THE INFLUENCE OF ULTRAVIOLET LIGHT ON PATHOGENICITY OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* (BALSAMO) VUILLEMIN TO THE EUROPEAN CORN BORER, *OSTRINIA NUBILALIS* HBN. (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

The influence of different doses of ultraviolet (UV) light on the pathogenicity of entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin to the European corn borer, *Ostrinia nubilalis* Hbn., and radial growth of fungus was studied in laboratory conditions. The suspensions of *B. bassiana* isolate SK99 were exposed to UV light. Four different doses of UV light were used in the experiment. The distance between exposed suspensions and UV light source was 0.3 m. Exposure duration was 15, 30, 45 and 60 minutes (as A, B, C and D variants). Control variant SK99 and obtained variants SK99A, SK99B, SK99C and SK99D were cultivated 21 days on Sabourard-dextrose agar. The larvae of *O. nubilalis* were infected with dry powder consisted of mycelia and spores from fungus cultures. During 10 days, the mortality of infected larvae was evaluated. It was ascertained that UV light exposition significantly influenced the mortality effect of *B. bassiana* isolates to *O. nubilalis* larvae. Variant SK99C showed the highest level of infectivity. Radial growth of UV variants was slower with rising time of exposure. The best ability to grow possessed non-irradiated isolate SK99 and the worse variant SK99D. The difference between these two variants was significant.

KEY WORDS: Beauveria bassiana, UV light, mutagenesis, Ostrinia nubilalis

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PATHOGENICITY OF ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA (BALSAMO) VUILLEMIN

DETAILED ABSTRACT

V laboratórnych podmienkach sa sledoval vplyv rôznych dávok ultrafialového (UV) žiarenia na radiálny rast a patogenicitu entomopatogénnej huby *Beauveria bassiana* (Balsamo) Vuillemin vo vzťahu k vijačke kukuričnej, *Ostrinia nubilalis* Hbn.. Suspenzie izolátu *B. bassiana* označeného SK99 boli vystavené UV žiareniu. Zdrojom UV žiarenia bola baktericídna výbojka Philips TUV 30 W s hladinou radiácie 83 $\mu W.s/cm^2$ a vlnovou dĺžkou 253,7 nm. V pokuse sa použili štyri rôzne dávky UV žiarenia. Vzdialenosť medzi suspenziami a zdrojom UV žiarenia bola 0,3 m. Dĺžka expozície bola 15, 30, 45 a 60 minút (varianty A, B, C a D). Kontrolný variant SK99 a získané varianty SK99A, SK99B, SK99C a SK99D sa kultivovali 21 dní on Sabourard-dextrózovom agare. Larvy *O. nubilalis* boli infikované práškom zloženým z mycélia a spór z kultúr entomopatogénnej huby. Počas 10 dní sa hodnotila mortalita infikovaných lariev.

Všetky varianty si zachovali virulenciu proti larvám škodcu. Desať dní po infekcii lariev bola minimálna úmrtnosť (varianty SK99A a SK99B) 86,67%. Potvrdilo sa, že vystavenie UV žiareniu preukazne ovplyvnilo účinnosť *B. bassiana* proti larvám *O. nubilalis*. Variant SK99C mal najväčšiu infekčnú schopnosť. Po desiatich dňoch od jeho aplikácie dosiahla mortalita lariev 100%. Infekčná schopnosť spór z variantu SK99C bola preukazne vyššia ako v prípade variantov SK99A a SK99B. Medzi kontrolou (SK99) a variantom SK99C bol rozdiel v infektivite nepreukazný. S predlžujúcim sa časom expozície bol radiálny rast UV variantov bol pomalší. Najlepšiu schopnosť rastu mal pôvodný variant nevystavený UV žiareniu, najhoršiu variant vystavený UV žiareniu najdlhšie t. j. SK99D. Rozdiel medzi týmito dvomi variantami bol preukazný.

Všeobecne sa mutagenéza pomocou UV žiarenia často používa na produkciu mutantov húb. Výsledky práce potvrdili, že je reálne používať UV žiarenie na tvorbu nových kmeňov entomopatogénnej huby *B. bassiana.* Experiment s larvami sa nerobil hneď po aplikácii žiarenia, ale až o 21 dní. Predpokladá sa, že aj keď mohla prebehnúť autoreparácia poškodenej genetickej informácie, 21 dní je dosť dlhá doba na stabilizáciu genetických zmien.

INTRODUCTION

The selection of entomopathogenic microorganism is an important link in the use of effective biopreparations for the protection of plants from insect pests of agricultural crops [29].

The entomopathogenic filamentous fungus *B. bassiana* attacks many species of insects [11,15], including the European corn borer, *Ostrinia nubilalis* Hbn. Many authors provided experiments in an interaction between *O. nubilalis* population and *B. bassiana* in the field and laboratory conditions. They found out that the fungus is very significant agent to suppress survival of pest's grubs [1, 2, 3, 6, 10, 18, 23].

The influence and interactions between UV radiation and properties of fungus *B. bassiana* were evaluated in a lot of works. Tobar et al. [27] selected *B. bassiana* isolates for resistance to UV light. Their Isolate Bb9218 was resistant to 10, 30 and 60 min exposures to UV light and it showed the highest *Hypothenemus hampei* percentage mortality throughout the evaluation time and was significantly different to the other treatments. Teng [26] ascertained that a long time exposure very significantly suppressed fructification of fungus, but on the other side different strains and isolates had different level of sporulation in addiction on different time of UV light exposure. Conidia survival of *B. bassiana* is a function of time exposure characterised by S-curve. Exposure of the fungus to UV light can change the form of its nutrition from prototrophy to auxotrophy.

Level of production of proteolytic, lipolytic and chitinolytic enzymes as well as organic acids recorded in exposed cultures was different from a level of non-irradiated ones caused different level of production of metabolites like proteolytic, lipolytic and chitinolytic enzymes and organic acids [7].

Volume of reversion mutant escalated with rising level of UV light exposure [17]. Kirsanova and Usenko [16] tested the infectivity of *B. bassiana* to drozophila (*Drosophila melanogaster)* and elicited that the influence of UV irradiation is asymmetrical.

UV light exposure to *B. bassiana* cultures can interfere with their physiological properties.

Hegedus and Khachatourians [12, 14] clarified relationship between UV radiation and influence of temperature on morphological, physiological and biochemical properties of the fungus. They affirmed non-linear dependence between pathogenecity of fungus and doses of UV exposure.

There are known positive results in the breeding of *Beauveria* species with the application of mutagens. Tests showed that laser treatment of *Beauveria* spores increased the mortality rate of pine moths (*Dendrolimus* sp.). Stable strains of *Beauveria* were selected which were more toxic to pine moths, grew faster and produced more spores, with a greater resistance to UV light [28]. Two strains of *B. bassiana*, isolated from *Carposina sasakii* and the scarabaeid *Holotrichia parallela*, were selected for their substantial spore production. Irradiation produced 3 mutant strains with increased spore production and increased infectivity towards *C. sasakii.* These new characters were retained in mass production of the mutants and field test results are described as satisfactory [30].

Because black-pigmented conidia were more tolerant to stimulated sunlight, it was recommended that it may be possible to incorporate, by selection or genetic engineering, this phenotypic character into potential mycopesticides [14].

The aim of this work was to determine the influence of different doses of UV light to the pathogenicity of *B. bassiana* to *O. nubilalis* larvae and its radial growth on Sabouraud-Dextrose agar.

MATERIAL AND METHODS

B. bassiana, isolate SK99, (Department of Plant Protection, Slovak Agricultural University in Nitra), and larvae of *O. nubilalis* originated from Slovakian population bred in laboratory for more than three generations on semi-artificial diet [22] were used in the experiment.

The fungus was grown on Sabouraud-Dextrose agar (SDA) at 25ºC for 21 days. Conidia of the 21-day culture of the fungus served as the material for irradiation. The suspension of conidia filtered through cotton and sterile gauze in ceramic filter was used in the experiment. Portions of 20 ml of an aqueous suspension containing 10^6 per ml conidia were placed in Petri dishes and irradiated. The source of ultra-violet light rays was a bactericidal lamp Philips TUV 30 W with a dose rate at the level of irradiation 83 $\mu W.s/cm^2$ and wavelength of 253.7

nm. The distance between exposed suspensions and UV source was 0.3 m. Exposure duration was 15, 30, 45 and 60 minutes (as A, B, C and D variants). The irradiated (marked as SK99A, SK99B, SK99C, SK99D) and non-irradiated (SK99) suspensions were inoculated on SDA in Petri dishes and incubated at 25ºC for 21-days.

Petri dishes with incubated *B. bassiana* suspensions were used for pathogenicity tests and for radial growth analysis.

Each irradiated *B. bassiana* suspension was tested in 3 replications (Petri dishes) for its patogenicity against *O. nubilalis* larvae. Each Petri dish contained 10 fifth-instar larvae. Control variant was organised in the same way. The larvae were put into the Petri dishes containing the fungus for 5 minutes. Then they were removed in dishes with segmented maize leaves and maintained at 25°C. The mortality of larvae was recorded at 24 hours intervals during ten days. Dead larvae were put into Petri dish on the moistured filter paper to confirm the growth of *B. bassiana*. Analysis of variance was used for statistic evaluation of the experiment.

Disks 5 mm in diameter were taken from incubated suspensions for radial growth analysis of *B. bassiana* variants (SK99A-SK99D) and control variant (SK99).
They were put on SDA in Petri dishes and cultivated 6 days at 25°C. Each experiment was performed in 3 repetitions.

Following equation was used for calculation of daily radial growth of fungus cultures:

$$G = G_a - G_b,$$

G = radial growth,
a = time of measurement
b = a – 24

Analysis of variance was used for used for statistic evaluation of the experiment.

RESULTS

Insect test

Table 1 shows the mortality of the European corn borer larvae caused by UV light variants of *B. bassiana*. All of the variants tested were virulenced against the pest larvae. Ten days after larvae had been treated by *B. bassiana*, the minimum of their mortality was 86.67 % by variants SK99A and SK99B. The 45 minutes exposure by UV light positively influenced the infectivity of *B. bassiana* (variant SK99C). Variant SK99C had the highest infectivity after 10 days and mortality of tested larvae was 100.0 %. The analysis of variance revealed significant differences between variants SK99A, SK99B and SK99C after 10 days. The relation between level of mortality and doses of exposure was non-linear.

Table 1. Infectivity of ultra-violet light variants of *Beauveria bassiana* (SK99A, B, C, D) toward *Ostrinia nubilalis* larvae. Exposure of ultra-violet light duration was 15, 30, 45 and 60 minutes (as A, B, C and D variants). For details see material and methods. Values are given at the average of three repetitions, each with 10 larvae. Means marked with the same letter are not significantly different (P = 0.05, Tuckey´s multiple range test).

Variant	% mortality of larvae									
	1.day	2.day	3.day	4.day	5.day	6.day	7.day	8.day	9.day	10.day
SK99	0	3.33	13.33	23.33	30.00	56.67	60.00	63.33	73.33	93.33 bc
SK99a	0	6.67	16.67	30.00	40.00	63.33	73.33	80.00	80.00	86.67 b
SK99b	0	10.00	13.33	20.00	36.67	43.33	43.33	56.67	63.33	86.67 b
SK99c	0	16.67	23.33	33.33	40.00	76.67	80.00	80.00	96.67	100.00 c
SK99d	0	3.33	13.33	20.00	36.67	60.00	63.33	76.67	86.67	93.33 bc
Control	0	0	0	0	0	0	0	0	0	0 a

Radial growth

Exposure of the isolate SK 99 to different UV light doses caused decreased fungus ability to growth (Table 2). The growth all of UV light variants lag behind control variant SK 99. The level of fungus growth was continually depleted with the escalated doses of exposure. The highest significant differences were between non-exposed SK 99 and exposed SK 99 B and SK 99 D.

The differences in the growth rate are illustrated in Fig. 2. The fastest growth was achieved by non-irradiated variant SK 99 and, similarly like radial growth ability, was decreased with increased UV doses.

Table 2. Growth of *Beauveria bassiana* variants on Sabouraud-Dextrose agar in mm. Average – average daily accessories of mycelium. Exposure of ultra-violet light duration was 15, 30, 45 and 60 minutes (as A, B, C and D variants). For details see material and methods. Means marked with the same letter are not significantly different (p = 0.05 and p= 0.01; Tuckey´s multiple range test).

Variant	Average	95 % (p= 0.05)	99 % (p=0.01)
SK 99	1.84	A	A
SK 99 A	1.74	B	A B
SK 99 B	1.69	B C	B
SK 99 C	1.65	C	B C
SK 99 D	1.55	D	C

DISCUSSION

It is interesting that increasing doses of UV light decreased the infectivity of fungus by the finite level (A, B - level). But the fungus gained the highest level of patogenicity at C – exposure (45 minutes). When the exposure duration increased to 60 minutes, the mortality of grubs fallen down.

Kirsanova and Usenko [16] discovered that with rising dose of UV-rays, the yield of variants of the fungus increased with reduced virulence, but the relation between fungus infectivity and UV irradiation was non-symmetrical. The curve was fluctuated, similar like in our case. However, the test insect was drozophila (*Drosophila melanogaster).*

But, different strains and isolates of *B. bassiana* can not have the same response and susceptibility to UV light influence [26].

Müller-Kögler [21] discovered that conidia of fungus were not damaged after long UV exposure (after 60 minutes). Conversely, longer exposures (1.5 hour) conducted to faster growth and better spore production of fungus. Fructification of fungus decreased after 4.5 hours of UV light irradiation and after 30-48 hours was completely stopped.

According to Levitin et al. [17], the dependence of the survival of *B. bassiana´*s conidia to UV rays is characterised by a sigmoid curve. The survival of conidia decreases with increased doses of UV lights. With the frequency of mutations it is similar, but exist threshold here. Amount of mutants fell down after exceeding this threshold. We discovered that the increasing time of exposure caused reduced radial growth and growth rate of variants. That is in agreement with conidia survival described above.

In the experiment of Farques et al. [9], conidia from 65 isolates of *B. bassiana*, 23 of *Metarhizium anisopliae*, 14 of *Metarhizium flavoviride* and 33 isolates of *Paecilomyces fumosoroseus* were irradiated by artificial sunlight (295 to 1,100 nm at an ultraviolet-B irradiance of 0.3 Wm^2) for 0, 1, 2, 4 and 8 hours. Exposure for 2 h or more was detrimental to all isolates tested. Their results are the most similar to ours. In our preliminary trial it was necessary to find correct exposure time. It is a reason why we used in our experiment not longer than 60 minutes exposure.

It is difficult to compare the results concerned to correct time of UV light necessary for induction of mutations. Sharma et al [24] found that when *B. bassiana* was exposed to UV 4 hours, toxin production was higher probably due to some cell mutation. Morley et al. [20] used conidia of 14 isolates of the entomopathogenic fungi *B. bassiana*, *M. flavoviride* and *M. anisopliae* which were exposed to 4, 8, 16 and 24 hours of UV light from a sunlight simulator at 40 degree. Conidial viability decreased markedly in all isolates with increasing UV exposure. Germination ranged between 10 and 50% after 24 hours exposure to UV.

Generally, fungal mutants are often generated by UV-mutagenesis [4, 5, 19, 25]. Our results show that it is real possibility to use UV irradiation for development of new *B. bassiana* strains. We did not treat growth experiments next to irradiation, but after

21 days. It could also come to autoreparations of damaged genetic information, but 21 days seems to be enough long time for stabilisation of genetic changes in developed UV variants.

The reduction of growth ability of fungus could be caused by many reasons. We tested the growth ability, but other authors attained the change manner in nutrition, from prototrophy to auxotrophy [17]. It was recorded production of new metabolites like enzymes, toxins, pigments etc. or their reduction [7, 12, 13, 17]. They also determined differences in quality and quantity of fungus metabolites. However no relation between germination rates, radial growth, conidial production, medial lethal time and other colony characteristics of *B. bassiana* strains and their virulence was found during the study in Argentine [8].

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