



The Main Pathogen of Radish (*Raphanus Sativus* L.) and Their Biocontrol in Adjara, Georgia

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Abstract

The antifungal activity of 18 different plant species known for their medicinal value was investigated. Medicinal plants and pathogenic fungi were collected in different geographical zones of Adjara, Georgia. Plant material were cut into small pieces and dried at room temperature for one week. Fungal species identification was based on the morphological characteristics of single spored isolates. 17 species of fungi have been identified. 7 species of fungi involved in forming of consortium on root vegetable of Radish plants have been specified as well. Long-term monitoring has shown us that the mycobionts found on Radish, the most dangerous and harmful 4 pathogen of which the dominant species is *Hyaloperonospora brassicae* F. sp. raphani. Among the 21 extracts evaluated, 5 extract showed mycelia inhibition 80.75-98.33%, 3 showed moderate effect of 75.56 - 79.32% and 6 showed least 31.4 % - 57.27%. The remaining 7 extracts from leaf of *Sambucus ebulus*, *Ricinus communis*, *Betonica officinalis*, *Jasminum officinal*, *Pimpinella anisum*, *Pyrethrum vulgare* and *Althae officinalis* showed no antifungal activity against the *H. brassicae* F. sp. Raphani. It was established that *Rubia coradifolia*, *Inula racemosa*, *Stevia rebaudiana* and *Panax ginseng* extracts can be emerged as safe alternatives to replace chemical fungicides against pathogens.

Keywords: *Hyaloperonospora brassicae* F. sp. raphani, Leaf extract, Antifungal activity, Inhibition effect, Biological control

INTRODUCTION

Radish (*Raphanus sativus* L.) is a vegetable cultivated mostly in the temperate areas for the root, which is succulent. It is very popular in Adjara (Georgia), mostly in the cold areas, due to its storage capacity. More recently, radish and other cruciferous vegetables (members of the Brassicaceae) have been recognized as important sources of chemoprotective phytochemicals in the diet. Radish is a productive vegetable based on biomass per area of cultivation. However, this crop is affected by many diseases, particularly those caused by fungi. Among fungi Radish downy mildew is an is an ecological and economically important disease in main production areas Georgia wide. We can find quite a lot of material about radish downy mildew is found in the works of various authors [1-7]. The orography of the Adjara and the edaphic-climatic conditions (Conditions conducive to the development and spread of pathogens) supports the widespread and development of Radish downy mildew causing agents.

Often, widespread diseases in the study area almost completely destroy the radish crop [8-10]. It is increasingly becoming a limiting factor for the successful cultivation of Radish in these regions. Several fungicides recommended for controlling these pathogens not only add up to human and environmental hazards but also be inconsistent and insufficient to deal with this disease. However, the application of this agrochemical is increasingly restricted due to the harmful effects of pesticides on biotic and abiotic

factors [11]. In many countries, the use of fungicides has been banned due to their polluting nature to have safe methods for plant disease control in sustainable agriculture there is a need for reducing the use of synthetics chemicals fungicides thereby replacing them with biocides with plant origin. The medical plants are rich sources of numerous bioactive secondary metabolites such as alkaloid, flavonoids, terpenoids, saponins, tannins and phenolic compounds which are the important sources of microbicides, antifungal activity and many pharmaceutical compound [12,13]. Many reports on the antiviral, antibacterial, antifungal, anthelmintic, anti-molluscal and anti-inflammatory properties of plants have reviewed. Thus, plant extracts which is a source of natural pesticides can be developed into new biocidal pesticides [14-176]. The goal of our research was intended to determine the composition of fungi on Radish in all agroecosystems in Georgia; study the pathogens and harmfulness of main fungal Radish pathogens and application of the biological method (various extracts of

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high medicinal plants) against the most common fungi *Hyaloperonospora brassicae* F. sp. Raphani in Adjara.

MATERIALS AND METHODS

Plant material

19 different plant species *Althaea officinalis*, *Betonica officinalis*, *Eucalyptus cinerea*, *Galium aparine*, *Inula racemosa*, *Hamamelis mollis*, *Jasminum officinale*, *Mentha sylvestris*, *Mentha longifolia*, *Panax ginseng*, *Panax ginseng*, *Pimpinella anisum*, *Pyrethrum vulgare*, *Ricinus communis*, *Rubia cordifolia*, *Rubia iberica*, *Salvia officinalis*, *Sambucus ebulus* and *Stevia rebaudiana* known for their medicinal value in traditional medicine, were cut into small pieces and dried at room temperature for one week.

Sample collection

This study was conducted between 2017 June and September 2020 in Adjara, Georgia. The objects of research were various varieties of Radish (*Raphus sativus*) and pathogenic fungi inhabited on Radish.

Isolation of fungi

For the isolation of fungi from *Raphus sativus* plants, the agar method was applied. Fungal pathogens were responsible for disease isolated from the leaves and fruit. From the samples obtained, serial dilutions (1:10) were prepared in test tubes with 9 ml sterile water, adding 1 g of fruit samples until dilutions 10⁻⁵, 10⁻⁶ and 10⁻⁷, of which 50 µL and shaken at 200 rpm for 3 h. Samples collected were seeded by diffusion technique on Petri dishes of 88 mm diameter containing one of the following artificial Potato Dextrose Agar (PDA) and Czapeck's agar medium. After incubation (23°C for 6 days) the number for each fungus was calculated. All fungi were cultured using a single spore technique on PDA and Czapeck's medium. After incubation, fungi were identified by their macroscopic and microscopic characteristics.

Identification of fungi

Fungal species identification was based on the morphological characteristics of single spored isolates [18]. Collections of the fungi have been examined by standard light microscopy (Pereval, Carl Zeiss, Jena and Olympus, BX50, Hamburg, Germany). The SEM micrographs have been prepared using a JSM-35 (Japan) SEM microscope.

Disease Assessment

Disease severity it was rated using a 0 to 9 disease scoring scale; where, 1 = no infections; 2 = 1-10% leaf area infected; 3 = 11-20% leaf area infected; 4 = 21-30% leaf area infected; 5 = 31-40% leaf area infected; 6 = 41-50% leaf

area infected; 7 = 51-60% leaf is infected; 8 = 61-70% leaf is infected; and 9 = 71-100 leaf are infected [19].

Disease severity scores were converted into percentage severity as follows:

$$\text{Disease Severity (\%)} = \frac{\text{Area of Diseased Tissue}}{\text{Total Tissue Area}} \times 100$$

The severity grades were converted into percentage severity index (PSI) for analyses as indicated by Wheeler [20]:

$$\text{PSI} = \frac{\text{Sum of numerical ratings}}{\text{No. of plants scored} \times \text{maximum disease score on scale}} \times 100$$

The relative disease severity reduction on untreated and treated plots of the treatment combination was calculated as follows.

$$\text{Relative disease severity reduction (\%)} = \frac{\text{untreated} - \text{treated}}{\text{untreated}} \times 100$$

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) and comparisons were carried out for each pair with the HSD Turkey test using SPSS statistical software (SPSS Inc., Chicago, IL, USA). All treatments were carried out in triplicate, and the values are given as means ± standard errors. Differences were considered to be significant when the P-value was less than or equal to 0.05.

RESULTS

Isolation and identification of the microorganisms

The microorganisms were isolated from Radish tissues showing spot symptom on leaf and root vegetable. On the Radish, 17 species of microorganisms (*Hyaloperonospora brassicae* F. sp. raphani, *Albugo candida*, *Pythium de baryanum*, *Aspergillus niger*, *Phytophthora* sp., *Erysiphe* sp., *Penicillium* sp., *Alternaria blight*, *Alternaria* sp., *Aphanomyces raphani*, *Cercospora cruciferarum*, *C. atrogrisea*, *Thielaviopsis basicola*, *Fusarium moniliforme* f. sp., *Sclerotinia sclerotiorum*, *Phoma* sp., *Rhizoctonia solani*) were identified as a result of phytopathological and mycological studies conducted in different places of agroecosystems in Georgia.

Formation of the consortium fungus on leaf and root vegetable

The infected leaf and root vegetable radish can be covered with a multi-colored mycelium which includes 7 species of fungi (*Hyaloperonospora brassicae* F. sp. raphani, *Aphanomyces raphani*, *Alternaria blight*, *Alternaria* sp., *Aspergillus niger*, *Sclerotium rolfsii*, *Rhizoctonia solani*) in **Figure 1**.



Figure 1. Symptoms of consortium on radish root vegetable.

Observations have shown that the formation of the consortium begins when air and soil temperatures are 25 to 31°C, and an optimum temperature is about 26-27°C. High humidity (89-96%) hastens the formation of the consortium.

Results of the studies showed that among the mycobionts found on radish, the most common, dangerous and harmful

are 4 pathogens: *Hyaloperonospora brassicae* F. sp. *Raphani* (51%), *Rhizoctonia solani* (28%), *Aphanomyces raphani* (23%) and *Sclerotinia sclerotiorum* (12%) in **Figure 2**.

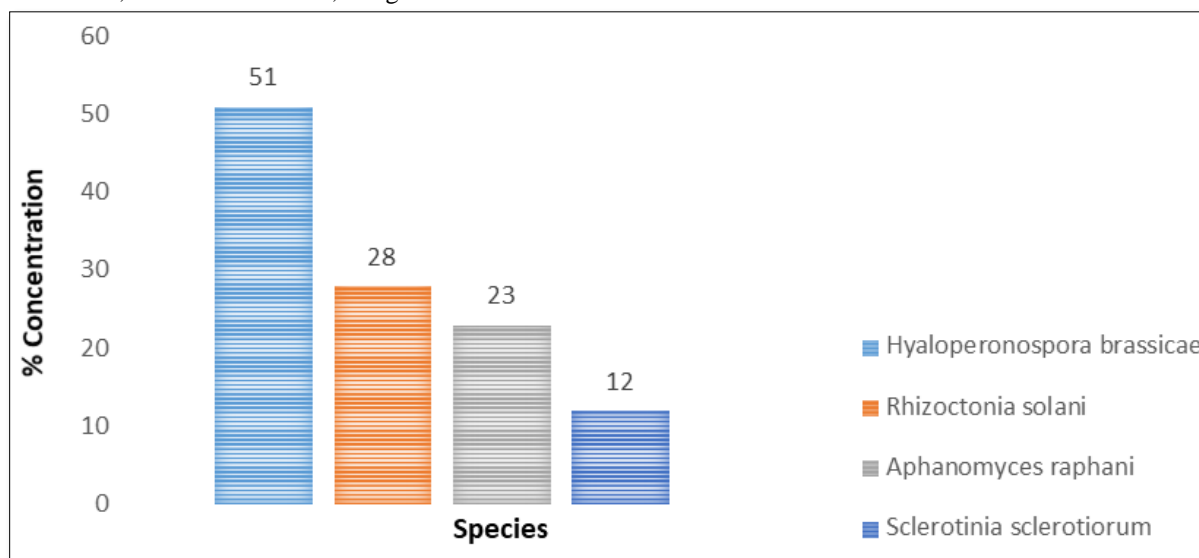


Figure 2. Spreads of the main pathogens of Radish in Georgia, %.

Analysis main pathogen of radish and symptoms diseases

As **Figure 2** shows fungi, the main species is *Hyaloperonospora brassicae* F. sp. *raphani* Syn.: *Peronospora brassicae*, *Peronospora parasitica*. It causes downy mildew. In the past, the cause of downy mildew in any plant in the family Brassicaceae was considered to be a single species *Peronospora parasitica*. However, this has recently been shown to be a complex of species with narrower host ranges, now classified in the genus *Hyaloperonospora*, from the perspective of plant pathology,

Hyaloperonospora brassicae F. sp. *raphani* is now the name of the most important pathogen in this complex. The pathogen is more common in seedbeds. In Adjara (favorable for the disease), yellow to pale brown spots develop rapidly into large irregular patches, on the upper surface of the young leaves. A greyish growth occurs on the underside of the leaves and may be present, too, on the upper surface during cool and moist conditions. The diseased areas turn brown and papery in dry weather. Severely affected seedlings are stunted and killed. The older leaves may have a speckled appearance in **Figure 3**.



Figure 3. Symptoms *H. brassicae F. sp. raphani* on leaves.

As **Figure 4** shows symptom on radish root vegetable is a white to gray dusty material. The fungus quickly spreads to completely cover the root vegetable surface. This disease is favored by high humidity, nearly similar symptoms appear

in the field and lead to the early death of the leaves; in moist weather the spots grow larger, join together and form large dead patches.



Figure 4. Symptoms of *H. brassicae F. sp.* on radish root vegetable.

Level of disease incidence and severity of Radish sowings

The distribution area of Downy mildew, level of disease incidence and severity were determined during observation of Radish sowings in **Table 1**.

Table 1. Spread of *H. Brassicae F. sp. raphani* in the different geographic zones in Georgia (2019-2022).

Years	Spread of <i>H. brassicae F. sp. raphani</i> in the different geographic zones (%)					Average
	Subtropical zone			Mountainous zone		
	Kobuleti	Xhelvachauri	Keda	Shuakhevi	Khulo	
2019	66.0	71.7	38.4	38.6	21.8	47.3
2020	40.5	56.7	36.5	31.9	25.8	38.3
2021	74.7	76.3	38.8	38.9	37.4	53.2
2022	34.3	44.4	35.4	29.5	24.5	33.6
Average	54.6	62.3	37.2	34.7	27.4	43.2

As shown in **Table 1**, the average values of the infected field were high (62,3-54,6%) in the subtropical zone (Xhelvachauri and Kobuleti). The lowest number of infected fields (27.4%) were in the mountainous zone (Khulo). A nearly equal number of infected fields (37.2-34.7%) were determined in Keda and Shuakhevi.

As shown in **Table 2**, the overall mean of incidence and severity were low (20,1% and 23,0%), especially in the non-irrigated region of Shuakhevi (12,2-15,3%). Drought and hot

dry weather during April and May limited the development of Downy mildew and that significantly contributed to the Downy mildew development. In 2020 and 2021 years overall mean of infected fields by Downy mildew were similar (53.2% and 53.4%, respectively) and lower, than in previous years. Also, there was a very low overall mean of incidence and severity of diseases - 20,1% and 23.0% in 2020, 23.7% and 25.3% in 2021, 26.1% and 28.8% in 2022 respectively.

Table 2. Overall mean values of incidence and severity levels of *H. brassicae F. sp. raphani*, %.

Geographical Zones	2019		2020		2021		2022		Average	
	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %
Kobuleti	8.9	4.3	7.2	1.60	0.9	2.4	34.4	6.7	0.35	33.75
Xhelvachauri	5.9	1.7	8.3	1.2	1.2	1.8	33.3	5.8	9.67	32.62
Keda	1.7	4.7	0.1	1.5	2.4	4.2	21.2	6.9	1.35	24.33
Shuakhevi	8.4	2.3	2.5	5.4	6.6	9.8	22.9	5.7	7.60	20.80
Khulo	9.5	1.6	2.2	5.3	7.2	8.5	18.8	9.7	4.90	16.93
Average	2.9	6.9	0.1	3.0	3.7	5.3	26.1	8.8	9.0	25.1

Antifungal activities of the extracts

Different concentration i.e. 1000ppm, 3000ppm, 5000ppm of 21 different metabolic extract from 18 different medicinal plant species was tested for their efficacy against *H. brassicae F. sp. raphani*. Antifungal activity of tested plant extract was investigated at different concentration against *H. brassicae F. sp. raphani*. The result revealed a highly significant percent inhibition.

The antifungal activity was observed to be dose-dependent i.e. with an increase in the concentration of plant extract percentage inhibition of mycelium growth increases. Antifungal activity of different plant extracts showed significant activity when compared with the leaf/root extract against *H. brassicae F. sp. raphani*. Root extract of *Inula racemosa* (R), *Rubia cordifolia* (R) and *Panax ginseng* (R) exhibit the highest activity.

Among the 21 extracts evaluated, 5 extract showed mycelia inhibition 80.75-98.33%, 3 showed moderate effect of 75.56-79.32% and 6 showed least 31,4 % - 57.27% (**Table 3**). The remaining 7 extracts from leaf extract of *Betonica officinalis*, *Althaea officinalis*, *Jasminum officinale*, *Pimpinella anisum*, *Pyrethrum vulgare*, *Ricinus communis* and *Sambucus ebulus* showed no antifungal activity against the *H. brassicae F. sp. raphani*.

This may be due to the lack of antifungal compound in the above mentioned 8 extracts. The percentage growth inhibition of *H. Brassicae F. raphani* was found maximum with *Stevia rebaudiana* (98.33%) and *Inula racemosa* (R) (91.86%). The plant *Galium aparine*, *Inula racemosa* (L),

Panax ginseng, *Rubia cordifolia* et al which inhibit the mycelia growth above 75% would probably be important candidates for plants for prevention of against Fungi. The investigation is an important step towards crop protection strategies for antifungal activity against important phytopathogen.

The value means of three replicates ± standard error. The values followed by different alphabets differ significantly when subjected to Tukey HSD at 0.5 subsets; R: Root; L: Leaf.

DISCUSSION

Study confirm that *H. brassicae F. sp. raphani* in Georgia can be a serious threat to the production of radish. Several phytopathogenic fungi including *H. brassicae F. sp. raphani* causes a significant decrease in nutritional value. Pathogen has been recorded as the dominant of Radish in Adjara. The use of many agriculturally important synthetic fungicide against this fungus has been banned by the World Health Organization (WHO) in several countries due to its toxicity effect against non-target organisms including human. Thus, there is an urgent need to search for an alternative method for the prevention of biodeterioration of vegetable without any toxicity to the consumer. Plants are rich sources of important organic compounds, pharmaceuticals and pesticide compounds. Many reports on the antiviral, antibacterial, antifungal, anthelmintic, anti-molluscal and anti-inflammatory properties of plants [15,16] have reviewed.

Table 3. Efficacy of herbal extracts of medicinal plants against *H. brassicae F. sp. Raphanin*.

Species	1000ppm		3000 ppm		5000 ppm	
	Colony Diameter	% Inhibition	Colony Diameter	% Inhibition	Colony Diameter	% Inhibition
<i>Eucalyptus cinerea</i> (L)	3.2±0.05	23.94±0.9	2.03±0.6	27.95±1.4	1.96±0.07	31.43±1.41
<i>Hamamelis mollis</i> (L)	3.1±0.08	25.83±0.8	2.09±0.9	32.86±1.5	1.97±0.05	34.5±1.33
<i>Panax ginseng</i> (L)	1.4±0.05	70.69±1.2	1.3±0.05	73.67±1.2	1.16±0.03	75.56±0.89
<i>Panax ginseng</i> (R)	2.5±0.04	75.84±0.7	1.3±0.04	77.82±1.2	1.16±0.05	80.75±0.90
<i>Mentha silvestris</i> (L)	3.12±0.7	30.65±1.4	3.2±0.02	31.63±0.7	2.73±0.04	38.60±0.75
<i>Mentha longifolia</i> (L)	3.4±0.04	32.3±0.8	2.9±0.05	37.6±1.37	2.3±0.04	46.9±1.12
<i>Galium aparine</i> (L)	3.3±0.07	65.37±1.1	1.75±0.0	76.34±0.8	1.52±0.04	79.32±0.82
<i>Rubia cordifolia</i> (L)	2.43±0.3	72.64±0.08	1.44±0.4	76.5±0.84	1.2±0.06	77.63±1.32
<i>Rubia cordifolia</i> (R)	2.35±0.4	76.59±0.6	1.21±0.7	79.48±0.9	1.1±0.7	83.41±1.27
<i>Rubia iberica</i> (R)	3±0.02	36.18±1.2	2.2±0.05	53.13±1.2	2.1±0.05	57.27±1.28
<i>Inula racemos</i> (L)	1.3±0.05	79.04±1.3	1.03±0.2	80.01±1.43	0.97±0.3	87.84±1.43
<i>Inula racemosa</i> (R)	1.4±0.7	82.91±1.3	1.04±0.7	88.47±1.44	0.95±0.5	91.86±1.35
<i>Salvia officinalis</i> (L)	3.7±0.8	29.52±0.5	3.5±0.06	31.52±1.5	3.04±0.6	37.12±1.53
<i>Stevia rebaudiana</i> (L)	2.37±0.4	83.74±1.3	2.03±0.8	87.76±1.7	1.7±0.8	98.33±1.72

Plant-derived secondary metabolites possessing antifungal activity. There is plenty of biologically active constituent which can be used as potential sources of commercially valuable pesticides which remain to be discovered [21-23]. This may be due to a lack of information on the screening/evaluation of diverse plants for their antifungal potential.

Among the mycobionts found on Radish, the most dangerous and harmful 4 pathogen of which the dominant species is *Hyaloperonospora brassicae F. sp. raphani*. It was found that effective leaf disease control may prevent root damage because roots are infected by conidia that come from the leaves. Similar findings were made by foreign researchers [3]. Among the 21 extracts evaluated, including 5 extract showed mycelia inhibition 80.75-98.33%, 3 showed moderate effect of 75.56 -79.32% and 6 showed least 31,4 % - 57.27%. The remaining 7 extracts from leaf extract of *Althaea officinalis*, *Betonica officinalis*, *Jasminum officinale*, *Pimpinella anisum*, *Pyrethrum vulgare*, *Ricinus communis* and *Sambucus ebulus* showed no antifungal activity against the *H. brassicae F. sp. raphani*. As studies have shown that *Stevia rebaudiana*, *Inula racemosa*, *Rubia coradifolia* and *Panax ginseng* extracts can be emerged as safe alternatives to replace chemical fungicides against pathogens. This may be due to the lack of antifungal compound in the above mentioned 7 extracts. The percentage growth inhibition of *Hyaloperonospora brassicae* was found maximum with *Stevia rebaudiana* (96.78%) and *Inula racemosa* (R) (92.86%). The plant *Inula racemosa* (L), *Rubia coradifolia*, *Galium aparine*, *Panax ginseng*, et al. which inhibit the mycelia growth above 75% would probably be important candidates for plants for

prevention of against Fungi. Almost similar findings were made by foreign researchers [24-26]. The investigation is an important step towards crop protection strategies for antifungal activity against important phytopathogen.

Based on a general analysis, it is very clearly shown that the investigation is an important step towards developing plant protection strategies for antifungal activity against the important phytopathogen *H. brassicae F. sp. raphani*. That can be beneficial for farmers and researchers who involve in agriculture. Lastly, by taking into consideration all the information provided in this review, the use of extracts evaluated should be promoted as a valid alternative to pesticides in the era of a green economy that aims at promoting human health and environmental safeguarding.

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