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IMPACT OF CORONA DISCHARGE ON *ASPERGILLUS NIGER* SPORES AND *CYATHUS OLLA* MYCELIUM GROWTH

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The article presents an investigation of the influence of the corona discharge with needle-ring electrode configuration on the efficiency of *Aspergillus niger* spore germination and *Cyathus olla* mycelium development. Current-voltage characteristics were measured for different distances between the needle and the metal ring. The plot of the dependence of conductance on voltage was built and the corona discharge breakthrough voltage was determined to be (2.7±0.1) kV. The treatment of *Aspergillus niger* spores was conducted at different development stages: at the germination stage (corona discharge treatment 1 day after the seeding) and dormant state (corona discharge treatment on the day of seeding). The growth rate of *Cyathus olla* mycelium in samples treated by corona discharge and in control group samples was investigated. The influence of the corona discharge on the potato dextrose agar growth medium before the seeding of *Cyathus olla* was studied. The pure cultures were obtained from the Culture Collection of Fungi at Kyiv University (FCKU) at the «Institute of Biology and Medicine» Educational and Scientific Centre of the Taras Shevchenko National University of Kyiv.

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INTRODUCTION

At present, the study of the interaction between plasma and fungi can provide mankind with improvements in two areas. The first area is the plasma-induced destruction of the spores and fungi for the disinfection of buildings, people, or plants. The second area is the enhancement of the mycelium growth rate via the cell stimulation or enrichment of the fungal growth environment with the plasma products [1-9].

Plasma-influenced inactivation of the microorganisms has 5 main mechanisms: temperature, electric field, UV light, direct chemical reactions of the neutral active forms, and interaction between charged particles and cell components [4]. In the case of the non-thermal atmospheric pressure plasma systems, the inactivation process is mainly driven by the active particles (e.g., atomic oxygen, metastable oxygen molecules, ozone, OH radicals).

The advantage of the corona discharge is the generation of the ion stream (ion wind) at a temperature close to the temperature of the environment [9-11]. This ability combined with the relatively low discharge power allows neglecting the influence of temperature on samples during the research.

Some species of fungi are used in the production of medicine and antibiotics. Thus, by improving the conditions and enhancing the growth rates of fungi sporocarps it is possible to decrease the price of some medicaments. For example, *Cyathus olla* fungi are used to synthesize salfredin, which is considered for the therapeutic use during cataract in people with diabetes. In addition, *Cyathus olla* has agricultural significance, as it can destroy dead rapeseed stems, infected by *Rhizoctonia solani*.

This work aims to study the corona discharge and its influence on the germination efficiency of *Aspergillus niger* spores and the development of *Cyathus olla* mycelium.

1. EXPERIMENTAL SET-UP

The influence of the corona discharge on the spores and fungi mycelium development was studied using an experimental set-up with the needle-ring electrode configuration (Fig. 1). The inner diameter of the ring was 12 mm, the needle diameter was 0.15 mm. The power supply provided voltage in the 0...7 kV range. The needle was set as a high-voltage cathode and the ring was set as a grounded anode. The ring was made of stainless steel and the needle was made of tungsten. The voltage was measured using a voltage divider composed of 1.5 GΩ resistance R_1 and 1.5 kΩ resistance R_2.

Samples were investigated using PrimoStar light microscope. Microphotographs were obtained using ScienceLabDCM 520 digital camera and Axiovision 4.3.7 image processing software.

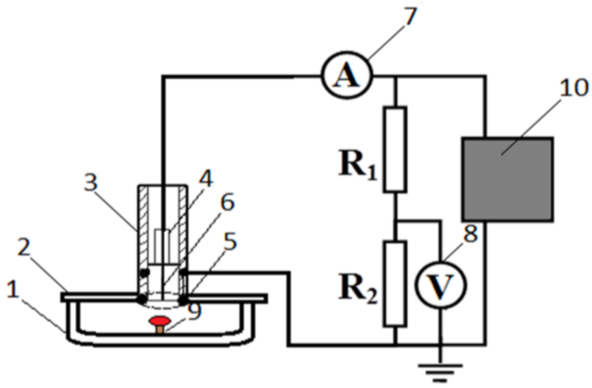


Fig. 1. Scheme of experimental set-up (needle-ring in dielectric): 1 – Petri dish; 2 – cover; 3 – dielectric (PTFE); 4 – ceramic; R_1 and R_2 – divider resistances; 5 – metal ring; 6 – tungsten needle; 7 – ammeter; 8 – voltmeter; 9 – studied sample; 10 – power supply

The experimental investigation of the influence of the corona discharge on the growth of the mycelial colonies of the higher fungi was conducted on the *Cyathus olla* culture. The pure cultures were obtained from the Culture Collection of Fungi at Kyiv University (FCKU) at the «Institute of Biology and Medicine» Educational and Scientific Centre of the Taras Shevchenko National University of Kyiv. The cultures were extracted from the basidiomata via the Buhalo method and incubated in a potato dextrose agar (PDA) at 23...25 °C. Mycelial colonies were treated on the 3rd day of the cultivation. The inoculated Petri dishes, which were not treated by corona discharge, were set as controls.

The influence indicators were determined from the difference between the average daily mycelium growth rates (mm/day) of the treated samples and the control group.

2. RESULTS AND DISCUSSION

The current-voltage characteristics (Fig. 2) of the corona discharge were measured for different distances between the ring plane and the needlepoint. In the case, when the needlepoint (see Fig. 1) was located between the metal ring and the ceramic isolator, the distance between the needle and the ring plane was marked with a "+" sign. When the metal ring was located between the needlepoint and the ceramic isolator, the distance was marked with a "-" sign. Fig. 3 shows the dependence of the conductance from the voltage between the needle and the grounded ring. The breakthrough voltage of the corona discharge was (2.7±0.1) kV, as determined from the plotted dependence between the conductance and voltage.

2.1. INFLUENCE OF CORONA DISCHARGE ON *ASPERGILLUS NIGER* SPORES

Aspergillus niger spores were sawn onto the potato dextrose agar growth medium. During the treatment of *Aspergillus niger* spores, corona discharge current was I_d = 25 μA, the voltage was U_d = 5.2 kV, and power was 0.13 W. The treatment with corona discharge lasted 1, 3, and 5 min with the energy spent on the discharge 7.8, 23.4, and 39 J respectively. The control group consisted of samples untreated by the corona discharge. *Aspergillus niger* spores were treated at different stages of development:

1) at the germination stage (treatment 1 day after seeding);

2) at the dormant state (treatment on the day of seeding).

The results of the experiment were recorded on the second day after the corona discharge treatment. Fig. 4 shows the photographs of the mycelial colonies of the *Aspergillus niger* in the case of spores treated by the corona discharge at the germination stage (1 day after seeding). Photographs were taken on the second day after the corona discharge treatment. In comparison to the control, corona discharge treatment inhibits the development of *Aspergillus niger* mycelial colony. An increase in the corona discharge treatment time led to a decrease in the mycelial colony development. Therefore, the increase of the treatment time and, correspondingly, of the energy spent on the discharge can achieve the significant inhibition of the *Aspergillus niger* mycelial colony development.

Fig. 5 shows the photographs of the mycelial colonies of the *Aspergillus niger* in the case of spores treated by the corona discharge at the dormant state (on the day of the spore seeding). Photographs were taken on the second day after the corona discharge treatment. Photographs show an insignificant difference between the control and the samples treated by the corona discharge. *Aspergillus niger* mycelial colonies are visibly less developed in the treated samples in comparison with control. Despite this, photographs show that corona discharge has less influence on the spores themselves, which still germinate after the treatment, than on the already germinated spores.

Fig. 6 shows photographs obtained during the microscopy of the *Aspergillus niger* mycelial colony.

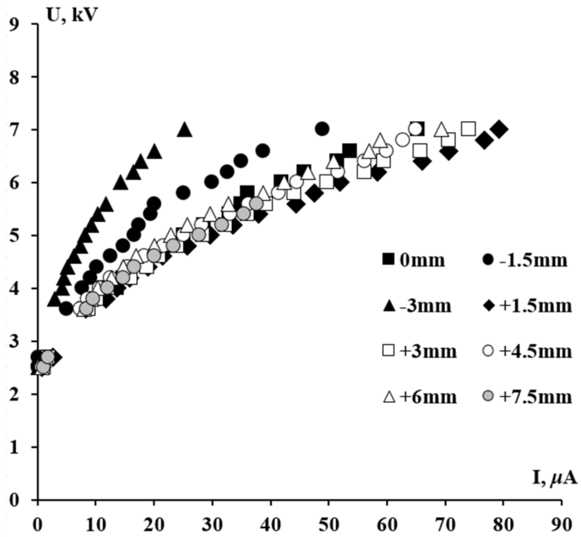


Fig. 2. Current-voltage characteristics of corona discharge with needle-ring electrode configuration

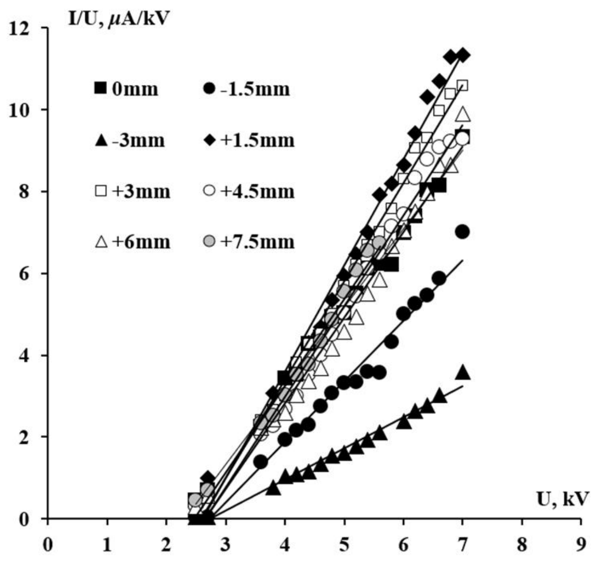


Fig. 3. Dependence of conductance on voltage during discharge

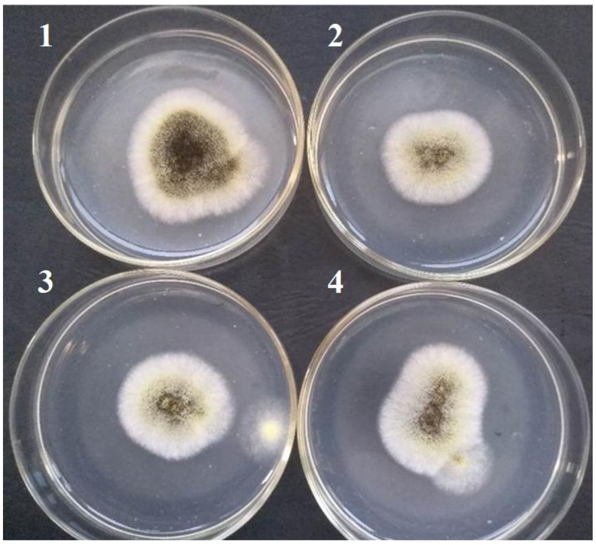


Fig. 4. Photographs of Aspergillus niger mycelial colony samples in case of spores treated with corona discharge during germination stage (1 day after seeding): 1 – control; 2 – 1 min; 3 – 3 min; 4 – 5 min

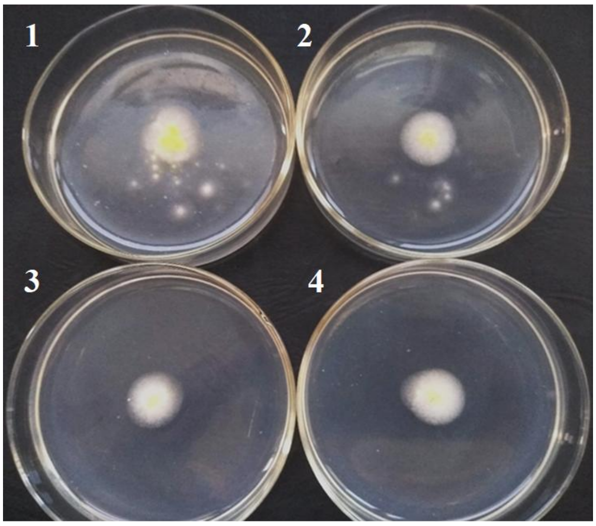
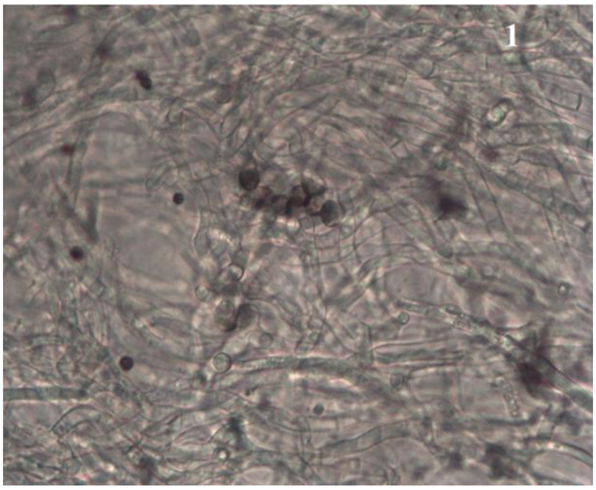


Fig. 5. Photographs of Aspergillus niger mycelial colony samples in case of spores treated with corona discharge during dormant state (at day of seeding): 1 – control; 2 – 1 min; 3 – 3 min; 4 – 5 min

Microscopic investigations showed the occurrence of spore germination and mycelial hyphae formation in all tested samples (see Fig. 6). Microscopy was conducted using PrimoStar light microscope, microphotography was done by ScienceLabDCM 520 digital camera, and Axiovision 4.3.7 image processing software.

As such, corona discharge impacts *Aspergillus niger* spores and mycelial colonies development only on the stage of spore germination, i.e., when the spores are already germinated. In addition, the increase of the time of the corona discharge treatment of the spores is corresponding to the inhibition of the *Aspergillus niger* mycelial colony development, but this time is not sufficient for the complete decontamination of the spore mass.

In further work with the corona discharge, the time of corona discharge treatment should be increased.



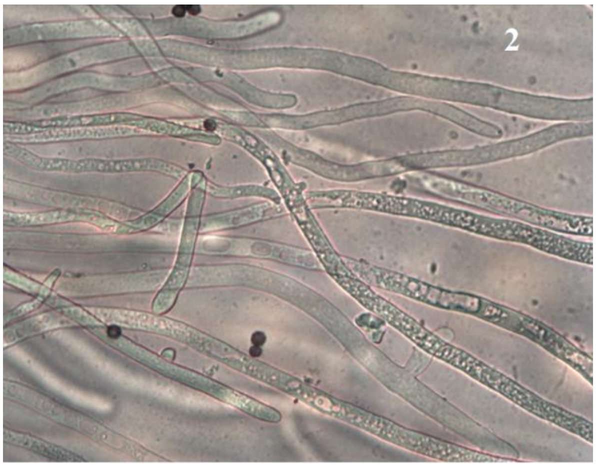


Fig. 6. Photographs of Aspergillus niger mycelial colony samples in case of spores treated with corona discharge during dormant state (at day of seeding): 1 – control; 2 – 5 min

2.2. INFLUENCE OF CORONA DISCHARGE ON *CYATHUS OLLA* MYCELIUM GROWTH

Cyathus olla test samples were grown on the potato dextrose agar growth medium at 23...25 °C. Samples were processed on the third day of the cultivation. The plasma stream was produced at 3...5 mm from the object surface at different time intervals (1, 3, 5, 10 min). During plasma treatment, colony diameter was 10...15 mm. Mycelial colony, which was not treated by plasma, was set as the control. The influence indicators were determined from the difference between the average daily mycelium growth rates (mm/day) of the treated samples and the control group. Fig. 7 shows the typical photograph of *Cyathus olla* culture.

Experiments were conducted in operating mode with I ≈ 21...26 μA, U ≈ 5.2 kV. The distance to the sample was controlled by moving needle-ring setup via regulating nut. To avoid contamination during the experiment, Petri dishes with samples were opened only before connection to the corona discharge and were closed immediately after disconnection from the discharge.

The results of the *Cyathus olla* mycelium growth investigation are shown in Fig. 8.

Fig. 7. Typical photograph of Cyathus olla mycelium

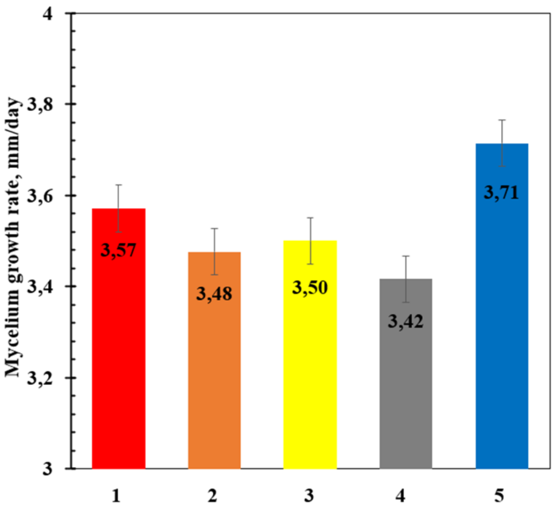


Fig. 8. Cyathus olla mycelium growth rate after different duration of plasma treatment: 1 – control; 2 – 1 min; 3 – 3 min; 4 – 5 min; 5 – 10 min

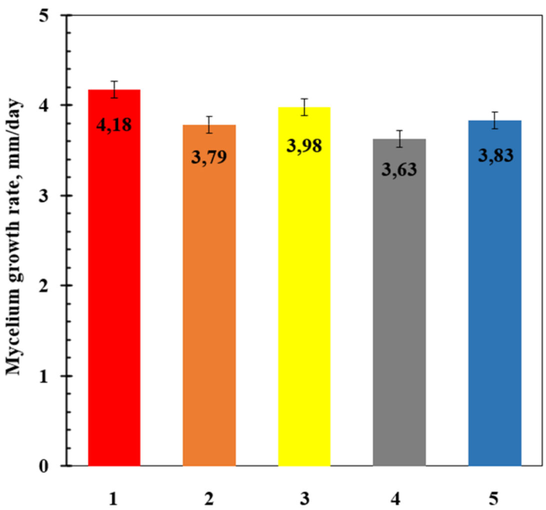


Fig. 9. Impact of plasma treatment time on pure potato dextrose agar growth medium: 1 – control; 2 – 1 min; 3 – 3 min; 4 – 5 min; 5 – 10 min

The treatment of *Cyathus olla* mycelium by the cold plasma of the corona discharge can speed up the culture growth without direct thermal damage. The dependence of the growth rate on the corona discharge treatment time showed a possibility for both accelerating and decelerating of the mycelium growth.

The experiments were conducted on the direct treatment of the growth medium using corona discharge in atmospheric air to investigate the influence of the specific mechanisms before the seeding of the mycelial colonies (Fig. 9). Direct corona discharge treatment of the growth medium does not influence the growth rate.

CONCLUSIONS

Set-up for the investigation of the corona discharge influence on the efficiency of the spore germination and fungi mycelium development was developed and built during the study. Current-voltage characteristics were measured for different distances between the needle and the metal ring. The plot of the dependence of conductance on voltage was built and the corona discharge breakthrough voltage was determined to be (2.7±0.) kV.

It was discovered that corona discharge influences the development of *Aspergillus niger* colonies only during the spore germination stage. The increase of the time of the spore treatment with the corona discharge corresponds to the inhibition of the development of *Aspergillus niger* mycelial colony. However, the time used in the experiments was not enough for the complete decontamination of the spore mass, thus the time of the spore treatment with the corona discharge should be increased.

The results obtained after the treatment of the fungi mycelium with the cold plasma of the corona discharge showed that such treatment can accelerate the culture growth without the direct thermal damage. It was determined during the experiment, that in the case of the corona discharge the dependence of the growth rate on the treatment time showed the possibility to both accelerate and decelerate the mycelium growth. It was shown that the direct treatment of only the growth medium using the corona discharge does not influence the growth rate of *Cyathus olla*.

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ВЛИЯНИЕ КОРОННОГО РАЗРЯДА НА СПОРЫ *ASPERGILLUS NIGER* И РАЗВИТИЕ МИЦЕЛИЯ *CYATHUS OLLA*

О.А. Недыбалюк, Ю.П. Веремий, Н.В. Цвыд, М.Н. Сухомлын, В.Г. Тищенко, А.В. Шевченко, И.И. Федирчик

Представлено исследование влияния коронного разряда с конфигурацией электродов острие-кольцо на эффективность прорастания спор *Aspergillus niger* и развитие мицелия грибов *Cyathus olla*. Измерены вольт-амперные характеристики для различных расстояний между острием и металлическим кольцом. Построен график зависимости обратного сопротивления от напряжения, с которого определено напряжение зажигания коронного разряда (2.7±0.1) кВ. Проведена обработка спор *Aspergillus niger* на разных стадиях развития: на стадии прорастания (обработка коронным разрядом через сутки после высевания) и стадии покоя (обработка коронным разрядом в день высевания). Исследована скорость роста мицелия *Cyathus olla* в образцах, обработанных коронным разрядом, и контрольной группы. Исследовано влияние коронного разряда на картофельно-глюкозный агар до высадки в эту среду *Cyathus olla*. Чистые культуры, получены из коллекции культур грибов FCKU Учебно-научного центра «Институт биологии и медицины» Киевского национального университета им. Тараса Шевченко.

ВПЛИВ КОРОННОГО РОЗРЯДУ НА СПОРИ *ASPERGILLUS NIGER* ТА РОЗВИТОК МІЦЕЛІЮ *CYATHUS OLLA*

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Представлено дослідження впливу коронного розряду з конфігурацією електродів вістря-кільце на ефективність проростання спор *Aspergillus niger* та розвиток міцелію грибів *Cyathus olla*. Виміряно вольт-амперні характеристики для різних відстаней між вістрям та металевим кільцем. Побудовано графік залежності оберненого опору від напруги, з якого визначено напругу запалювання коронного розряду (2.7±0.1) кВ. Проведено обробку спор *Aspergillus niger* на різних стадіях розвитку: на стадії проростання (обробка коронним розрядом через добу після висіву) та стадії спокою (обробка коронним розрядом в день висіву). Досліджено швидкість росту міцелію *Cyathus olla* в зразках, оброблених коронним розрядом, та контрольної групи. Досліджено вплив коронного розряду на картопляно-глюкозний агар до висадження в це середовище *Cyathus olla*. Чисті культури отримані з Колекції культур грибів FCKU Навчально-наукового центру «Інститут біології та медицини» Київського національного університету ім. Тараса Шевченка.