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THE ACTION OF SOME TOTAL PLANT EXTRACTS ON THE GERMINATION AND GROWTH OF *FUSARIUM OXYSPORUM* FUNGUS

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Abstract: In our experiments, we tested the action of some plant extracts (*Berberis vulgaris, Solanum nigrum*, a mixture of five equally combined plant extracts and walnut peels extract) on the germination and growth of *Fusarium oxysporum*, isolated from *Opuntia* spp.

Plant extract from *Berberis vulgaris* (0.05%) had a fungicide effect on the studied species. Plant extract from *Solanum nigrum* (0.1%) had the same effect.

The mixture of five plant extracts (Thymi herba, Juglandis folium, Querci cortex, Primulae rhizoma et radix, Chelidonii herba) had a fungistatic effect on the development of fungus'colonies.

Walnut peels extract had a less powerful effect on *Fusarium oxysporum* fungus.

Total plant extracts'inhibitory action depends on the type of active substances and their concentration in the nutritive medium.

Introduction

Fusarium genus (order **Hyphomycetales**) has many pathogen species, such as: *Fusarium oxysporum, Fusarium culmorum, Fusarium moniliforme,* etc.

Fusarium oxysporum attacks plants and causes plant wilt.

Fungus'sporulation consists of conidiophores, microconidia of 5 – 12 × 2.2 – 3.5 μm and macroconidia of 33 – 60 × 3 – 5 μm [8].

There are used different methods, like chemical, biological, sanitary methods, for the control of plant wilt caused by *Fusarium oxysporum* fungus.

There is evidence of some plant extracts that have antimicrobial activity. They are, for example, plant extracts from *Chelidonium majus* and *Pastinaca sativa* that inhibited 90 % the development of *Botrytis cinerea* fungus, isolated from vine [6].

Sixteen volatile compounds occuring naturally in fruits were tested for their effects on spore germination and growth of *Monilinia fructicola* and *Botrytis cinerea*. Nine of these compounds (benzaldehyde, benzyl alcohol, δ-7 decalactone, γ-caprolactone, γ-decalactone, γ-octalactone, methyl salicylate and γ-valerolactone) greatly inhibited spore germination of both fungi at 1.250 μl/L. Benzaldehyde totally inhibited spore germination of *Botrytis cinerea* at 25 μl/L and germination of *Monilinia fructicola* at 125 μl/L. Three of the compounds (benzaldehyde, methyl salicylate and ethylbenzoate) completely inhibited growth of *Monilinia fructicola* and *Botrytis cinerea* at 370 μl/L. Ethyl benzoate was fungicidal against *Monilinia fructicola* and fungistatic against *Botrytis cinerea*, whereas methyl salicylate and benzaldehyde were fungistatic against both fungi [12].

Also, plant extracts from *Petroselinum hortense* and *P. sativa* had a total inhibitory effect on the development of *Botrytis cinerea*, isolated from *Lycopersicum esculentum* and *Cinnamomum* spp. [7].

Material and Method

In our experiments, we tested the action of some total plant extracts on the germination and growth of *Fusarium oxysporum* fungus.

We tested plant extracts from *Berberis vulgaris*, *Solanum nigrum*, a mixture of plant extracts (Thymi herba, Juglandis folium, Querci cortex, Primulae rhizoma et radix, Chelidonii herba) and walnut peels extract.

Plant extract from *Berberis vulgaris* contains 1% alkaloids appreciated in berberin and the one from *Solanum nigrum* contains 1% alkaloids appreciated in solanin. Walnut peels extract has iuglon as the active substance.

There were done many experimental variants with different concentrations for each plant extract type and six repetitions were done for each experimental variant.

Plant extract was added to the sterilized nutritive medium (Czapek-agar, beer must-agar, malt-agar) and was distributed in Petri plates (70 mm in diameter). Petri plates were inoculated in central part with *Fusarium oxysporum* fungus and were incubated at 22 °C [10].

There were done observations on the development of fungus'colonies at regular times (3, 6, 9 and 12 days).

Estimation of colonies' diameters on nutritive medium with plant extract was done in comparison with the control (without plant extract).

Experimental results are represented in tables 1 – 4.

Results and Discussion

The action of plant extract from *Berberis vulgaris* on *Fusarium oxysporum* fungus was tested in different concentrations (0.025%; 0.03%; 0.04%; 0.05%).

Active substances from *Berberis vulgaris* are represented by different alkaloids, such as: berberin, berbamin, oxyacanthin, magnoflorin, berberubin, etc. [11].

These alkaloids are mostly present in root's bark and leaves; they cause digestive, hepatic and nervous troubles [11].

Ripe fruits of *Berberis vulgaris* don't contain alkaloids; they contain glucose, fructose, gums and C vitamin [1].

Recent studies showed that berberin has bacteriostatic activity, especially against Koch bacillus [2].

Table 1: The action of *Berberis vulgaris* total plant extract on the germination and growth of *Fusarium oxysporum* fungus

No. Crt.	Plant extract concentration (%)	Colony's diameter (mm) after 3 days	6 days	9 days	12 days	Inhibition after 12 days (%)
1.	0.025	8	20	31	44	60.49
2.	0.03	8	20	31	41	65.70
3.	0.04	0	3	10	15	95.41
4.	0.05	0	0	0	0	100
5.	Control	23	46	70	70	-

Legend: 0 = absence of fungus colony
- = absence of inhibition

It is obvious that at concentrations of 0.025% and 0.03% plant extract from *Berberis vulgaris* has a fungistatic effect. On the contrary, at concentrations of 0.04% and 0.05% it has a fungicide effect (Table 1).

Total plant extract from *Berberis vulgaris* has an inhibitory effect on "in vitro" germination and growth of *Fusarium oxysporum*. Inhibitory effect depends on plant extract's

concentration in the nutritive medium. At a concentration of 0.05%, there is evidence of total inhibition and the fungus doesn't germinate (Table 1).

We also tested the action of *Solanum nigrum* plant extract on the studied species. There were used many concentrations: 0.02%; 0.04%; 0.06%; 0.08%; 0.1% (Table 2).

Table 2: The action of *Solanum nigrum* total plant extract on the germination and growth of *Fusarium oxysporum* fungus

No. Crt.	Plant extract concentration (%)	Colony's diameter (mm) after 3 days	6 days	9 days	12 days	Inhibition after 12 days (%)
1.	0.02	18	34	70	70	-
2.	0.04	7	16	33	44	60.49
3.	0.06	2	9	17	33	77.78
4.	0.08	0	3	11	21	91
5.	0.1	0	0	0	0	100
6.	Control	24	41	70	70	-

Legend: see Table 1

Solanum nigrum plants are toxic because of solanin. The highest toxicity have unripe fruits and plants grown in sunny places [11].

Beside solanin, *Solanum nigrum* also contains solasonin, solamargin, solasodamin, chloragenic acid, sugars, fats, C vitamin.

Beside alkaloids, there are great ammounts of saponins especially in fruits; they have antimicrobial activity [3].

Total plant extract from *Solanum nigrum* had an inhibitory effect on *Botrytis aclada* fungus. The inhibitory action depended on the type of plant extract and its concentration in the nutritive medium [9].

At low concentrations (0.02%) of plant extract, fungus' mycelium is white and abundant. After 9 days from inoculation, mycelium occupies all Petri plate. It has a good development even at higher concentrations (0.04%). At a concentration of 0.06%, plant extract has a fungicide effect; after 12 days from inoculation, colony's diameter is about 33 mm (Table 2).

Fungicide effect is evident at higher concentrations (0.08% and 0.1%). Plant extract from *Solanum nigrum* has a fungicide effect (100% inhibition) at a concentration of 0.1 % (Table 2).

We also tested the action of a mixture of plant extracts (Thymi herba 20%, Juglandis folium 20%, Querci cortex 20%, Primulae rhizoma et radix 20%, Chelidonii herba 20%) for the control of *Fusarium oxysporum* fungus.

Thymi herba represents the terrestrial part of *Thymus vulgaris*.

It contains volatile oil, tanin and a bitter substance. It has antifungic and antimicrobial activity [5].

Juglandis folium represents the leaves of *Juglans regia* and contains active subtances like iuglon and hidroiuglon.

Querci cortex is the dry bark of *Quercus* trees. It mainly contains a mixture of tanins (20 – 80%) [11].

Primulae rhizoma et radix is the vegetal product of *Primula* species and it contains a lot of saponins.

Chelidonii herba represents the flowering upper part of *Chelidonium majus*. Plant extract contains alkaloids like chelidonin, oxychelidonin, sanguinarin, berberin, etc. [4].

Table 3: The action of total plant extracts mixture on the germination and growth of *Fusarium oxysporum* fungus

No. Crt.	Plant extract concentration (%)	Colony's diameter (mm) after 3 days	6 days	9 days	12 days	Inhibition after 12 days (%)
1.	1	13	42	65	68	5.64
2.	2	5	30	57	62	21.56
3.	4	3	23	53	57	33.7
4.	6	1	14	39	54	40.49
5.	8	1	13	29	51	46.92
6.	Control	25	41	70	70	-

Legend: see Table 1

In case of experimental variant with a concentration of 1%, fungus'mycelium is white, abundant and radial; after 3 days from cultivation mycelium's diameter is about 13 mm. After 6 days it becomes of 42 mm, after 9 days 65 mm and after 12 days is about 68 mm (Table 3).

At a concentration of 2%, there is evidence of an insignificant inhibitory effect on fungus' colonies.

The same thing happens in case of 4% and 6% concentrations.

At a concentration of 8%, the mixture of plant extracts has a weak inhibitory effect on the development of the fungus' colonies (Table 3).

Based on the experimental results, we may say that the mixture of plant extracts has a weak inhibitory action on the germination and growth of *Fusarium oxysporum* fungus.

We used the following concentrations 1%; 2%; 4% and 6% for testing the action of walnut peels extract.

In this case, there is evidence of a very weak inhibition even at a concentration of 4% (Table 4).

Plant extract has a powerful inhibitory effect in case of experimental variant with a concentration of 6%. On such a nutritive medium, the inhibition of fungus' colonies was about 49% (Table 4).

Table 4: The action of walnut peels total extract on the germination and growth of *Fusarium oxysporum* fungus

No. Crt.	Plant extract concentration (%)	Colony's diameter (mm) after 3 days	6 days	9 days	12 days	Inhibition after 12 days (%)
1.	1	19	50	70	70	-
2.	2	18	51	70	70	-
3.	4	7	32	62	68	5.64
4.	6	0	13	44	50	48.98
5.	Control	24	57	70	70	-

Legend: see Table 1

Conclusions

According to the experimental results we obtained, some conclusions can be drawn:

- Plant extract from *Berberis vulgaris* (0.05%) had a fungicide effect on the germination and growth of *Fusarium oxysporum* phytopathogen fungus;
- Total inhibitory effect of plant extract from *Solanum nigrum* was achieved at a concentration of 0,1 %;
- The mixture of plant extracts had a weak inhibitory effect on the studied fungus;

- Walnut peels total extract had a very weak fungistatic action on *Fusarium oxysporum* fungus;

- Inhibitory action of plant extracts on the germination and growth of *Fusarium oxysporum* fungus depended on the type of active substance and its concentration in the nutritive medium.

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ACŢIUNEA UNOR EXTRACTE VEGETALE TOTALE ASUPRA GERMINĂRII ŞI CREŞTERII CIUPERCII *FUSARIUM OXYSPORUM*

(Rezumat)

Genul *Fusarium* (familia **Tuberculariaceae**, ordinul **Hyphomycetales**, clasa **Hyphomycetes**, subîncrengătura **Deuteromycotina**, încrengătura **Eumycota**) cuprinde specii parazite comune pe plante cultivate şi spontane.

Una dintre speciile parazite este *Fusarium oxysporum*, care produce ofilirea vasculară a plantelor sau fusarioza. Această specie a fost izolată de pe plante de *Opuntia* spp.

În cercetările efectuate s-a studiat acţiunea unor extracte vegetale totale asupra germinării şi creşterii ciupercii *Fusarium oxysporum* cultivată pe medii nutritive (Czapek-agar, Must de bere-agar, Malţ-agar). Efectul extractelor totale a fost apreciat comparativ cu colonia martor.

Extractul de *Berberis vulgaris* a avut efect inhibitor total asupra germinării şi creşterii ciupercii *Fusarium oxysporum* la concentraţia de 0,05 %, iar cel de *Solanum nigrum* la 0,1 %.

De asemenea, s-a testat acţiunea unui amestec de cinci extracte combinate în proporţie egală (Thymi herba, Juglandis folium, Querci cortex, Primulae rhizoma et radix, Chelidonii herba) şi s-a obţinut un efect fungistatic începând cu concentraţia de 4%.

Efect inhibitor asemănător a avut şi extractul din frunze de nuc. Extractul din coji de nucă a avut un efect fungistatic "in vitro" la o concentraţie de 6%.

Activitatea inhibitoare a extractelor vegetale totale este determinată de tipul de substanţe active şi concentraţia acestora în mediul de cultură.