was only 20.08% (Fig. 5).

Table 2. Test fungi sporulation depending on the examined factors
Tab. 2. Zarodnikowanie grzybów testowych w zależności od badanych czynników

Fungi	Concentration [$mm^3 \cdot cm^{-3}$]	Aqueous extracts of:			Mean	$LSD_{0,05}$
		Inflorescences	Leaves	Stalks		
Botrytis cinerea	25	0,90	3,10	1,30	1,8	0,28
	10	1,20	4,10	1,80	2,4	
	1,0	3,20	4,20	2,90	3,4	
	0,1	3,40	2,00	3,00	2,8	
	Control	2,80			2,8	
	Mean	2,30	3,24	2,36		
	$LSD_{0,05}$	0,11				
	$LSD_{0,05}$ for interaction	0,37				
Fusarium poae	25	13,7	13,25	2,30	9,75	2,30
	10	2,8	12,60	10,50	8,63	
	1,0	2,3	9,30	5,35	5,65	
	0,1	12,35	8,50	2,80	7,88	
	Control	8,40				
	Mean	7,9	10,41	5,87		
	$LSD_{0,05}$	1,35				
	$LSD_{0,05}$ for interaction					
Phoma exiqua var. exiqua	25	21,05	19,50	17,80	19,45	1,02
	10	19,60	11,00	15,90	15,50	
	1,0	10,90	8,00	12,30	10,40	
	0,1	8,5	7,80	11,50	9,26	
	Control	14,95			14,95	
	Mean	15,00	12,25	14,49		
	$LSD_{0,05}$	1,50				
	$LSD_{0,05}$ for interaction					
Sclerotinia clerotiorum	25	3,60	3,00	1,50	2,70	0,51
	10	2,60	2,30	1,85	2,25	
	1,0	1,90	2,00	1,70	1,80	
	0,1	1,60	1,70	1,90	1,73	
	Control	2,10			2,10	
	Mean	2,36	2,22	1,81		
	$LSD_{0,05}$	0,40				
	$LSD_{0,05}$ for interaction	n.s.				

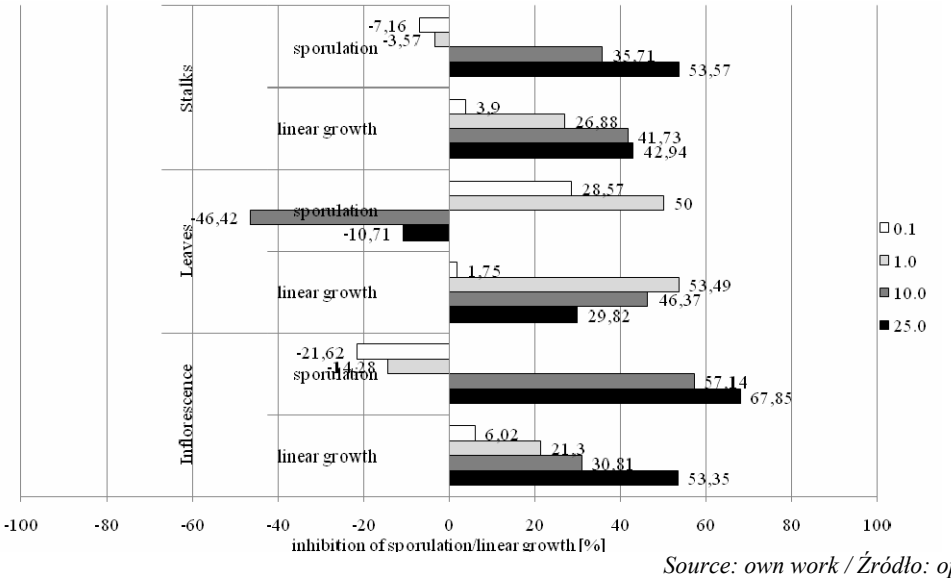
Source: own work / Źródło: opracowanie własne



Source: own work / Źródło: opracowanie własne

Fig. 1. Coefficients of *Sclerotinia sclerotiorum* linear growth and the production of ascospores inhibition [H]

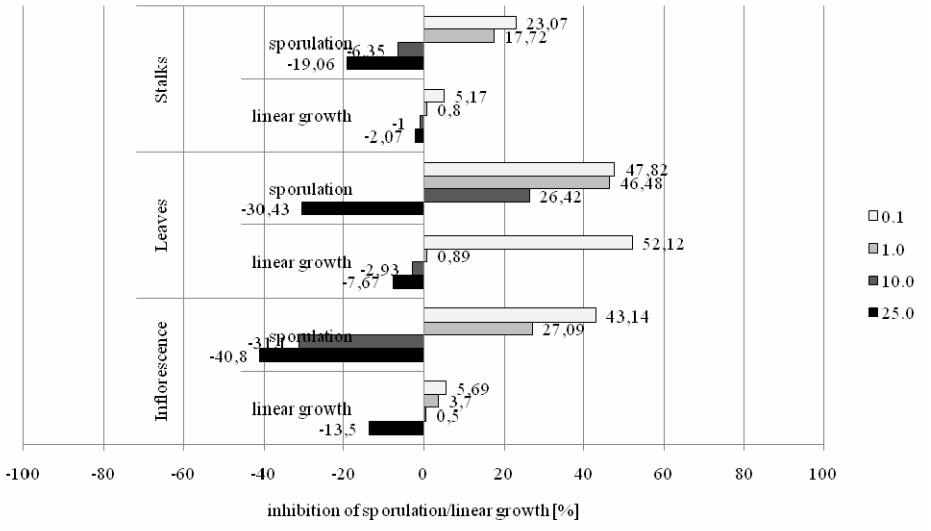
Rys. 1. Współczynniki zahamowania wzrostu liniowego i wytwarzania zarodników workowych Sclerotinia sclerotiorum [H]



Source: own work / Źródło: opracowanie własne

Fig. 2. Coefficients of *Botrytis cinerea* linear growth and sporulation inhibition [H]

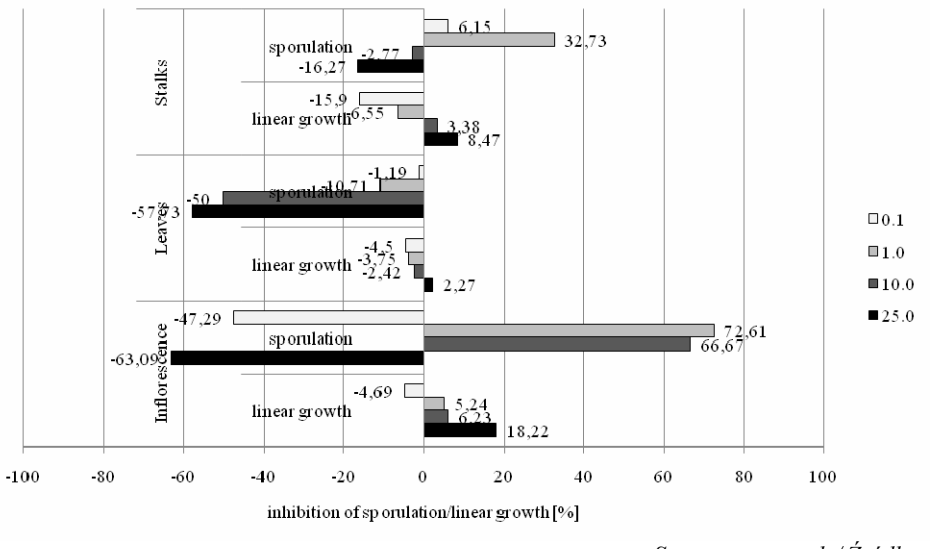
Rys. 2. Współczynniki zahamowania wzrostu liniowego i zarodnikowania Botrytis cinerea [H]



Source: own work / Źródło: opracowanie własne

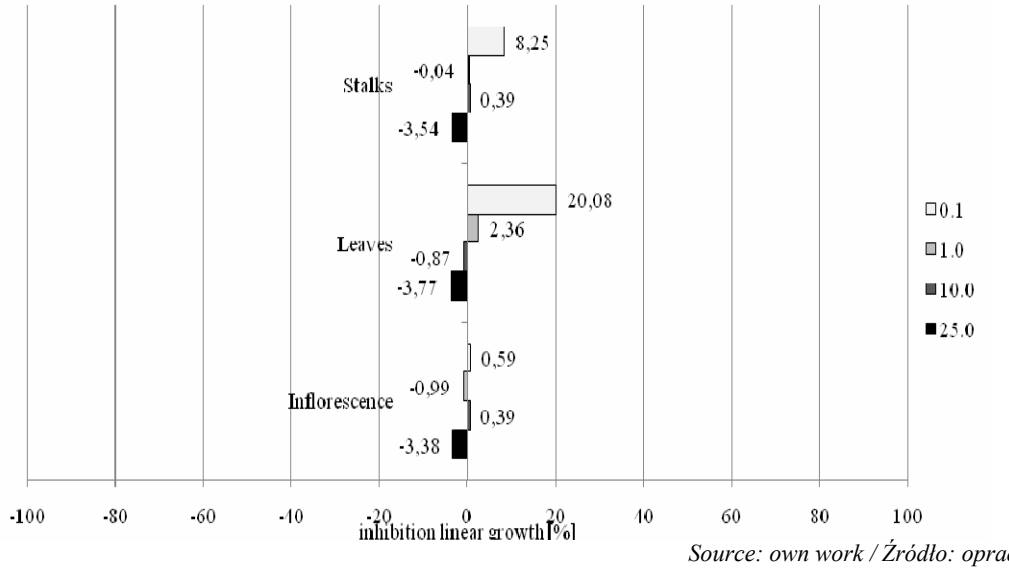
Fig. 3. Coefficients of *Phoma exiqua var. exiqua* linear growth and sporulation inhibition [H]

Rys. 3. Współczynniki zahamowania wzrostu liniowego i zarodnikowania Phoma exiqua var. exiqua [H]



Source: own work / Źródło: opracowanie własne

Fig. 4. Coefficients of *Fusarium poae* linear growth and sporulation inhibition [H]
Rys. 4. Współczynniki zahamowania wzrostu liniowego i zarodnikowania Fusarium poae [H]



Source: own work / Źródło: opracowanie własne

Fig. 5. Coefficients of *Rhizoctonia solani* linear growth inhibition [H]
Rys. 5. Współczynniki zahamowania wzrostu liniowego Rhizoctonia solani [H]

Bokhari et al. [4] reported that the activity of plant extracts depends not only on the part of the plant which was used for its preparation and the concentration, but mainly on the type of solvent used. This study examined aqueous extracts, which activity is certainly weaker compared to the extracts of organic solvents, like e.g. n-hexane, chloroform, methanol.

4. Conclusions

The obtained results indicate the differentiated reaction of particular test fungi on aqueous *Chenopodium quinoa* extracts. Fungicidal activity evaluated based on the surface growth of fungal colonies and sporulation process depends on both part of the plant which was used for extract preparation, and its concentration. Aqueous extracts of stems and inflorescences in the highest concentration (25), the most limited (42.9–53.35%) the linear growth and sporulation (53.57–67.85%) of *B. cinerea*. Very intense inhibition of *Sclerotinia sclerotiorum* colonies growth on the media containing higher concentration of all types of extracts, unfortunately, is accompanied by stimulation of the production of ascospores. The lowest concentration of *C. quinoa* leaves extract demonstrates fungicidal activity on *P. exiqua,* inhibiting colony growth by 43.4%, and sporulation by 52.1%. *R. solani* and *F. poae* demonstrated the highest resistance to applied aqueous extracts, which is reflected in very low coefficients of colonies growth inhibition and stimulation.

5. References

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This Research was financed by the Ministry of Science and Higher Education of the Republic of Poland.