ENHANCEMENT OF *Beauveria bassiana* VIRULENCE AND EFFICACY TO CONTROL *Diatraea saccharalis* IN SUGARCANE CROP FOR PANELA PRODUCTION

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INTRODUCTION

The crop of sugarcane for *panela* production is of great importance in Latin America, Central America and India. *Panela* yield depends on sucrose content in sugarcane, characteristic seriously affected by the presence of the stem borers complex of the genus *Diatraea* sp. In Colombia, studies in the Cauca River Valley indicate a reduction in cane weight of 0.83% for each percentage unit of internodes bored, and a further loss of 0.26% of sugar yield at milling (Goméz *et al*., 2009). The use of insecticides has not been efficient due to the cryptic nature of this pest that develop its larval cycle inside sugarcane stalks. In a previous work, a Colombian isolate of *Beauveria bassiana* (Bv062) demonstrated high potential to develop a bioinsecticide for the control of *Diatraea* sp. The present work aimed to evaluate potential virulence enhancers for fungal conidia and determine efficacy of formulated conidia under laboratory and field conditions.

Courtesy. Yuly Paola Sandoval

EXPERIMENTS AND RESULTS

1. Evaluation of enhancers of insecticidal activity of *B. bassiana* Bv062

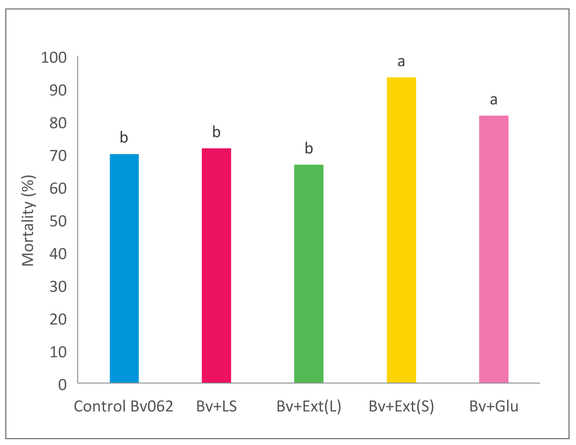
Enhancers substances (ES):
- Enzymatic extract from liquid fermentation of *Trichoderma koningiopsis* 2%
- Enzymatic extract from solid state fermentation of *T. koningiopsis* Th003 2%
- Glucose 2%
- Sodium Lignosufonate 2%

+ Preparation of Bv062 conidial suspensions $5x10^7$ con/mL

Inoculation: 2 µL on 2° instar *D. saccharalis* larvae.

Incubation: each larvae in a plastic cup with a corn grain. 25°C±2°C – RH:60%

Mortality was recorded every two days



- EXPERIMENTAL DESIGN: Completely randomized with three replicates and 10 larvae per replicate.
- DATA ANALYSIS: Efficacies were calculated according to Schneider – Orelli (Zar 1999):
- ANOVA and Tukey test (95%). All analyses were carried out using the Statistix (8.0) program.

Figure 1. Mortality (%) of *D. saccharalis* larvae caused by *B. bassiana* Bv062 alone and in mixture with enhancer substances.
Bv= *Beauveria bassiana*; LS=Sodium Lignosulfonate; Ext(S): Enzymatic extract from solid state fermentation; Ext(L): Enzymatic extract from liquid fermentation; Glu= Glucose. Treatments with the same letter do not present significant differences according to ANOVA test (95%). The analyzes were performed in pairs comparing each treatment with the control.

SELECTION

Glucose ✗ — Affected formulations process

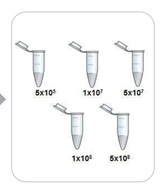
Enzymatic Extract ✓ — Enzymes are able to degrade insect cuticule and peritrophic matrix of the intestine (Molnar et al. 2010)

2. Insecticidal activity of formulation prototypes based on *B. bassiana* under controlled conditions.

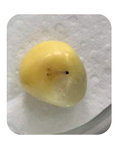
Formulation prototypes

Emulsifiable concentrate (EC)

Wettable Granulate (WG)



Preparation of conidial suspensions



Insecticidal activity on 2°instar *D. saccharalis* larvae.

- Experimental design: Completely randomized with three replicates and 10 larvae per replicate.
- Data analysis: lethal concentration – Probit Analysis – POLO PLUS

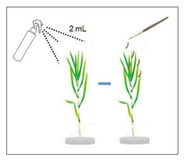
Table 1. Lethal concentrations LC_{50} y LC_{90} of prototypes based on *B. bassiana* Bv062, on *D. saccharalis* larvae

Treatments	LC_{50} (con/mL)	Confidences Limits (con /mL)		LC_{90} (con/mL)	D.F	X^2	P
		Lower	Higher				
EC	$1,2x10^6$	$8,4x10^3$	$4,3x10^6$	$1,0x10^9$	3	0,276	0,964
EC + enzymatic extract	$4,7x10^5$	$4,3x10^4$	$1,2x10^6$	$1,2x10^7$	3	0,119	0,989
WG	$1,6x10^6$	$3,2x10^5$	$3,4x10^6$	$8,0x10^7$	3	0,086	0,993
WG + enzymatic extract	$4,9x10^5$	$2,8x10^4$	$1,5x10^6$	$2,8x10^7$	3	0,290	0,962

Potency: increased 3 times with the addition of enhancers under laboratory conditions

3. Determination of insecticidal activity of formulation prototypes based on *B. bassiana* under semicontrolled conditions.

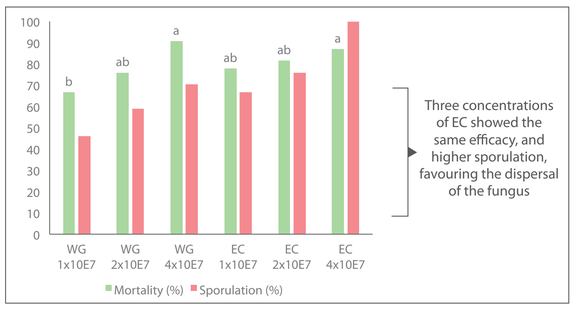
Treatments
T1: $1x10^7$ con/mL
T2: $2x10^7$ con/mL
T3: $4x10^7$ con/mL



Application of 2 mL of each treatment in sugarcane plants (15 cm approx.) and infestation with five *D. saccharalis* larvae.

After 8 days of application Destructive sampling: Recovery of live and dead larvaes.

Live larvae: Observations under laboratory conditions.



Three concentrations of EC showed the same efficacy, and higher sporulation, favouring the dispersal of the fungus

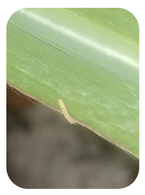
- Experimental design: completely randomized with four replicates and 10 plants per replicate.
- Data analysis: Mortality was corrected according to Abbott's formula. ANOVA and Tukey test (95%). All analyses were carried out using the Statistix (8.0) program

Figure 2. Effect of the application of formulations prototypes on based *B. bassiana* Bv062 on the mortality of *Diatraea saccharalis* larvae under semi-controlled conditions.
Treatments with the same letter do not differ significantly according to Tukey's test (95%).

4. Dose selection under field conditions.

Sugarcane plants (RD7711) – two months older

Treatments were applied using a backpack hydraulic sprayer
Treatments: three doses of the biopesticide and plants sprayed with wáter (control)



Manual infestation: six second instar larvae/plant

Day 7: larval recovery and evaluation under laboratory conditions

- Experimental design: Randomized complete blocks with three replicates.
- Each plot consisted of four rows of 1 m in length, spaced of 60 cm
- Sample size: 10 plants of the two internal rows of each plot
- Data analysis: Mortality was corrected according to Abbott's formula. ANOVA and Tukey test (95%). All analyses were carried out using the Statistix (8.0) program

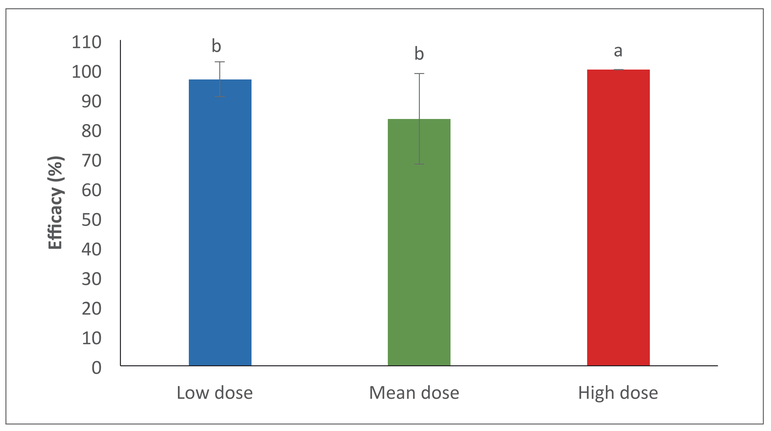
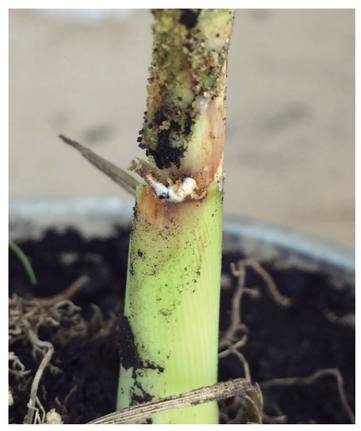


Figure 3. Efficacy of biopesticide (EC) based on *Beauveria bassiana* Bv062 to control *D. saccharalis* larvae in sugarcane plants under field conditions. High dose: $1,6x10^{12}$ con/ha, Mean dose: $3,2x10^{12}$ con/ha, High dose: $3,4x10^{12}$ con/ha
Treatments with the same letter do not differ significantly according to Tukey's test (95%). The error bars represent the standard deviation



Although with the high dose a significantly greater efficacy was obtained, the mean and low doses presented an efficacy over 80%, with high potential for the control of the pest.

CONCLUSIONS

- The use of enhancers substances (glucose and enzymatic extract) increased the mortality of the larvae caused by unformulated and formulated fungi under laboratory conditions
- Results demonstrated the high potential of the isolate Bv062 to control the predominant pest in sugarcane crops for *panela* production.

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