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PATHOGENICITY OF *BEAUVERIA BASSIANA* FOR ADULTS OF *TRIBOLIUM CASTANEUM* (*COL.: TENEBRIONIDAE*) IN STORED GRAINS

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Conidia of *Beauveria bassiana* were evaluated for activity against adults of *Tribolium castaneum*, under laboratory conditions closely resembling the stored-grain conditions.

Three doses of the fungus were applied in the treatments: 0.1 g, 0.5 g and 1 g per 20 insects/dose/flask. Treated insects and untreated were incubated in broken wheat. There were 5 replications for each test.

A total of 1600 treated and control insects used were kept in a climatic chamber ($70 \pm 5\%$ and $27 \pm 2°C$) for 45 days. Observations were performed daily and the recording of infected cadavers began when the percentage of mortality exceeded 50%. The evaluation of the efficiency of each dose of *B. bassiana* was made 14, 21, 28 and 45 days after inoculation.

Mortality of adults that were exposed to the beetles treated with 0.5 g and 1 g of conidia of *B. bassiana* / 20 adults was 87 and 85% respectively, within 21 days of exposure. Onset of mortality was further delayed at lower dosage of the fungus; 55% after 21 days. After 28 days there were no difference in mortality between the 0.5 g and 1 g dosages of *B. bassiana.* The most efficient treatment was 0.5 g/20 insects, which resulted in a control higher than 50% 14 days post treatment.

KEY-WORDS: *Beauveria bassiana, Tribolium castaneum,* microbial control

Pest insects of stored-grains can cause reduction in weight, quality, commercial value and germinative power of seeds (Campanella & Diaz, 1978; Marsans, 1987). *Tribolium castaneum* Herb. is a cosmopolitan pest that attacks mainly the germ of stored grains and farinaceous products and causes flours to change their coloration and have unpleasant odours. It also results in qualitative-quantitative losses in dried fruits, chocolates and other vegetable-origin foodstuffs (Gallo *et al.*, 1978; Gonzales, 1989).

Frequent use and exposure to increasing dosages of insecticides has caused *T. castaneum* strains to appear with the Kdr gene, which makes them resistant to some pesticides such as pyrethroids and D.D.T.. Twenty seven out of 29 *T. castaneum* stains were resistant to malathion, 18 to dichlorvos, 10 to chlorpyrifos-methyl and 10 to phosphine (Zettler, 1991).

A promising strategy with good potential to minimize the adverse effects of insecticides is the use of entomopathogenic fungi and other microbial control agents. Fungal pathogens

penetrate through the cuticle of the host and may produce epizootics which decimate populations of insects, which, after their death, help the fungus to sporulate and spread conidia as infectious propagules (Mier *et al.*, 1994).

Out the 700 entomopathogenic species of fungi reported from insects, only 10 have been widely known as biocontrol agents (Hajek & St. Leger, 1994). Among them, one of the most frequently used is *Beauveria bassiana* (Bals.) Vuill. (Hyphomycetes). Its potential as a mycoinsecticide has been demonstrated on a huge number of different insect hosts (Inglis *et al.*, 1993; Feng *et al.*, 1994; Padín *et al.*, 1995). This is related to the different modes of infection of the fungus, either through the intersegmental membranes, the respiratory system, the feeding channel and the bucal cavity (Inglis *et al.*, 1993).

The aim of this study has been to determine the efficiency of *B. bassiana* for the control of *T. castaneum* Herb. under conditions closely resembling the grain-stored environment.

MATERIALS AND METHODS

INSECTS

Tribolium castaneum was mass produced in a climatized chamber under controlled conditions (temperature: 27 ± 2°C; relative humidity: 70 ± 5%) with alternating light-dark cycles of 12 h, started with insects found in wheat grains free from pesticides. The diet used for the production of the insects consisted of a mixture of 85% wheat flour, 5% powdered beer yeast, 5% skim milk powder and 5% wheat germ.

FUNGUS

One *B. bassiana* strain has been used. Fungus was initially isolated from dead larvae *Diatraea saccharalis* Fab. (Lepidoptera: Pyralidae), collected in a corn field near Pergamino (Buenos Aires, Argentina).

Beauveria was passed through a stored-grain beetle, *T. castaneum,* by spraying the adult insects with a concentrated conidiospore suspension. Twenty exposed beetles were placed in a flask containing 50 g of broken wheat grains. Flask was incubated at 27°C. Ten days after exposure, dead insects collected from the flask were surface desinfested with ethylic alcohol for 1 min, rinsed with sterile distilled water for 5 min and placed on sterile filter paper in a moist chamber for 5 days at 25°C. *Beauveria* developing from infected insects was isolated by spreading spores on PDA. For bioassays the fungus was cultured at 25°C on PDA during 21 days and harvested by scraping mycelial mats with a sterile spatula.

Three different doses of spore inocula were weighed out for each replicate. This mycelium was stored in small sterile flasks at 5°C for 7 days before the insects were inoculated.

The viability was assessed by recording the percentage germination of spores. Conidia were suspended in distilled water and germination percentages were determined on PDA.

After 24 h at 25 ± 1°C, conidia were fixed with lactophenol and germination percentages were determined by examination of a minimum of 100 conidia from each of 2 replicate cultures. Conidia were considered viable if germ tubes were greater than 3 μm in length.

PATHOGENICITY TESTS

The contact between the insects and the fungus was made in 500 ml glass flasks containing 50 g of broken wheat where the 15-day-old *T. castaneum* adults were transferred.

Before the insects were added to the flasks, wheat grains were carefully mixed with the corresponding dosage of inocula. Untreated grains were used in controls. The vessels were covered with a metal mesh to allow air circulation.

In total, 1600 adult insects were simultaneously inoculated with the fungus in 80 flasks. The treatments included three doses of the inocula: 0.1 g, 0.5 g and 1 g per 20 insects/treatment flask and the controls without the pathogen. There were 5 replications for each treatment and untreated grain control. The spore count was ca. 6×10^6 conidia/mg. There were a second control with 5 flasks containing grain + fungus (1 g) to determine the mycelial growth on wheat 45 days after application.

All flasks were kept in a climatized chamber with controlled humidity and temperature ($70 \pm 5\%$ and $27 \pm 2°C$).

Observations of each of the flasks, excepting the second control, were made daily by emptying the contents of each flask onto a white paper to identify the dead individuals.

The confirmation of infection began when the percentage of mortality exceeded 50%, that was 14 days post treatment. At that moment 20 randomly selected flasks were evaluated, 5 per each dose and control, corresponding to the first period. The dead adults of *T. castaneum* collected in the indicated periods were immediately submerged in ethylic alcohol for 1 min and washed in sterile distilled water for 5 min. They were then put in a humid chamber and incubated at 25°C until the onset of the illness. The presence of the cotton-white mycelium and the sporulation over the cuticle of the cadaver was the evidence of the death by mycosis. After having been analyzed, the samples were discarded. The same procedure was repeated 21, 28 and 45 days after the treatment, corresponding to the second, third and fourth period respectively.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

In the bioassay, a completely randomized factorial design was used with 5 replicates. The factors were the doses of the fungus (0; 0.1; 0.5 and 1 g) and the storage period after inoculation (14, 21, 28 and 45 days) with 4 treatments for each of them. The results for percentage insect disease for each treatment were analyzed using an ANOVA and Tukey's test to separate treatment means at $P \leq 0.05$ (table 1).

TABLE 1

Cumulative mortality of T. castaneum *adults due to fungal infection in stored grains 14, 21, 28 and 45 days after treatment*

Date	Dosage *			
	0 g	0.1 g	0.5 g	1 g
14	0 a	38.00 (± 1.99) a	49.73 (± 2.78) a	55.05 (± 2.09) a
21	0 a	47.93 (± 2.92) a	69.83 (± 3.50) b	67.74 (± 2.72) b
28	0 a	64.90 (± 3.50) a	78.56 (± 3.05) bc	72.90 (± 1.88) bc
45	0 a	72.90 (± 1.88) a	87.42 (± 2.58) c	82.25 (± 3.16) c

* Means followed by the same letter in the same column are not significantly different at the 0.05 level as determined by Tukey's studentized multiple range test. Five replicate tests for each dosage conducted on each of 4 separate dates.

RESULTS

The daily observations showed the relationship between the doses of the fungus, the contact time with it and the percentage of mortality of *T. castaneum.*

Between 24-72 h of incubation of cadavers, for the majority of the insects fungal infection was established as the cause of death. The characteristic sign of *B. bassiana* infection, a cotton-white mycelium, emerged through the intersegmental membranes, oral and anal orifices with a tendency eventually to cover the whole body.

The effect of dosage on mortality 14, 21, 28 and 45 days following application of fungus is given in table 1.

At the highest dosages, 0.5 g and 1 g of conidia/20 adults, there was a sharp increase in mortality 14 days after treatment. Onset of the majority of mortality was delayed at lower dosage.

Beauveria bassiana was significantly more lethal to *T. castaneum* at 45 days of exposure than 14 and 21 days, for any dose ($P \leq 0.05$).

For the 3 doses there were no difference in mortality between 28 and 45 days, and the major percentages were recorded with 0.5 g of the fungus/20 insects. This treatment, which was the most effective, produced, in addition, a higher control at 50% 14 days later.

After 45 days the fungus did not show mycelial growth on the wheat grains under storage conditions.

All of the insects that were killed with *B. bassiana* and subsequently transferred to moist chambers manifested signs of patent infection with the fungus with subsequent production of conidia. There was no evidence of mycosis in any control cadavers.

DISCUSSION

Efficiency of biocontrol of pest insects with entomopathogenic fungi is mainly due to their capacity to produce epizootics (Young, 1974; Leathers *et al.,* 1993) from the infected cadavers. These cadavers can be constituted in a source of inocula that allows for the dissemination of the microbial agent in the environment (Gottwald & Tedders, 1982; Lacey *et al.,* 1994). When invading the haemocoel, the fungus proliferates and under moist conditions, it emerges and produces a layer of aerial conidia on the surface of host cadavers (Feng *et al.,* 1994).

The study carried out with *B. bassiana* and *T. castaneum* indicated that dry conidia of the fungus could achieve high levels of kill without the requirement of high humidity in the management of stored grain pests, supporting the results of previous works (Bateman *et al.,* 1993; Adane *et al.,* 1996). The percentages of mortality showed that there was a progressive increase of the mycosis until the fourth week of the treatments. However, the external signs of disease was only evidenced when the dead insects remained in a humid chamber. It is probable that the humidity was insufficient in the grain mass of the assay so as to produce the development of the pathogen through the exoskeleton.

Ferron (1977) reported humidity-independent infection of *Acanthoscelides obtectus* Say by *B. bassiana,* due to a boundary layer of moist air which envelops the insect integument. The infection of *A. obtectus* occurred between humidities of 0 and 100%, but that the development of the fungus on the cadavers was only possible at humidity near saturation. A cadaver may show no external signs of disease, despite having been killed by a fungal infection (Adane *et al.,* 1996).

Speed of kill is an important factor when considering entomopathogenic fungi as biocontrol agents since the death of reproducing adults will be quicker when the insecticide effect of the treatment is more virulent.

Mortality higher than 50% of the insects was obtained within 14 days of inoculation, and maximum lethality was obtained after 28 days. Since the vital cycle of *T. castaneum* from egg to adult is 30-45 days, the time interval between treatment and death is too long as it allows mature females to oviposit.

Nevertheless results of this and previous studies (Padin *et al.*, 1995) indicate that *B. bassiana* has considerable potential for the control of *T. castaneum.*

Future work will be directed towards the development of a combination of *B. bassiana* formulations with sub-lethal doses of storage-grain chemical insecticides.

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RÉSUMÉ

Pathogénicité de *Beauveria bassiana* vis-à-vis d'adultes de *Tribolium castaneum* dans des grains stockés

L'activité des conidies de *B. bassiana* vis-à-vis d'adultes de *Tribolium castaneum* a été évaluée dans des conditions de laboratoire similaires à celles existant dans des grains stockés.

Les traitements ont consisté en une application de trois doses de champignon: 0,1 g, 0,5 g et 1 g dans des flacons contenant 20 insectes par dose, avec du blé concassé pour les témoins comme pour les individus traités. Les insectes utilisés (au nombre de 1600) ont été placés dans une chambre climatisée (70 ± 5% et 27 ± 2°C) pendant 45 jours. Des observations quotidiennes ont été faites et le contrôle des individus morts infectés a commencé quand le pourcentage de mortalité a dépassé les 50%. L'évaluation de l'efficacité de chaque dose de *B. bassiana* a été faite 14, 21, 28 et 45 jours après l'inoculation. La mortalité des adultes traités avec 0,5 g et 1 g de conidies de *B. bassiana*/20 adultes a été respectivement de 87 et 85%, dans les 21 premiers jours d'exposition. Le début de la mortalité a été retardé avec des doses plus faibles du champignon: seulement 55% après 21 jours d'exposition. A partir de 28 jours, il n'y a pas de différence de mortalité observée entre les doses de 0,5 g et de 1 g de *B. bassiana*. Le traitement le plus efficace a été obtenu avec une dose de 0,5 g / 20 insectes et a produit une mortalité supérieure à 50% après 14 jours.

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REFERENCES

Adane, K., Moore, D. & Archer, S. A. — 1996. Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the laboratory. — *J. stored Prod. Res.*, 32, 105-113.

Barson, G., Renn, N. & Bywater, A. F. — 1994. Laboratory evaluation of six species of entomopathogenic fungi for the control of the house fly (*Musca domestica* L.), a pest of intensive animal units. — *J. Invertebr. Pathol.*, 64, 107-113.

Bateman, R. P., Carey, M., Moore, D. & Prior, C. — 1993. The enhanced infectivity of *Metarhizium flavoviride* in oil formulations to desert locusts at low humidities. — *Ann. Appl. Biol.*, 122, 145-152.

Campanela, C.P. & Diaz, E. A. — 1978. Almacenaje y conservación de granos. — *Información Agropecuaria,* Buenos Aires, 2, 18-21.

Feng, M. G., Poprawski, T. J. & Khachatourians, G. G. — 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: Current status. — *Biocontrol Sci. & Technol.,* 4, 3-34.

Ferron, P. — 1977. Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (Fungi Imperfecti, Moniliales) in imagines of *Acanthoscelides obtectus* (Col.: Bruchidae). — *Entomophaga,* 22, 393-396.

Gallo, D., Nakano, O., Silveira Neto, S., Carvalho, R. P. L., Batista, G. C., Berti Filho, E. Parra, J. R. P., Zucchi, R. A. & Alves, S. B. — 1978. Manual de Entomologia Agrícola. — *Ed. Agronomica Ceres Ltda.,* São Paulo, Brasil.

Gonzalez, R.H. — 1989. Insectos y ácaros de importancia agrícola y cuarentenaria en Chile. — *Univ. Chile, BASF,* Chile, 310 pp.

Gottwald, T. R. & Tedders, W. L. — 1982. Studies on conidia release by the entomogenous fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) from adult pecan weevil (Coleoptera: Curculionidae) cadavers. — *Environ. Entomol.,* 11, 1274-1279.

Hajek A. E. & St. Leger, R. J. — 1994. Interactions between fungal pathogens and insect hosts. — *Annu. Rev. Entomol.,* 39, 293-322.

Inglis, D. G., Goettel, M. S. & Johnson, D. L. — 1993. Persistence of the entomopathogenic fungus, *Beauveria bassiana,* on phylloplanes of crested wheatgrass and alfalfa. — *Biol. Control,* 3, 258-270.

Lacey, L. A., Martins, A. & Ribeiro, C. — 1994. The pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* for adults of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). — *Eur. J. Entomol.,* 91, 313-319.

Leathers, T. D., Gupta, S. C. & Alexander, N. J. — 1993. Mycopesticides: Status, challenges and potential. — *J. Industrial Microbiology,* 12, 69-75.

Marsans, G. J. — 1987. Manejo y conservación de granos. *Ed. Hemisferio Sur,* Buenos Aires, 266 pp.

Mier, T., Rivera, F., Rodríguez Ponce, M. P., Carrillo Farga, J. & Toriello, C. — 1994. Infectividad del hongo entomopatógeno *Verticillium lecanii* en ratones y cobayos. — *Rev. Lat. Amer. Microbiol.,* 36, 107-111.

Padín, S. B., Dal Bello, G. M. & Vasicek, A. L. — 1995. Potencial bioinsecticida de hongos entomopatógenos de plagas en granos almacenados. — *Rev. Fac. Agronomía UBA,* 15, 1-7.

Young, E. C. — 1974. The epizootiology of two pathogens of the coconut palm rhinoceros beetle. — *J. Invertebr. Pathol.,* 24, 82-92.

Zettler, J. L. — 1991. Pesticide resistance in *Tribolium castaneum* and *T. confusum* (Coleoptera: Tenebrionidae) from flour mills in the United States. — *J. Econ. Entomol.,* 84, 763-767.