30th International Conference Ecological Truth & Environmental Research 2023

Proceedings

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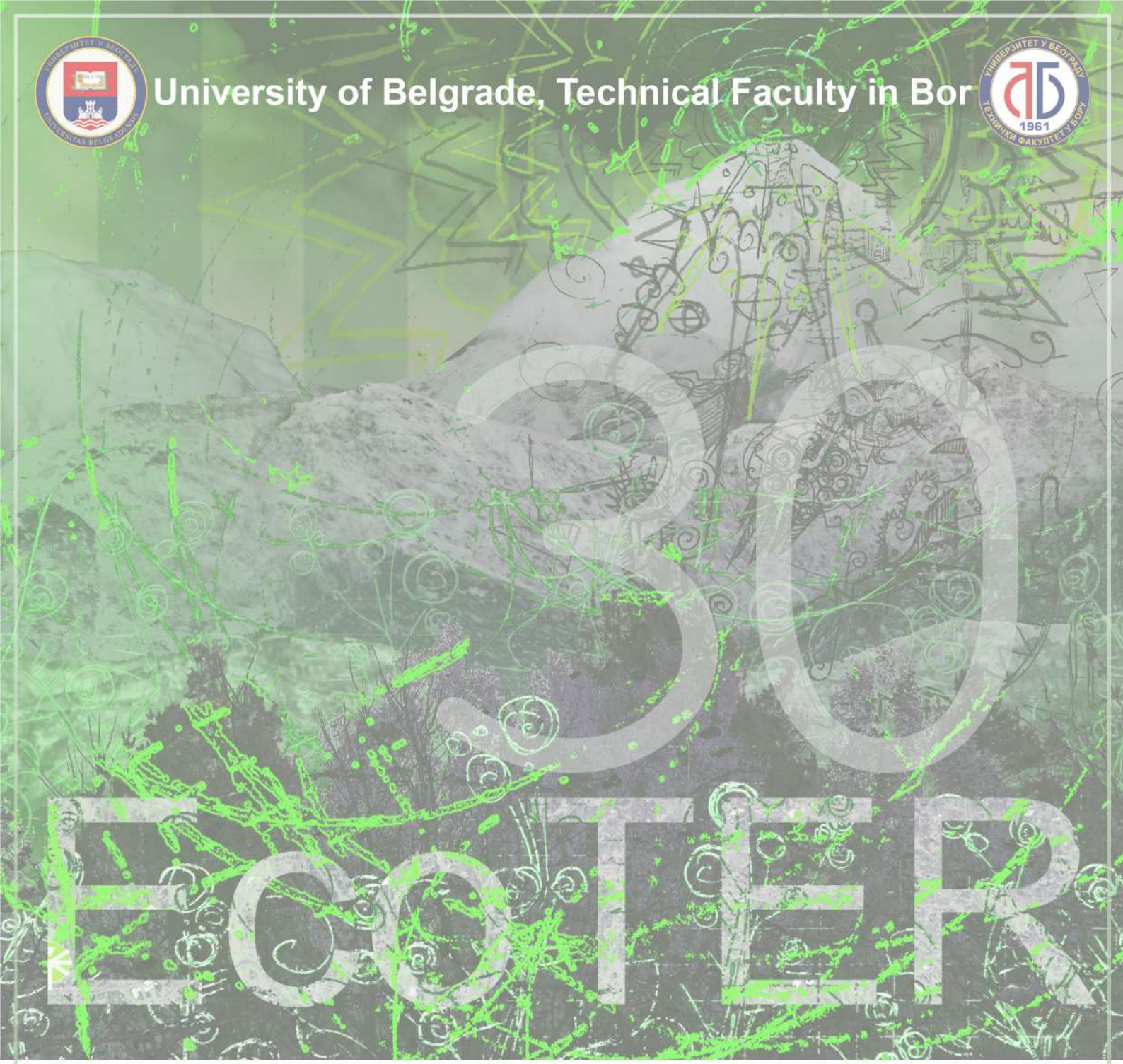

University of Belgrade, Technical Faculty in Bor

30th International Conference Ecological Truth & Environmental Research 2023

Proceedings

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PROCEEDINGS

30th INTERNATIONAL CONFERENCE

ECOLOGICAL TRUTH AND ENVIRONMENTAL RESEARCH – EcoTER'23

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PREFACE

The 30th international conference Ecological Truth & Environmental Research – EcoTER'23 kept three areas in focus: ecology, environmental protection and sustainable development. The conference will be held on Mt Stara Planina in hotel Stara Planina, Serbia, 20–23 June 2023. The monograph is published on the occasion of the 30th anniversary of the conference. On behalf of the scientific and organizing committee, it is a great honor and pleasure to wish all the participants a warm welcome to the conference.

The monograph is published on the occasion of the 30th anniversary of the conference.

We hope to convey the message of the conference, which is that a transformation of attitudes and behavior would bring the necessary changes. This is also an opportunity for the participants who are experts in this field to exchange their experiences, expertise and ideas, and also to consider the possibilities for their collaborative research.

The 30th international conference Ecological Truth & Environmental Research – EcoTER'23 is organized by the University of Belgrade, Technical Faculty in Bor, and co-organized by the University of Banja Luka, Faculty of Technology, the University of Montenegro, Faculty of Metallurgy and Technology – Podgorica, the University of Zagreb, Faculty of Metallurgy – Sisak, the University of Pristina, Faculty of Technical Sciences – Kosovska Mitrovica and the Association of Young Researchers, Bor.

These Proceedings 103 papers from the authors coming from the universities, research institutes and industries in 11 countries: Australia, USA, Brazil, Spain, Portugal, Libya, Italy, Bulgaria, Bosnia and Herzegovina, North Macedonia, and Serbia.

As a part of this year's conference, the 5th Student Session – EcoTERS'23 is being held. We appreciate the contribution of the students and their mentors who have also participated in the conference.

The support of the Gold donor and their willingness and ability to cooperate has been of great importance for the success of the EcoTER'23. The organizing committee would like to extend their appreciation and gratitude to the Gold donor of the conference for their donation and support.

We appreciate the effort of all the authors who have contributed to these Proceedings. We would also like to express our gratitude to the members of the scientific and organizing committees, reviewers, speakers, chairpersons and all the conference participants for their support to the EcoTER'23. Sincere thanks go to all the people who have contributed to the successful organization of the EcoTER'23.

Prof. Snežana Šerbula,

President of the scientific and organizing committee

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ESTIMATION OF THE ANTIFUNGAL ACTIVITY OF THE TWO DIFFERENT CARBON DOTS AGAINST *Aspergillus flavus*

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Abstract

Environmental occurrence of the pathogenic fungus Aspergillus flavus (A. flavus) has hazardous effects on the health status of plants, humans, and animals. It is important to explore novel antifungal agents to control the growth of this fungus. In this study, we aimed to evaluate the antifungal contact activity of two different types of carbon dots (CD_S) nanomaterials; the one was coated with vitamin B_{12} ($CD_S@VB_{12}$) and the other was obtained from folic acid (FA@CDs). We used fluorescence spectroscopy to obtain their fluorescence fingerprinting profile. The CDs were pipetted on the PDA medium with sterilised filter paper placed in the center of the Petri dish. Then, pure A. flavus cultures were subcultured on PDA and incubated at 25°C for 7 days, and the diameters of mycelium growth were daily evaluated. The percentage of inhibition by $CD_S@VB_{12}$ ranged from 38.09% to 60.9% and from 46.03% to 71.39% for the amounts of 30 µl and 50 µl, respectively. On the other hand, FA@CDs did not show any inhibitory effect on the first day, and after that more progressive growth of A. flavus was noticed. The obtained results showed that $CD_S@VB_{12}$ could be considered as a potential new antifungal material against toxic and pathogenic A. flavus.

Keywords: carbon nanodots, antifungal activity, *A. flavus.*

INTRODUCTION

Aspergillus spp. includes a variety of environmental filamentous fungi found in diverse ecological niches worldwide [1]. *Aspergillus flavus* (*A. flavus*), after *Aspergillus fumigatus,* is the most important Aspergillus species which leads to a variety of allergic reactions and infectious diseases in immunocompromised individuals [1–3]. *A. flavus* grows at temperatures in the range of 12 to 48°C, with an optimum temperature at 37°C, contributing to pathogenicity in humans. Mainly, clinical syndromes related to this fungus include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and others [2]. Furthermore, *A. flavus* produces aflatoxin B_1 (AFB_1) which have many hazardous health effects to human and animals [4], and has been classified as a first-class human carcinogenic compound [5]. The exposure of humans and animals to this toxic compound mainly occurs via the consumption of contaminated foods, as well as inhalation of toxigenic spores and by dermal contact [6]. The most commonly used methods for determination of aflatoxins are

high-performance liquid chromatography (HPLC) and enzime linked immunosorbent assay (ELISA) [7]. Further, optical methods, such as fluorescence spectroscopy, are widely used for non-invasive screening the aflatoxin-contaminated cereal seeds [8,9]. Different pre and post-harvest strategies are used for preventing and controling fungal growth and their toxic compounds such as aflatoxins [10–12]. Phytopathogenic fungi have mostly been controlled by cheap chemicals which are easily obtained and indiscriminate use [13]. That may cause several problems regarding environmental pollution, disease in humans and animals, ecological imbalances, as well as developing fungi resistance [13,14]. Among the Aspergillus species, *A. flavus* seems to be more virulent and resistant to antifungal drugs [2]. Therefore, there is an urgent need to control the growth of pathogenic fungus. To overcome the fungus resistance, it is crucial to develope novel antifungal agents [13,15]. It has been demonstrated that many kinds of essential oils obtained from plants or herbs exhibit antifungal effects against *A. flavus* [16,17]. Nanomaterials have received great attention in agriculture, medicine and other fields of science [18,19]. Nowdays, different types of nanomaterials are considered a good alternative to chemical fungicides for the growth control of phytopathogenic fungi [13,20]. Carbon-based nanoparticles are known as carbon dots (CDs). They are nanoclusters of amorphous carbon or composed of small crystalline structure, with sizes bellow 10 nm [21]. CD_S have wide applications due to their excellent chemical stability, easy surface modification, high water solubility, good biocompatibility, photoluminescence and low toxicity [21,22].

In this study, we aimed to estimate the contact effects of two different types of CD_S, one coated with vitamin B_{12} and the other synthesized from folic acids, on the growth of the *A. flavus* colony. To our knowledge, the analysed CD_S have not been tested before as antifungal agents on toxigenic fungus. Therefore, obtained results may provide a useful approach for further designing and exploiting carbon dots as new antifungal materials in environmental protection.

MATERIALS AND METHODS

Materials

Folic acid (≥97%), D-Lactose (≥98%), phosphoric acid (85 wt.%, 99.99%) were purchased from Sigma-Aldrich Química (Spain). The Ultrapure Millipore water was used for all measurements. The isolate *A. flavus* No. 4219 was used as a toxigenic strain, and potato dextrose agar (PDA) as growth culture media.

Synthesis of CD_S coated with vitamin B_{12} (CD_S@VB_{12})

A hydrothermal method was used for the sythesis of CD_S@VB_{12}. Briefly, 12 mg lactose was dissolved in 200 µl deoinised water, and than 10 mg vitamin B_{12} and 800 µl 85% H_3PO_4 were added in the Tefon-lined strainless steel vessel and heated at 150°C for 2 h [23].

Synthesis of CD_S obtained from folic acid (FA@CDs)

The synthesis of CD_S from folic acid (FA@CDs) was carried out according to work [24]. The detailed synthesis and characterisation of these CD_S were described in the previous studies [23,24].

Fluorescence spectroscopy

Fluorescence characteristics of CDS@VB_{12} and FA@CDs were determined by FL3-221 spectrofluorimeter (Jobin Yvon Horriba, France), equipped with 450 W Xe lamp. The fluorescence emission spectra of CDs were measured in a right-angle (RA) configuration. All measurements were done at room temperature. Experimental parameters for CD_S@VB_{12} and FA@CDs were done at excitation wavelengths 385 nm and 350 nm, respectively. The emission range for both types of CDs was 365 nm to 650 nm, with an integration time of 0.1 s and bandpass for excitation and emission slits around 2 nm.

Evaluation of the antifungal activities of CD_S@VB_{12} and FA@CDs

A schematic illustration of antifungal test is shown in Figure 1. The pure *A. flavus* culture were subcultured to three places around the filter paper (Whatman No. 4) on the potato dextorse agar (PDA) using a microbiological method. Aliquots of 30 µl and 50 µl of the analysed CD_S were pipetted on the sterilized round filter paper, that was placed in the center of the Petri dish. The PDA medium with sterilised round filter paper without addition of CQDs was used as control. Each CDs was tested in three replicates. Further, the Petri dishes were covered and incubated at 25°C for 7 days in dark. The diameters of mycelium growth were evaluated daily during seven days, and their percentage of inhibition (IG) was calculated by the following formula: IG (%) = [(C−T)/C] × 100 where C and T shows colony diameter of control and CD_S-treated group, respectively [16].

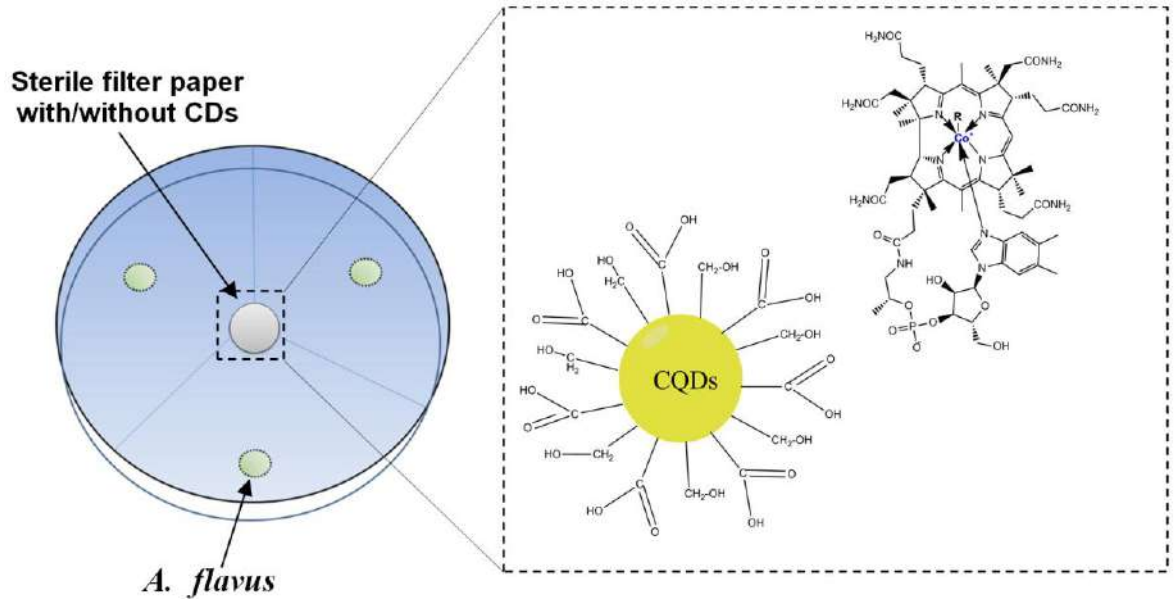

Figure 1 *Schematic illustration of antifungal contact test of A.flavus-CD_S@VB_{12}*

Statistical analyses

All data are expressed as mean values of the three repetitions per treatment. The differences were considered to be statistically significant at $p \leq 0.05$ according to non-parametric Mann-Whitney *U* test.

RESULTS AND DISCUSSION

Figure 2 and 3 shows fluorescence emission spectra of CDs@VB_{12} and FA@CDs, respectively. As displayed in Figure 2, CDs@VB_{12} exhibits two emission maxima at 450 nm and 560 nm in the range from 400 to 600 nm. This result is in agreement with our previous studies [23]. On the other hand, FA@CDs show one emission maximum with a position around 450 nm, similar to the results obtained in the previous study [24]. The observed difference in spectral characteristics results from the difference in their respective structures.

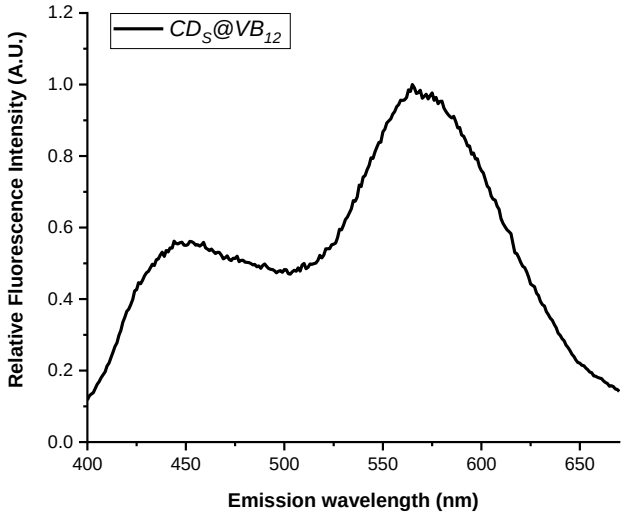

Figure 2 *Fluorescence emission spectrum of CDs@VB_{12}, after excitation at 385 nm*

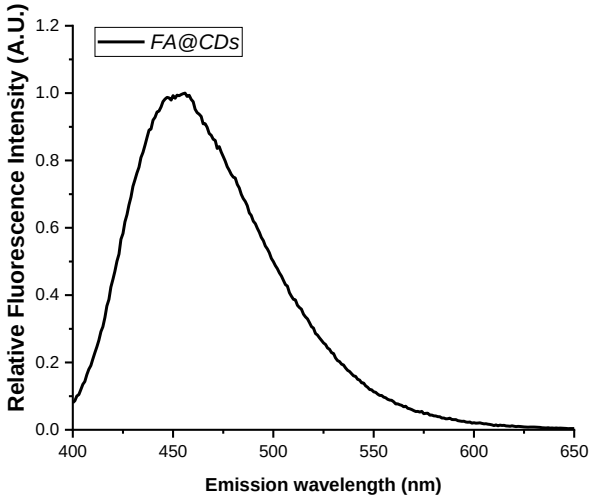

Figure 3 *Fluorescence emission spectrum of FA@CDs, after excitation at 350 nm*

Estimation of contact effect between CDs and *A. flavus*

Representative pictures of the colony morphology of *A. flavus* on PDA without (Figure 4a) and with the treatment (Figure 4b) after seven days of incubation are shown in Figure 4. The results of macroscopic characterisation show yellow-green colour of mycelia of the *A. flavus* [25]. The results related to the diameters of mycelium growth (mm) during seven days are presented in Table 1 and Table 2. During the studied period, one can see that increasing the dose of CDs@VB_{12} leads to a significant decrease of the diameters of mycelium growth. On the other hand, the FA@CDs did not show any significant inhibition effect on fungi growth after the first day. After that, more progressive growth of *A. flavus* was observed. Obtained results indicate that mycelium growth was stimulated with FA@CDs, and is not dependent on the applied doses.

a) *b)*

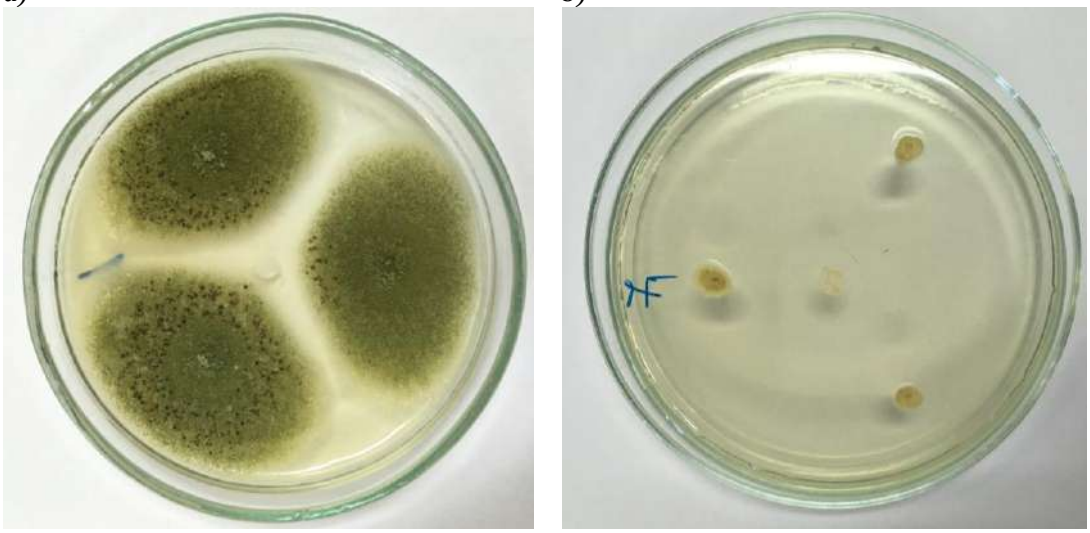

Figure 4 *Representitive pictures of the colony morphology of A. flavus (Isolate No.4219) on PDA a) without and b) with the treatment*

Table 1 *Exposure time of CD_S@VB12 and A.flavus contact during seven days*

Exposure time (days)	**Diameter of mycelium growth (mm)**		
	Control	**CD_S@VB_{12} (30 µl)**	**CD_S@VB_{12} (50 µl)**
1st	7	4.3±0.33	3.8±0.38
2nd	18.3±0.57	9.5±0.6938	7.4±1.1097
3rd	27.6±0.5773	12.7±0.962	9.4±1.2619
4th	35.83±0.763	15.22±1.07	11.3±1.45
5th	43.66±0.577	17.4±0.693	12.61±1.397
6th	49.33±1.155	19.22±1.17	14±1.764
7th	54±3	21.1±0.694	15.4±1.895

**Data are means ± standard (± SD) deviation of the three replicates (n=3).*

Table 2 *Exposure time of FA@CDs and A.flavus contact during seven days*

Exposure time (days)	Diameter of mycelium growth (mm)		
	Control	*FA@CDs* (30 µl)	*FA@CDs* (50 µl)
1st	7#	7±0.33#	7#
2nd	18.3±0.57	20	20
3rd	27.6±0.5773	29.2±0.192	29.2±0.19
4th	35.83±0.763	38.55±1.07	37.8±0.96
5th	43.66±0.577	47.8±0.192	45.6±0.33
6th	49.33±1.155	55.88±0.69	54.3±0.333
7th	54±3	61.4±0.838	60.3±0.66

**Data are means ± standard (± SD) deviation of the three replicates (n=3);*
 # – no significant difference.

As shown in Figure 5, the percentage inhibition by CDs@VB_{12} on *A. flavus* varied from 38.09% to 60.9% for 30 µl, and 46.03% to 71.39% for 50 µl. The calculated IG for *A. flavus* was 1.2-fold higher for 50 µl CDs@VB_{12} than for 30 µl. The studied CDs@VB_{12} reached a maximum value of IG against *A. flavus* on the 5th day. However, similar values of IGs were found with prolonged time exposure (6th and 7th day). The results imply that applied CDs@VB_{12} exhibited antifungal activity to fungus *A. flavus*. Studied FA@CDs did not show inhibitor effects on *A. flavus.* Moreover, it was obvious that after the first day, this CDs lead to stimulative effects of fungus growth (Figure 6). It has been reported that the effect of CDs on microorganisms, and their interaction, appears to be dependent on numerous factors such as surface chemistry, composition, size, and shape among others [21,22]. Further, the mechanism of antimicrobial effects of CDs could be activated under visible natural illumination to promote the generation of high reactive oxygen species [26].

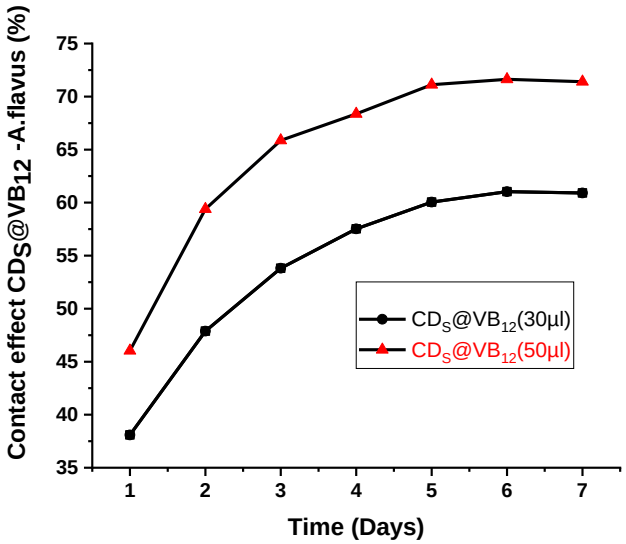

Figure 5 *Contact effect of CD_S@VB_{12} on A. flavus growth during 7 days exposure*

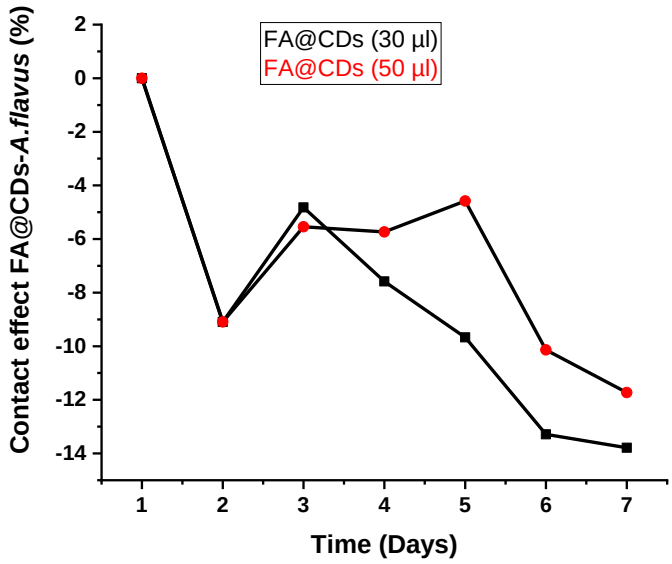

Figure 6 *Contact effect of FA@CDs on A. flavus growth during 7 days exposure*

CONCLUSION

This study provides new information about the contact effects of the two types of carbon dots on *A. flavus*. Our results imply that antifungal effects of studied carbon dots depend on their compositions; however, further research will be needed for a deep understanding of the mechanisms of their actions. The FA@CDs did not show effectiveness against growth *A. flavus*, on the other hand, the CD_S@VB_{12} showed a good inhibitory effect and thus could be considered a promising antifungal agent, with its potential application in agriculture, food and medicine.

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