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Liquid production of entomopathogenic fungi and ultraviolet radiation and temperature effects on produced propagules

Produção líquida de fungos entomopatogênicos e efeitos da radiação ultravioleta e da temperatura sobre os propágulos produzidos

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ABSTRACT: The purpose of this paper was to evaluate the liquid culture media for the production of *Metarhizium anisopliae* (IBCB 425) and *Beauveria bassiana* (IBCB 66), as well as the tolerance of these seedlings to the ultraviolet action and to the temperature. Twelve treatments composed of combinations between carbon and nitrogen concentrations were assessed. In order to determine the effect of ultraviolet radiation, plates with blastospores were exposed to it for 25 and 50 seconds. To determine the temperature effect, blastospores from culture media were exposed to 20, 25, 30 and 35ºC. For the virulence experiments, caterpillars of *Diatraea saccharalis* were sprayed with 2 mL of fungal suspension with the aid of a Potter tower. The best media for *M. anisopliae* are 16.00 g (carbon) + 7.00 g (nitrogen) and 14.40 g (carbon) + 7.00 g (nitrogen), whereas for *B. bassiana*: 20.00 g (carbon) + 6.30 g (nitrogen) and 20.00 g (carbon) + 7.00 g (nitrogen). The longer the exposure to ultraviolet radiation, the smaller the number of colonies. At 35ºC, there is a significant decrease in the formation of colonies. The produced seedlings of fungi are pathogenic to *D. saccharalis*.

KEYWORDS: *Beauveria bassiana*; *Metarhizium anisopliae*; fungi production; microbial control; sugarcane borer.

RESUMO: O objetivo deste trabalho foi avaliar os meios de cultura líquidos para a produção de *Metarhizium anisopliae* (IBCB 425) e *Beauveria bassiana* (IBCB 66), bem como a tolerância desses propágulos à ação dos raios ultravioleta e à temperatura. Foram avaliados 12 tratamentos compostos pelas combinações entre carbono e nitrogênio. Para determinar o efeito da radiação ultravioleta, placas com blastósporos foram expostas à radiação por 25 e 50 segundos. Para conhecer o efeito da temperatura, os blastósporos de meios de cultura foram expostos a temperaturas de 20, 25, 30 e 35ºC. Para os experimentos de virulência, as lagartas *Diatraea saccharalis* foram pulverizadas com 2mL de suspensão fúngica, com o auxílio de uma torre de Potter. Os melhores meios para *M. anisopliae* são 16,00 g (carbono) + 7,00 g (nitrogênio) e 14,40 g (carbono) + 7,00 g (nitrogênio), e para *B. bassiana*, 20,00 g (carbono) + 6,30 g (nitrogênio) e 20,00 g (carbono) + 7,00 g (nitrogênio). Quanto mais tempo de exposição à radiação ultravioleta, menor o número de colônias. Aos 35ºC, há uma diminuição significativa na formação de colônias. Os propágulos produzidos pelos fungos são patogênicos a *D. saccharalis*.

PALAVRAS-CHAVE: *Beauveria bassiana*; *Metarhizium anisopliae*; produção de fungos; controle microbiano; broca da cana-de-açúcar.

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INTRODUCTION

The liquid media have been increasingly used for the large-scale fungi production because they allow better control of physical and nutritional conditions required by the microorganism (Jackson et al. 1996; Jackson, 1997). However, the industrial-scale entomopathogenic fungi production represents a critical and limiting step in the development of a microbial control program for a particular pest. Thus, the search for new methods of production systems is very important to control microbial pests economically feasible when applied to large areas (Tanzini, 2002).

According to Leite et al. (2003), the diversity of carbon (C) and nitrogen (N) sources has been widely exploited in the development of culture media, especially the complex one made by natural products. Nevertheless, more recent studies have shown that the ratio C : N is also an important factor to be considered in the development of culture media, especially of liquid medium that aims to produce submerged forms.

More susceptible to conditions in the field, entomopathogenic fungi are exposed to biotic and abiotic factors that influence their survival and propagation, as well as host infection (Goettel et al., 2000). Among abiotic factors, solar radiation is the most important (Fargues et al., 1996; Braga et al., 2001a; Cagan; Svercel, 2001) for being able to inactivate conidia and cause lethal damage to DNA and mutations (Nicholson et al., 2000). In general, the effects of ultraviolet (UV) radiation reduce the fungal efficiency in the field (Braga et al., 2001b). It is also worth mentioning temperature as another factor that acts on pathogens and affects the production, storage stability, and pathogenicity under field conditions. This factor becomes even more important in view of the pathogens inability of protecting themselves from temperature fluctuations through physiological systems (Alves, 1982).

Thus, the objective of this study was to evaluate the different culture media on the net production of fungal seedlings of *Metarhizium anisopliae* (*M. anisopliae*) and *Beauveria bassiana* (*B. bassiana*), and to investigate the effect of UV radiation and of different temperatures on the produced seedlings, as well as the virulence of fungi to *Diatraea saccharalis* (*D. saccharalis*).

MATERIAL AND METHODS

The experiments were carried out at the Laboratory of Biological Control of the Biological Institute in Campinas, State of São Paulo, Brazil.

Strains of *M. anisopliae* (IBCB 425) and *B. bassiana* (IBCB 66) are found deposited in the Collection of Entomopathogenic Microorganisms "Oldemar Cardim Abreu," held at the Laboratory of Biological Control. IBCB 425 was obtained from a soil sample from Iporanga, in São Paulo, and IBCB 66 comes from the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae), collected in São José do Rio Pardo, also in São Paulo. The strains are found stored in a freezer at -20ºC as pure conidia, packed in plastic tubes like "Eppendorfs." For the bioassays, fungal strains were transferred in Petri dishes (9 cm in diameter) containing PDA (Potato Dextrose Agar) culture medium (200 g of potato, 20 g of agar, and 20 g of dextrose in 1,000 mL of distilled water).

In the laminar flow hood, each fungus was inoculated at three points on the plates by using a platinum loop, which was previously flambéed. The plates were kept in a BOD (Biochemical Oxygen Demand) incubator, at 25 ± 1ºC, RH (Relative Humidity) 70 ± 10% and 12-hour photophase, for ten days. The produced conidia were removed from the surface of the culture medium, with the aid of a metal spatula, which had been previously flambéed for preparing the suspension in sterile water with wetting agent (Tween® 80 – 0.1 mL^{-1}) containing 1 x 10^8 conidia mL^{-1}. The prepared suspensions were used for fungi net production.

A total of 12 treatments with six replicates for each was assessed, consisting of combinations between the concentrations of C in the form of D-glucose anhydrous (40% of C), and sucrose (42.11% of C) with N in the form of brewer's yeast (7.31% N) per liter of medium. The proportions were based on the work done by Sano (2005), as seen in Table 1.

Each treatment consisted of six Erlenmeyer flasks containing 100 mL of the medium, sealed with hydrophobic cotton cap covered by aluminum foil, and autoclaved at 1 atm, at 120ºC, for 20 minutes. With a pipette, it was added 1 mL of the suspension at a concentration of 1 x 10^8 conidia mL^{-1} in each vial, in a vertical laminar flow hood. Then, the flasks were put to continuous agitation at 40 rpm, at 26 ± 1ºC and in a 12-hour photoperiod, in which they remained for four, six, and eight days. The experiments were divided into two parts, due to the capacity to accommodate Erlenmeyer shakers flasks. After statistical analysis, the best media of each part were selected.

Concentration of blastospores

Measurements of blastospores were performed at four, six, and eight days after inoculation by taking, in aseptic conditions (laminar flow), a sample of 5 mL/vial for counting the blastospores. From this sample, it was added 1 mL for 9 mL of sterile water and wetting agent (Tween 80®). The suspension was quantified in Neubauer chamber, under microscope (400 times magnification). To assess the quality of the fungus with regard to the presence of contaminants, two samples of 0.1 mL in each flask were transferred to Petri dishes containing PDA culture medium. The plates were kept in a BOD incubator, at 25 ± 1ºC, RH 60 ± 10%, and 12-hour photophase for a 7-day period. Contaminated vials were discarded.

Table 1. Amounts of carbon and nitrogen sources to prepare 1.0 L of each culture medium.

Treatment (40% C + 7,31% N)		C source (g)	N source (g)
		D-glucose anhydrous	Brewer's yeast
1	16.00 g of C + 6.30 g of N	40.00	86.13
2	16.00 g of C + 7.00 g of N	40.00	95.70
3	16.00 g of C + 7.69 g of N	40.00	105.27
Treatment (42,11% C +7,31% N)		**C source (g)**	**N source (g)**
		Sucrose	**Brewer's yeast**
4	20.00 g of C + 6.30 g of N	47.50	86.13
5	20.00 g of C + 7.00 g of N	47.50	95.70
6	20.00 g of C + 7.69 g of N	47.50	105.27
Treatment (40% C + 7,31% N)		**C source (g)**	**N source (g)**
		D-glycose anhydrous	**Brewer's yeast**
7	14.40 g of C + 7.00 g of N	36.00	95.70
8	16.00 g of C + 7.00 g of N	40.00	95.70
9	17.60 g of C +7.00 g of N	44.00	95.70
Treatment (42,11% C +7,31% N)		**C source (g)**	**N source (g)**
		Sucrose	**Brewer's yeast**
10	18.00 g of C + 7.00 g of N	42.75	95.70
11	20.00 g of C + 7.00 g of N	47.50	95.70
12	22.00 g of C + 7.00 g of N	52.25	95.70

C: carbon; N: nitrogen.

The counting of colonies

Ultraviolet

After quantifying blastospores, the effect of UV radiation on the plants was evaluated by putting them in 9 cm plastic plates containing BDA and pentabiotic (0.5 $g.L^{-1}$) – 100 mL of each treatment/plate, with the aid of a micropipette in laminar flow. Then, the plates were subjected to a germicidal lamp (UV radiation of 253.7 nm) — 25.0 cm far from the radiation source — and exposed to radiation for 25 and 50 seconds. A control treatment without radiation exposure was also performed. The plates were kept in BOD incubator at 25ºC, for three days. After this period, the colonies of *M. anisopliae* and *B. bassiana* formed on the plates were counted.

Temperature

The same methodology as in the previous item was used to evaluate the temperature effect. The treatments were exposed to different ones: 20, 25, 30, and 35ºC.

Virulence to *D. saccharalis*

Five treatments with eight replications and ten caterpillars were used, totaling 80 larvae per treatment and 400 others for the virulence experience. The third-instar larvae of *D. saccharalis* were obtained from a population reared in the laboratory of São João mill in Araras, São Paulo, Brazil. Each batch of 10 larvae was placed on a Petri dish of 12 cm in diameter in order to be treated with the aid of a Potter tower. They were sprayed with 2 mL of the fungal suspension of each treatment, at a concentration of 1.0 x 10^7 conidia mL^{-1}. The insects of control treatment were sprayed with 2 mL of sterile water.

The wetting agent was added in every treatment (0.01% Tween 80®). Later, the larvae were transferred to plastic pots of 6.5 x 5.0 cm in diameter, with a screw cap, after fasting for a period of 24 hours. After such time, some sugarcane stalks of 3 cm were offered.

Each dead insect was washed in 70% alcohol and distilled water for surface disinfection. The insects were transferred then to plastic plates containing cotton soaked in water to form a moist environment. The plates were stored in an incubator at 25 ± 1ºC, with 12-hour photophase, and relative humidity of 70 ± 10%. The confirmation of mortality caused by the pathogen was achieved with this procedure, by observing mycelial growth and conidiogenesis on insect cadaver.

Statistical analysis

A randomized experimental design was used, and the analysis was performed with the ESTAT (Statistical Analysis Systems) software, developed in FCAV/UNESP in Jaboticabal, São

Paulo, Brazil. The productivity average of blastospores was subjected to variance analysis by F test at a 5% probability, and the averages compared by Tukey's test at a significance level of 5%. Some analyses demanded data transformation.

RESULTS AND DISCUSSION

Production in net medium of *M. anisopliae* and *B. bassiana*

Concentration of blastospores, i.e. the sum of the blastospores production (x 10^8 mL^{-1}), was made in three evaluations. Then, the two most productive culture media of each stage were chosen (Tables 2 and 3).

The best media of each of the two steps for *M. anisopliae* and *Beauveria bassiana* (Table 4) were selected. For the first, the best culture media showed D-glucose anhydrous (C source) in its composition, and for the latter, the best culture media presented sucrose (C source) in it. It was noticed that both treatments showed 10% more and 10% less of D-glucose anhydrous, and the same amount of brewer's yeast (Table 4).

With regard to *B. bassiana* productivity, it must be stated that the 11th treatment (20.00 g C + 7.00 g N) had the greatest amount of blastospores at the 4th day of evaluation (Table 4). Thus, the productivity was distributed throughout the analysis — except on day 4, which stood out from others. Also, both treatments selected for *B. bassiana* have the same amount of sucrose as well as 10% more and 10% less of brewer's yeast. Bearing in mind that the liquid media selected to *M. anisopliae* and *B. bassiana* have a larger amount (in grams) of N in its composition, this study has taken the brewer's yeast as a N source, while Sano (2005) has considered the yeast extract. From an economic standpoint, the former is more advantageous than the latter, presenting itself as a viable alternative of N source.

It was assessed that the best media to produce blastospores are formed by a lower concentration of N and a

Table 2. Average concentration of *Metarhizium anisopliae* (IBCB 425), obtained from liquid culture medium with different concentrations of nitrogen and carbon at four, six and eight days after inoculation (at 26 ± 1°C and 12-hour photophase).

Treatment (g)	Average concentration (x 10^8 blastospores/mL^{-1})					
	4th day	6th day	8th day	S (3 evaluations)	F test	CV (%)
16.00 C + 6.30 N	1.16 A a[1]	1.18 A a	1.31 A a	3.65	2.40^{ns}	13.42
16.00 C + 7.00 N	1.21 A b	1.22 A b	1.48 A a	3.91	9.45*	11.75
16.00 C + 7.69 N	1.11 A b	1.19 A ab	1.29 A a	3.59	6.54*	10.21
20.00 C + 6.30 N	1.19 A a	1.15 A ab	1.01 B b	3.35	5.65*	8.65
20.00 C + 7.00 N	1.21 A a	1.16 A a	1.05 B a	3.42	1.90^{ns}	22.80
20.00 C + 7.69 N	1.22 A a	1.13 A ab	1.00 B b	3.35	7.50*	8.67
F test	0.83^{ns}	0.96^{ns}	14.49*	–	–	–
CV (%)	10.04	18.27	10.70	–	–	–
Standard error of the mean	0.0485	0.0886	0.0520	–	–	–
Treatment (g)	**Average concentration (x 10^8 blastospores/mL)**					
	4th day	6th day	8th day	S (3 evaluations)	F test	CV (%)
14.40 C + 7,00 N	1.58 A a[1]	1.55 A a	1.49 A a	4.62	0.36^{ns}	12.50
16.00 C + 7,00 N	1.30 A a	1.37 A a	1.51 A a	4.18	1.57^{ns}	15.13
17.60 C + 7,00 N	1.44 A a	1.51 A a	1.55 A a	4.50	0.20^{ns}	20.47
18.00 C + 7,00 N	1.31 A b	1.45 A ab	1.55 A a	4.31	4.03*	10.18
20.00 C + 7,00 N	1.42 A a	1.44 A a	1.54 A a	4.40	1.03^{ns}	10.11
22.00 C + 7,00 N	1.44 A a	1.41 A a	1.38 A a	4.23	0.12^{ns}	14.37
F test	0.74^{ns}	0.89^{ns}	1.36^{ns}	–	–	–
CV (%)	18.86	9.96	12.86	–	–	–
Standard error of the mean	0.1112	0.0611	0.0752	–	–	–

Averages followed by the same letter do not differ by Tukey's test at probability of 5%. Data transformed in $\sqrt{x}+0,5$.[1] capital letters for comparing averages between columns and lowercase ones for comparing averages between lines; *significant at 5% of probability by F test; ^{ns}not significant at 5% of probability by F test; CV: coefficient of variation; C: carbon; N: nitrogen.

Table 3. Average concentration of *Beauveria bassiana* (IBCB 66), obtained from liquid culture medium with different concentrations of nitrogen and carbon at four, six and eight days after inoculation (at 26 ± 1°C and 12-hour photophase).

Treatment (g)	Average concentration (x 10^8 blastospores/mL)					
	4th day	6th day	8th day	S (3 evaluations)	F test	CV (%)
16.00 C + 6.30 N	1.4 A a[1]	1.00 B b	0.87 C b	3.31	25.47*	12.11
16.00 C + 7.00 N	1.29 A a	0.98 B b	0.81 C c	3.08	26.21*	11.74
16.00 C + 7.69 N	1.24 A a	1.04 B b	0.89 C c	3.17	15.46*	11.15
20.00 C + 6.30 N	1.36 A a	1.43 A a	1.74 A a	4.53	1.56ns	26.24
20.00 C + 7.00 N	1.13 A b	1.16 BC b	1.52 A a	3.81	8.99*	13.31
20.00 C + 7.69 N	1.20 A b	1.48 A a	1.48 AB a	4.16	6.46*	9.92
F test	1.99ns	20.30*	12.86*	–	–	–
CV (%)	14.58	11.30	22.06	–	–	–
Standard error of the mean	0.0763	0.0573	0.1057	–	–	–
Treatment (g)	**Average concentration (x 10^8 blastospores/mL)**					
	4th day	**6th day**	**8th day**	**S (3 evaluations)**	**F test**	**CV (%)**
14.40 C + 7.00 N	1.18 A a[1]	1.19 A a	1.21 AB a	3.58	0.22ns	12.67
16.00 C + 7.00 N	1.23 A a	1.29 A a	1.16 B a	3.68	0.22ns	13.51
17.60 C + 7.00 N	1.17 A a	1.22 A a	1.22 AB a	3.61	0.19ns	12.15
18.00 C + 7.00 N	1.21 A a	1.24 A a	1.25 AB a	3.70	0.48ns	7.64
20.00 C + 7.00 N	1.18 A b	1.30 A ab	1.46 A a	3.94	9.67*	8.56
22.00 C + 7.00 N	1.12 A b	1.13 A a	1.13 B b	3.38	11.82*	7.82
F test	0.91ns	2.08ns	3.30*	–	–	–
CV (%)	9.08	10.32	13.10	–	–	–
Standard error of the mean	0.0440	0.0531	0.0657	–	–	–

Averages followed by the same letter do not differ by Tukey's test at probability of 5%. Data transformed in $\sqrt{x}+0,5$;[1] capital letters for comparing averages between columns and lowercase ones for comparing averages between lines; *significant at 5% of probability by F test; nsnot significant at 5% of probability by F test; CV: coefficient of variation; C: carbon; N: nitrogen.

Table 4. Average concentration of *Metarhizium anisopliae* (IBCB 425) and *Beauveria bassiana* (IBCB 66), obtained from the liquid culture media in different concentrations of nitrogen and carbon, at four, six and eight days after inoculation (at 26 ± 1°C and 12-hour photophase).

Treatments (g)	Average concentration (x 10^8 blastospores/mL)									
	Metarhizium anisopliae					Beauveria bassiana				
	4th day	6th day	8th day	F test	CV (%)	4th day	6th day	8th day	F test	CV (%)
2 16.00 C + 7.00 N	1.45 A a[1]	1.47 A a	1.50 A a	0.67ns	10.31	–	–	–	–	–
7 14.40 C + 7.00 N	1.54 A a	1.43 A a	1.38 A a	1.31ns	12.88	–	–	–	–	–
4 20.00 C + 6.30 N	–	–	–	–		1.34 B a	1.57 A a	1.59 A a	3.57ns	12.08
11 20.00 C + 7.00 N	–	–	–	–		1.56 A a	1.59 A a	1.60 A a	0.54ns	9.97
F test	0.72ns	0.30ns	0.11ns	–		9.09*	0.04ns	0.62ns	–	–
CV (%)	10.25	14.32	9.34	–		8.98	10.62	12.94	–	–
Standard error of the mean	0.0631	0.0882	0.0575	–		0.0566	0.0670	0.0816	–	–

Averages followed by the same letter do not differ by Tukey's test at probability of 5%. Data transformed in $\sqrt{x}+0,5$;[1] capital letters for comparing averages between columns and lowercase ones for comparing averages between lines; *significant at 5% of probability by F test; nsnot significant at 5% of probability by F test; CV: coefficient of variation; C: carbon; N: nitrogen.

higher one of C. This was also reported by Oliveira (2000) and Sano (2005), who have added different concentrations of yeast extract to the liquid media in the production of *Sporothrix insectorum* and *M. anisopliae*, respectively, and have gotten the best yields with lower concentrations of N.

The counting of colonies

Ultraviolet

It was observed that there was no significant difference between treatments of *M. anisopliae* and those of *B. bassiana* (Table 5). However, it was seen that the longer the exposure to UV radiation, the lower the number of colonies; with a very low amount of them after 50 seconds of exposure. These results are in agreement with the comments reported in several papers related to the influence of UV radiation on sporulation (Leach, 1965), germination (Zimmerman, 1982), viability (Corrêa, 1983), and inactivation of entomopathogens microorganisms (Ignoffo; Batzer, 1971; Broome et al., 1974; Jaques, 1985; Griego et al., 1985; Ali; Sikorowski, 1986).

Temperature

Table 6 summarizes the numbers of colonies obtained after incubation at 20, 25, 30, and 35ºC. Fungi have germinated at 25 and 30°C, yielding the expected number of colonies. However, at a 20°C temperature, the growth of colonies was significantly lower than the one observed at 25 and 30ºC. It was virtually null at a 35ºC temperature.

According to Alves (1986), *M. anisopliae* and *B. bassiana* have favorable T ranges for development, from 24 to 30°C and 22 to 26ºC, respectively, while Walstad et al. (1970) have reported that the limit from 24 to 30ºC is the best for germination of *M. anisopliae* spores. Bastos;Matta (1976) concluded that 25ºC was the best temperature for sporulation of *M. anisopliae*.

Similar results were stated by Santos (1978), when the number of colonies after incubation of *M. anisopliae* conidia reached 98 at 28ºC. The same author emphasized that 37ºC inhibits spore germination, with no found growth in plates at such temperature.

Among *B. bassiana* treatments, 20.00 g C + 6.30 g N was significantly different from 20.00 g C + 7.00 g N, when compared to the number of colonies at 35ºC. However, this variation was not consistent along the temperature ranges, indicating that this is only an occasional variation. For *M. anisopliae*, the best gradient for growth of colonies was between 25 and 30ºC, while for *B. bassiana* the optimum temperature was at 25ºC.

Virulence to *D. saccharalis*

After analyzing the confirmed mortality of infected larvae, there was no difference between treatments for both species (Table 7).

Table 5. Number of colonies obtained from *Metarhizium anisopliae* (IBCB 425) and *Beauveria bassiana* (IBCB 66) after exposure of blastospores to ultraviolet radiation for 25 and 50 seconds, and incubation of three days (at 25 ± 1°C and 12-hour photophase).

Treatments (g)	Number of colonies (0.1 mL/ Petri's plate)									
	M. anisopliae					*B. bassiana*				
	Without exposure	25 s	50 s	F test	CV (%)	Without exposure	25 s	50 s	F test	CV (%)
2 16.00 C + 7.00 N	11.13 A a[1]	6.27 A b	1.11 A c	358.30*	10.49	–	–	–	–	–
7 14.40 C + 7.00 N	10.64 A a	6.39 A b	1.31 A c	151.74*	15.18	–	–	–	–	–
4 20.00 C + 6.30 N	–	–	–	–		10.55 A a	5.36 A b	1.95 A c	295.54*	10.36
11 20.00 C + 7.00 N	–	–	–	–		10.89 A a	6.13 A b	1.83 A c	235.77*	11.51
F test	1.14^{ns}	0.04^{ns}	0.83^{ns}	–		0.47^{ns}	4.36^{ns}	0.20^{ns}	–	–
CV (%)	7.33	17.06	29.51	–		8.02	11.04	24.48	–	–
Standard error of the mean	0.3259	0.4410	0.1505	–		0.3510	0.2591	0.1888	–	–

Averages followed by the same letter do not differ by Tukey's test at probability of 5%. Data transformed in $\sqrt{x}+0,5$;[1] capital letters for comparing averages between columns and lowercase ones for comparing averages between lines; *significant at 5% of probability by F test; ^{ns}not significant at 5% of probability by F test; CV: coefficient of variation; C: carbon; N: nitrogen.

Results point out that the blastospores have caused a *D. saccharalis* mortality rate between 26.00 and 29.00% for both *M. anisopliae* and *B. bassiana* on evaluated media. The blastospore is a low-resistance structure in the environment because the cell wall has the same constitution of the hypha one (Leite et al., 2003). Formation of such also makes the seedlings turn into hygroscopic ones, a fact that discourages their membership in the host (Boucias et al., 1981).

The choice for production of blastospores comes from their possibility of being produced in submerged culture medium. This facilitates the production process, reduces the time of collection or filling, and allows an increase in the number of infective units produced per average volume (Torre; Cárdenas-Cota, 1996). Thus, the higher production of propagules may compensate for their lower pathogenicity.

Table 6. Number of colonies of *Metarhizium anisopliae* (IBCB 425) and *Beauveria bassiana* (IBCB 66) obtained after inoculation of blastospores produced in liquid media and exposed to different temperatures.

Treatment (g) *M. anisopliae*	Temperature					
	20ºC	25ºC	30ºC	35ºC	F test	CV (%)
2 16.00 C + 7.00 N	8.87 A b	11.13 A a	10.19 A a	0.88 A c	237.87*	9.58
7 14.40 C + 7.00 N	8.57 A b	10.64 A a	10.08 A a	0.79 A c	367.82*	7.76
F test	0.67^{ns}	1.14^{ns}	0.05^{ns}	0.38^{ns}	–	–
CV (%)	7.26	7.33	8.21	28.80	–	–
Standard error of the mean	0.2584	0.3259	0.3397	0.0984	–	–
Treatment (g) *B. bassiana*	**Temperature**					
	20ºC	**25ºC**	**30ºC**	**35ºC**	**F test**	**CV (%)**
4 20.00 C + 6.30 N	8.11 A b[1]	10.55 A a	8.90 A b	0.97 A c	245.26*	9,53
11 20.00 C + 7,00 N	7.93 A c	10.89 A a	9.69 A b	0.71 B d	250.16*	9.67
F test	0.14^{ns}	0.47^{ns}	4.09^{ns}	5.00*	–	–
CV (%)	10.42	8.02	7.23	23.97	–	–
Standard error of the mean	0.3413	0.3510	0.2741	0.4851	–	–

Averages followed by the same letter do not differ by Tukey's test at probability of 5%. Data transformed in $\sqrt{x}+0,5$;[1] capital letters for comparing averages between columns and lowercase ones for comparing averages between lines; *significant at 5% of probability by F test; ^{ns}not significant at 5% of probability by F test; CV: coefficient of variation; C: carbon; N: nitrogen.

Table 7. Confirmed mortality (%) of third-instar larvae (*Diatraea saccharalis*) at eight days after the application of different suspensions of *Metarhizium anisopliae* and *Beauveria bassiana*, obtained from liquid culture media (blastospores) in different concentrations of nitrogen and carbon (temperature at 26 ± 1°C, RH 70 ± 10% and 12-hour photophase).

Treatment (g)	Confirmed mortality (%)	
	M. anisopliae	*B. bassiana*
2 16.00 C + 7.00 N	26.82 A	–
7 14.40 C + 7.00 N	26.17 A	–
4 20.00 C + 6.30 N	–	29.58 A
11 20.00 C + 7.00 N	–	28.32 A
Control (distilled water)	7.72 B	9.17 B
F test	103.59*	70.27*
CV (%)	14.89	17.26
Standard error of the mean	0.1066	0.1364

Averages followed by the same letter do not differ by Tukey's test at probability of 5%. Data transformed in $\sqrt{x}+0,5$;[1] capital letters for comparing averages between columns and lowercase ones for comparing averages between lines; *significant at 5% of probability by F test; ^{ns}not significant at 5% of probability by F test; CV: coefficient of variation; C: carbon; N: nitrogen.

CONCLUSIONS

The best media to produce blastospores are formed by a lower concentration of N and a higher one of C. The most appropriate culture media for *M. anisopliae* contain D-glucose anhydrous (C source) in its composition, where *B. bassiana* has sucrose (C source).

The exposure to UV radiation and to temperatures at 35°C is detrimental to fungi growth. *M. anisopliae* and *B. bassiana* are virulent to third-instar larvae of *D. saccharalis*, whatever is the liquid medium in which they were produced.

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