Susceptibility of *Corythaica cyathicollis* Costa to a native isolation of *Beauveria bassiana* (Vuillemin)

Susceptibilidad de *Corythaica cyathicollis* Costa a un aislamiento nativo de *Beauveria bassiana* (Vuillemin)

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ABSTRACT

The research aimed to evaluate a native isolation of *B. bassiana* under laboratory conditions as a biological alternative control for the eggplant lace bug (*C. cyathicollis*). The experiments were conducted in the Pathology and Entomology Laboratories at the Universidad de Córdoba, Monteria (Colombia). Initially, the pathogenicity of *B. bassiana* on adult insects was studied using a concentration of $1{\cdot}10^7$ spores/mL, in which mortality and intrinsic mortality were determined, in addition, the presented symptomatology was described. Subsequently, the concentrations $1{\cdot}10^0$ (control treatment), $1{\cdot}10^3$, $1{\cdot}10^5$, $1{\cdot}10^6$, $1{\cdot}10^7$ and $1{\cdot}10^8$ spores/mL were evaluated to determine the lethal concentrations of CL_{50} and CL_{90} through Probit analysis. The results obtained showed that the native isolation was pathogenic since post-inoculation was observed, between the fifth and sixth days, with 50% mortality (and 87.7% at 12 days), in addition, the intrinsic mortality was 92.4%. The symptomatology showed loss of mobility and lack of appetite between 11 and 12 hours post-inoculation, approximately, and the presence of mycelium 2 days after death. The percentages of mortality for the evaluated concentrations were 0, 46.6, 73.3, 83.3, 90.0 and 96.6% respectively, and the lethal concentrations of CL_{50} and CL_{90} were $1.8{\cdot}10^3$ and $6.5{\cdot}10^6$ spores/mL respectively. These results indicate the potential of entomopathogenic fungus as an alternative that could be articulated into integrated pest management.

Key words: eggplant, lace bug, entomopathogenic fungus, biological control, pathogenicity, lethal concentrations.

RESUMEN

La investigación tuvo como objetivo evaluar bajo condiciones de laboratorio un aislamiento nativo de *B. bassiana* como alternativa de control biológico al chinche de encaje de la berenjena (*C. cyathicollis*). Los experimentos se desarrollaron en instalaciones de los Laboratorios de Fitopatología y Entomología de la Universidad de Córdoba, Monteria (Colombia). Inicialmente se estudió la patogenicidad de *B. bassiana* sobre adultos del insecto, usando una concentración de $1{\cdot}10^7$ esporas/mL, en éstos se determinó la mortalidad, mortalidad intrínseca y se describió la sintomatología presentada. Posteriormente, se evaluaron las concentraciones $1{\cdot}10^0$ (testigo), $1{\cdot}10^3$, 110^5, $1{\cdot}10^6$, $1{\cdot}10^7$ y $1{\cdot}10^8$ esporas/mL, con las cuales se determinaron las concentraciones letales CL_{50} y CL_{90} a través del análisis Probit. Los resultados obtenidos mostraron que el aislamiento nativo fue patogénico al presentar entre el quinto y sexto día post-inoculación, mortalidad del 50% y a los 12 días del 87,7%, además, la mortalidad intrínseca para esta lectura fue del 92,4%. La sintomatología mostrada fue pérdida de movilidad e inapetencia aproximadamente entre las 11 y 12 horas post-inoculación y aparición de micelio a los 2 días después de muerto. Los porcentajes de mortalidad para las concentraciones evaluadas fueron de 0, 46,6; 73,3; 83,3; 90.0 y 96,6% respectivamente y las concentraciones letales CL_{50} y CL_{90} fueron de $1{,}8{\cdot}10^3$ y $6{,}5{\cdot}10^6$ esporas/mL respectivamente. Estos resultados indican el potencial del entomopatógeno como alternativa para ser articulada en el manejo integrado de la plaga.

Palabras clave: berenjena, chinche de encaje, entomopatógeno, control biológico, patogenicidad, concentraciones letales.

Introduction

In Colombian departments such as Córdoba and Sucre, eggplant is one of the main vegetable cultivations that are part of the rural economy. In this country, the Plan Hortícola Nacional (PHN) prioritizes eggplant cultivation since it is one of the vegetables which receives more benefits as an alternative of agricultural diversification and a prospectus of export (Acopaflor, 2007), by being one of the ten most consumed vegetables worldwide (MADR, 2006) and providing health benefits (Gonçalves *et al.,* 2006; González *et al.*, 2007; Duran *et al.,* 2007), especially with its antioxidant contribution (Whitaker and Stommel, 2003; Marion, 2004; Luthria and Mukhopadhyay, 2006; Sadilova *et al.,* 2006; San José *et al.,* 2007; Raigon *et al.,* 2008). In addition, the Colombian Caribbean has competitive advantages for

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eggplant cultivation, such as its climatic factors, as well as the physical and chemical characteristics of the land, which allow for its production year round.

In Córdoba and Sucre, agricultural treatment problems are seen as the principal limits of eggplant cultivation, especially land pathogens associated with the illness know as "wilt" and arthropods like *Bemisia tabaci* (Gennadius), *Tetranychus* sp., *Leptinotarsa decemlineata* (Say) and *Corythaica cyathicollis* Costa (Aramendiz *et al*., 2008). The latter one, apart from attacking eggplant (*Solanum melongena* L.), has been associated with solanaceas species like *S. atropurpureum* Schrank, *S. fastigiatum* Willd., *S. granuloso-leprosum* Dunal, *S. mauritianum* Scopoli (Pedrosa-Macedo *et al.,* 2003; Olckers *et al.,* 2002) and cultivated species like *S. lycopersicum* L., *S. tuberosum* L. (Kogan, 1960) and *Solanum gilo* Raddi (Torres, 1995).

In the Colombian departments previously mentioned, the dry periods generally favor a high population of *C. cyathicollis* in eggplant cultivation (Torres, 2003). The mechanical damage is caused by nymphs as well lace bug adults when they suck up the leaf sap, which is initially presented as chlorotic spot joining, eventually causing a yellowish color, drying, and detachment of the leaves. This process also influences production in a significant manner; the loss of leaves favors damage in the fruits known as sunburn, which deteriorates quality (Kogan, 1960; Salgado and Regino, 2001).

In eggplant cultivations in Brazil, some management practices like fertilization and covering levels have been evaluated to control *C. cyathicollis* (Da Silva, 2006; Da Silva *et al*., 2008). In vegetable cultivation in Colombia, particularly for tomatoes, pest management based exclusively on chemical control has led to an important increase in production costs, a negative impact on the environment and a potential toxicity risk for workers and consumers (Guerrero, 2003; Fuentes and Barreto, 2006).

In contrast to this outlook, national and international specialized markets constantly emphasize products obtained under Good Agricultural Practices (GAP), as well as in an agro-ecological_manner, especially in vegetables characterized by a low cooking times or no cooking requirement at all. An alternative to this problem is organic control through the use of natural enemies like entomopathogenic fungi. The most common fungi are *Paecilomyces*, *Hirsutella*, *Metarhizium* y *Beauveria* (France *et al*., 1999). The latter one has shown good results in controlling other tropical pests (Mena *et al*., 2003; Lucero *et al*., 2004; Pariona *et al*., 2007; Cova *et al.,* 2009), meaning it should be considered as a potential agent for mycoinsecticides development (Alves, 1986).

Currently, vegetable and fruit cultivation continues to grow significantly, and customers are searching for the best purveyors, who have the capacity to provide them with products all year long. There is great opportunity in mass production and export of vegetables, hence, producers who meet the technological requirements to satisfy the markets will make the most of the commercial and competitive advantages (Acopaflor, 2007).

With the aim of contributing to the search for management alternatives compatible with food safety terms, sustainability and competitiveness in eggplant cultivation, this research had the goal of evaluating the potential of a native isolation of *B. bassiana* as a biological control for the eggplant lace bug (*C. cyathicollis*) under laboratory conditions.

Materials and methods

The research was conducted in the Phytopathology and Entomology Laboratories at the Universidad de Córdoba, Monteria (Colombia) under controlled conditions with a temperature of 26°C and 85% relative humidity. The host was the *C. cyathicollis* (Costa) species, with biological states obtained from offspring developed in eggplant plants available in the Entomology Laboratory of the Faculty of Agricultural Science. The *C. cyathicollis* population was started with the adults and nymphs harvested from commercial eggplant cultivations. The source of the entomopathogenic fungus came from an isolation of the *C. cyathicollis* species collected from the field (native isolation). The inoculum was obtained through the methodology suggested by Antia *et al.* (1992) and Vélez *et al.* (1997). The generic identification of the entomopathogenic fungus was conducted through the taxonomical key proposed by Barnet and Hunter (1998) for imperfect fungi.

Pathogenicity test

The pathogenic of the native isolation of *B. bassiana* was executed on *C. cyathicollis* adults 5 d after emergence, all of them were active and healthy, guaranteeing the homogeneity of the biological material, using the $1{\cdot}10^7$ spores/mL concentration of the entomopathogenic fungus. The main solution of the native isolation was prepared with 10 g of rice boiled with the *B. bassiana* inoculum in 100 mL of sterile distilled water, plus two drops of spores dispersant (Tween®). The solution concentration was calculated at 10^{-3} dilution through cell counting in a Neubauer chamber.

Subsequently, it was adjusted to the $1{\cdot}10^7$ spores/mL concentration. The utilized control treatment was ($1{\cdot}10^0$ spores/mL) sterile distilled water plus the dispersant, in order to avoid the adverse effects of these components on insects that may influence the *B. bassiana* pathogenic action.

The infection with *B. bassiana* was made by direct spraying with an atomizer at an approximate distance of 10 cm on *C. cyathicollis* adults using a volume of 5 mL for the concentrations $1{\cdot}10^0$ (control treatment) and $1{\cdot}10^7$ spores/mL. Three populations of thirty of insects were used for each concentration. Each population was insolated in plastic trays and fed with eggplant leaves, which were changed daily for 11 d.

The symptoms of the illness caused by *B. bassiana* were described through daily observations of the changes and behaviors shown by the *C. cyathicollis* adults (lack of appetite, loss of mobility and coloration). The dead insects were placed in a moist chamber to elicit mycelium emission and fungal sporulation on the bodies. The evaluated variables were mortality, through the daily counting of post-infection dead adults until the 12^{th} d, and intrinsic mortality (number of dead insects with the presence of the fungus), determined by counting the total number of dead insects that presented the signs and symptoms of the illness caused by *B. bassiana* until the 12^{th} reading.

Determination of lethal concentrations CL_{50} and CL_{90}

The concentrations $1{\cdot}10^3$, $1{\cdot}10^5$, $1{\cdot}10^6$, $1{\cdot}10^7$ and $1{\cdot}10^8$ spores/mL and a control treatment $1{\cdot}10^0$ (ADE + dispersant) were evaluated, which were obtained through the procedure described in the pathogenicity test. Each concentration constituted a treatment which was distributed in a random design with three repetitions; 60 adults were infected by the treatments. Mortality counts were done until the 12^{th} d. With the mortality data, the fifty (CL_{50}) and ninety (CL_{90}) lethal concentrations were calculated through Probit analysis (Finney, 1972; Raymons, 1985).

Results and discussion

Pathogenicity of the native isolation of *B. bassiana*

The native isolation was pathogenic and showed high levels of virulence, between the fifth and sixth d post-inoculation, with 50% mortality (and 87.7% at 12 d) in adults. In addition, the intrinsic mortality for this reading was 92.4%, which confirms that the insect deaths were caused by the entomopathogenic attack (Tab. 1). The high virulence might be due to the natural specificity between the inoculum and the host, since the native fungus isolation was applied to the host were it was isolated; which coincides with the views of authors like Roberts and Humbre (1984); Torres and López (1997); France *et al*. (1999) who recommend the utilization of native isolations extracted from the insect, since these stocks have suffered natural selection processes and coevolution with the pathogenic action. Moreover, Alves (1986) pointed out that the pathogenicity is one of the entomopathogenic microorganism genetic characteristics, which makes it go straight through the insect and cause the illness, as long as the virulence is of the degree of pathogenicity of isolations toward a specific host.

Symptomatology of infection in *C. cyathicollis*

The symptoms of infection produced by *B. bassiana* in *C. cyathicollis* in adults begin with the changing color of affected individuals, from black to reddish in the abdominal part, and the lack of appetite between 11 and 12 h post-inoculation, approximately. Once the insect is dead, it suffers dehydration, turns rigid; and 2 d after that, myce-

TABLE 1. Pathogenicity of the *B. bassiana* native isolation in *C. cyathicollis* adults.

Post-inoculation time (h)	Accumulated mortality (No.)		Mortality (%)		Intrinsic Mortality (%)	
	$1{\cdot}10^0$ (spores/mL)	$1{\cdot}10^7$ (spores/mL)	$1{\cdot}10^0$ (spores/mL)	$1{\cdot}10^7$ (spores/mL)	$1{\cdot}10^0$ (spores/mL)	$1{\cdot}10^7$ (spores/mL)
24	0	0	0	0	0	0
48	0	2	0	2.2	0	2.5
72	0	27	0	30.0	0	31.6
96	0	34	0	37.8	0	39.2
120	0	39	0	43.3	0	45.6
144	0	47	0	52.2	0	53.2
168	0	49	0	54.4	0	55.7
192	0	55	0	61.1	0	63.3
216	0	67	0	74.4	0	78.5
240	0	70	0	77.7	0	82.3
264	0	75	0	83.3	0	87.3
288	0	79	0	87.7	0	92.4

TABLE 2. Mortality of *C. cyathicollis* adults caused by CL_{50} and CL_{90} concentrations of a *B. bassiana* native isolation under laboratory conditions.

***B. bassiana* (spores/mL)**	**$1·10^0$**	**$1·10^3$**	**$1·10^5$**	**$1·10^6$**	**$1·10^7$**	**$1·10^8$**
C. cyathicollis mortality (%)	0	73.3	83.3	90.0	96.6	46.6
CL_{50} (spores/mL)				$1.8·10^3$		
CL_{90} (spores/mL)				$6.5·10^6$		

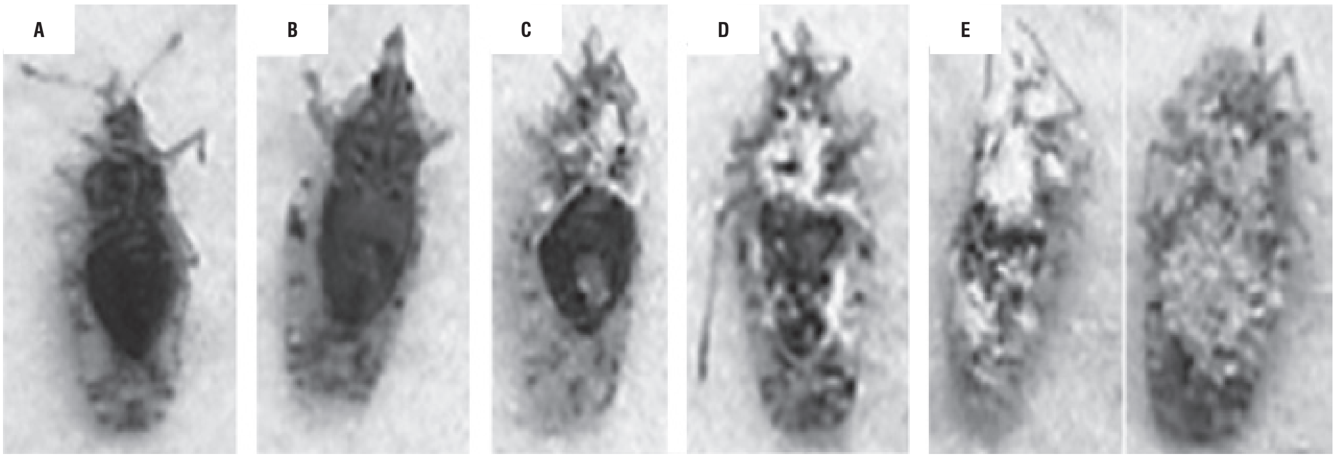

FIGURE 1. *C. cyathicollis* symptomatology affected by *B. bassiana*. A, healthy insect; b, affected insect; c, rigid and dehydrated insect; d, mycelium in sutures; e, fungal sporulation in the insect's body.

lium appears between the abdominal sutures. The external emission of fungus mycelium is observed the third day after the insect's death, initiating between the abdominal sutures, thorax, and head of the adult. Consequently, the insect is adhered to the surface where it dies. Then, mycelium continues with the legs, wings, antennas, and finally it covers the entire body of the insect (Fig. 1).

These symptoms match the ones described in *Schistocerca piceifrons peruviana* (Pariona *et al.,* 2007), as far as the invasion of the entomopathogenic in the hemocele, producing paralysis in the insect, which may explain the immobility and rigidity observed in *C. cyathicollis* adults.

In other tingidae like the lace bug of avocado (*Pseudacysta perseae*), *B. bassiana,* under laboratory conditions, produced a high level of mortality with mycelium presence starting with the fourth d of application. Furthermore, symptom appearance and verification through sporulation was clear (Almaguel *et al.,* 1997).

Average and ninety lethal concentrations of native isolation of *B. bassiana*

The mortality caused by the native isolation of *B. bassiana* in e ach treatment $1·10^3$, $1·10^5$, $1·10^6$, $1·10^7$, $1·10^8$ spores/mL) for 7 d post-inoculation was 46.6, 73.3, 83.3, 90.0, and 96.6% respectively, while in the control treatment there was no *C. cyathicollis* mortality of *C. cyathicollis*. The CL_{50} and CL_{90} concentrations, obtained through mortality data in each treatment, were $1.8·10^3$ and $6.5·10^6$ spores/mL respectively, according to the Probit analysis (Tab. 2).

These results indicate that there is a direct relationship between the spores' concentration in the native isolation of *B. bassiana* and the mortality of *C. cyathicollis* adults under the study conditions. Even though the *B. bassiana* fungus causes high mortality in the lace bug *C. cyathicollis* under laboratory conditions, it is not always reflected in the field due to the fungi structures and, particularly, to the dissection or inhibition spores may suffer by other microorganisms when they are exposed to the environment; among other factors such as humidity, solar radiation and temperature.

Conclusions

The native isolation of *Beauveria bassiana* presented as pathogenic in adults of the *Corythaica Cyathicollis* species under laboratory conditions, causing an intrinsic mortality of 92.4% at 12 d post-inoculation for the concentration $1·10^7$ spores/mL.

The average lethal concentration (CL_{50}) and the ninety lethal concentration (CL_{90}) of the native *B. bassiana* isolation against *C. cyathicollis* under laboratory conditions were $1.8·10^3$ and $6.5·10^6$ spores/mL respectively.

C. cyathicollis adults with symptoms of the infection produced by *B. bassiana* under field conditions can be recognized by the change in abdominal coloration, from black to reddish, which presents before the external emission of the fungus mycelium.

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