Biological Activity of Extracts from Xylotrophic Fungi: Application in Agricultural Fields

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Abstract.

Eleven species (dried fruit bodies) *Fomitopsis pinicola, Cerrena unicolor, Piptoporus betulinus, Daedaleopsis tricolor, Stereum subtomentosum, Funalia trogii, Phellinus cinereus, Trametes versicolor, T. pubescens, T. gibbosa* and *Ganoderma applanatum* were used to extract biologically active compounds (BACs) in crude extracts (CFE) which may further be studied to evaluate their impact on rate of seeds germination, biosynthesis of photosynthetic pigments, biomass formation and of length of tomato seedlings (*Solanum lycopersicum*, cv. Dubrava). The results showed that xylotrophic mushrooms are source of potential biologically active compounds useful for agriculture.

MATERIALS AND METHODS

Fungi Species

Fruiting bodies of 11 xylothrophic mushroom species - *Fomitopsis pinicola, Cerrena unicolor, Piptoporus betulinus, Daedaleopsis tricolor, Stereum subtomentosum, Funalia trogii, Phellinus cinereus, Trametes versicolor, T. pubescens, T. gibbosa* and *Ganoderma applanatum* were picked up at the biological station of Ural Federal University, 60 km from Ekaterinburg city, Middle Urals in Russia Federation. They were harvested from five species of plant substrates which are *Betula pendula, Pinus Sylvestris, Prunus padus, Populus balsamifera* and *Picea obavata*

Extraction of CFEs and Quantification of BACs

A series of extraction steps were carried out using consequently 80%, 60%, 40 % ethanol and diH_2O from 500 mg of dried biomass for each fungus' sample. The aliquots of these CFEs were used for the quantitative measurement of free amino acids using ninhydrin method,of total phenolic compounds, of total soluble proteins and of qualitative assay of amino acids which was performed using two techniques, ascending paper chromatography and LC-MS chromatography (UPLC-QTofXevo Waters).

Effect of CFEs on Seed Germination and Early Growth of Tomato Plantlets

Seeds were sown on Petri dishes containing filter paper, twenty seeds on each petri dish. The petri dishes were moistened with CFEs except the control seeds which were only moistened with diH_2O. Twice a day, we assessed the rate of seed germination, growth and moisture and length of seedlings; moistening of seeds and seedlings was performed at the same time; the temperature of the laboratory room was 20°C-25°C. After thirteen days, every Petri dish was moistened 9 ml CFE alongside with 20 ml diH_2O

Root and Shoot Biomass

After 13 days from the sowing time, ten seedlings were harvested from every Petri dish. We carefully washed them, separated roots from shoots and dried them in oven at 80° C for 48 hours. Root and shoot of the same seedling were held in labeled piece of aluminum foil. The dried biomass of root and shoot were separately determined using an analytical balance. Statistical calculations were made.

Determination of Chlorophylls a & b and Carotenoids

After 13 days of growth from sowing time, we harvested primary fresh leaves or cotyledons of seedlings; 100 mg were ground and homogenized with 80% acetone, $CaCO_3$ and glass sand; the mixtures were centrifugated; the supernatant (extracts) were separated from solid wastes. The concentration of photosynthetic pigments was determined by spectrophotometry using Apel UV-VIS spectrophotometer, Japan; the optical density of extracted chlorophylls a, b and carotenoids were measured at 663 nm, 646 nm and 470 nm of wavelength respectively, their concentrations (mg/g) were calculated based on leaf mass

RESULTS AND DISCUSSION

Germination Process of Seeds

The germination process has showed that there was little difference in germinated number of seeds every day. The most active process of seeds germination was observed between the second and ninth days, after nine days there was no new germinated seed. For a period of thirteen days from sowing time there were between 88-99% germinated seeds

Growth characteristics - Roots' and shoots' Biomass

All CFEs have showed stimulatory effect on biomass synthesis by seedlings (Fig.1), CFEs from *T. versicolor* [a]* and *G. applanatum* have showed the lowest and highest stimulatory effects respectively.

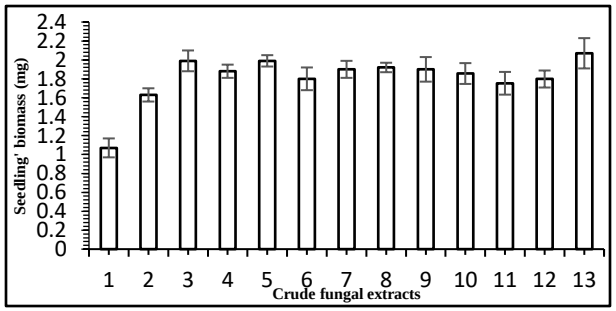

Figure 1. Average of Dried Biomass (mg) of Seedling.

1. diH_2O, 2. *T. versicolor* [a]*, 3. *C. unicolor*, 4. *F. pinicola*, 5. *T. versicolor b****, 6. *P. betulinus*, 7. *D. tricolor*, 8. *S. subtomentosum*, 9. *F. trogii*, 10. *P. cinereus*, 11. *T. pubescens*, 12. *T. gibbosa* and 13. *G. applanatum*. *T. versicolor* was picked up from two different plant substrates - *Betula pendula* (a*) and *Prunus padus* (b**)

Growth characteristics - Roots' and shoots' Biomass

All CFEs have showed stimulatory effect on biomass synthesis by seedlings (Fig. 2), CFEs from *T. versicolor* [a]* and *G. applanatum* have showed the lowest and highest stimulatory effects respectively.

Content of Photosynthetic Pigments

Several CFEs have showed stimulatory activity on biosynthesis of photosynthetic pigments; all CFEs have showed stimulatory effect on chl a, the concentration (mg/g) of chl a was between 0.93±0.11 and 1.38±0.13 equivalent to +2.2 (%) and +51.65 (%) (Table 1) showed by CFEs from *F. pinicola* and *F. trogii* respectively. The effect (%) on chl b biosynthesis was between -19.35% and +58.06% which were synthesized by seedlings moistened with CFEs from *T. pubescens* and *F. trogii* respectively (Table 1). Nine out of twelve CFEs (75%) have displayed stimulatory effect on chl b and carotenoids biosynthesis. CFEs from *F. pinicola, T. pubescens* and *G. applanatum* have showed inhibitory whereas CFEs from *F. pinicola, T. versicolor* [a] and *T. pubescens* showed did inhibit carotenoids' biosynthesis. CFE from *F. trogii* showed the highest stimulatory effect equivalent to +50%.

TABLE 1. Chlorophyll a, b and Carotenoid Content (mg/g Fresh Weight). Data Presented are the Mean values ± Standard Error (SEM) from 5 Replicates.

Fungus	Chl a	Effect (%)	Chl b	Effect (%)	Car	Effect (%)
DiH$_2$O	0.91±0.10	0	0.31±0.05	0	0.20±0.02	0
T. versicolor [a]*	1.00±0.06	+9.89	0.42±0.03	+35.48	0.19±0.01	-5
C. unicolor	1.01±0.09	+10.99	0.41±0.02	+32.26	0.22±0.03	+10
F. pinicola	0.93±0.02	+2.2	0.30±0.01	-3.23	0.18±0.04	-10
T. versicolor [b]**	1.06±0.11	+16.48	0.38±0.04	+22.58	0.22±0.02	+10
P. betulinus	1.19±0.14	+30.77	0.38±0.06	+22.58	0.26±0.02	+30
D. tricolor	1.22±0.02	+34.07	0.37±0.02	+19.35	0.29±0.01	+45
S. subtomentosum	1.19±0.18	+30.77	0.35±0.06	+12.90	0.29±0.05	+45
F. trogii	1.38±0.13	+51.65	0.49±0.07	+58.06	0.30±0.02	+50
P. cinereus	0.93±0.11	+2.2	0.32±0.04	+3.22	0.21±0.03	+5
T. pubescens	0.99±0.13	+8.79	0.25±0.06	-19.35	0.18±0.20	-10
T. gibbosa	0.95±0.08	+4.4	0.38±0.04	+22.58	0.24±0.03	+20
G. applanatum	0.93±0.06	+2.2	0.26±0.03	-16.13	0.22±0.10	+10

T. versicolor was picked up from two different plantsubstrates - *Betula pendula* (a*) and *Prunus padus* (b**

CONCLUSION

From our study, we got interesting results which have showed that there are various wood decaying fungi with potential effect on plant growth (Table 2):

TABLE 2. Summary of Different Effects CFEs on Early Growth of Barley, Cucumber and Tomato (Yellow – Negative Effect, Green – Positive Effect)

Fungus	Ger.[1]	Bio.[2]	Chl a	Chl b	Car	Total (+)
F. pinicola	0	+	+	-	-	2/5 – 40 %
T. versicolor [a]*	+	+	+	+	-	4/5 – 80 %
T. versicolor [b]**	-	+	+	+	+	4/5 – 80 %
C. unicolor	+	+	+	+	+	5/5 – 100 %
P. betulinus	+	+	+	+	+	5/5 – 100 %
D. tricolor	+	+	+	+	+	5/5 – 100 %
S. subtomentosum	+	+	+	+	+	5/5 – 100 %
F. trogii	+	+	+	+	+	5/5 – 100 %
P. cinereus	0	+	+	+	+	4/5 – 80 %
T. pubescens	+	+	+	-	-	3/5 – 60 %
T. gibbosa	0	+	+	+	+	4/5 – 80 %
G. applanatum	+	+	+	-	+	4/5 – 80 %
Total (+)	8/12	12/12	12/12	9/12	9/12	50/60
Total (+; %)	66.66	100	100	75	75	83.33 %

T. versicolor was picked up from two different plant substrates - *Betula pendula* (a*) and *Prunus padus* (b**)

Out of twelve CFEs, eight have stimulatory effect; only CFE from *T. versicolor* [c] has shown inhibitory effect on seed germination rate whereas CFEs from *F. pinicola, P. cinereus* and *T. gibbosa* did not reveal neither stimulatory nor inhibitory effect on germination rate. All of ten studied CFEs have shown positive effect on biomass growth in seedlings. Finally, all CFEs did stimulate chlorophyll a biosynthesis, while nine CFEs did show stimulatory effect in chlorophyll b and carotenoids; inhibitory effect was shown by CFEs from *F. pinicola*, *T. pubescens* and *G. applanatum* on chlorophyll b biosynthesis whereas CFEs from *F. pinicola* and *T. versicolor* [c] did it in carotenoid biosynthesis.

These results suggest that wood decaying mushrooms could be used as the source of BACs to boost, promote and stimulate plants yield and production.

ACKNOWLEDGMENTS

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