EVALUATION OF BACTERIAL ISOLATES IN THE CONTROL OF *Plutella xylostella* L. IN BROCCOLI†

[EVALUACIÓN DE LOS AISLADOS DE BACTERIAS EN EL CONTROL DE *Plutella xylostella* L. EN BROCCOLI]

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SUMMARY

The diamondback moth (*Plutella xylostella* L.) is an insect that causes great losses in Brassica plantations, which may reach 100% loss in some cases. The most common method to control this pest is still the use of insecticide. However, its successive and arbitrary use has contributed to the development of insecticide resistance, and the study of alternative methods has become essential to successfully control the diamondback moth in broccoli. The objective of this study was to select isolates of rhizobacteria that act to control diamondback moth in broccoli. The experimental design was in randomized blocks, with three bacterial isolates [1 - *Kluyvera ascorbata* (EN4); 2 - *Bacillus subtllis* (R14); 3 - *Bacillus cereus* (C210) and 4 - Control (distilled water + spreader sticker)] tested with 5 repetitions in 20 m^2 plots. Larval viability (LV), pupal viability (PV), larval stage duration (LD), pupal stage duration (PD), pupal weight (PW) and total mortality (TM) were evaluated. The rhizobacteria isolates performed better to reduce larval and pupal viability, larval and pupal duration, and increase total mortality, and can be used as a management option to complement the control strategies of the diamondback moth in broccoli.

Keywords: *Brassica oleraceae* var. *italica*; entomopathogens; biological control.

RESUMEN

La Palomilla dorso diamante (*Plutella xylostella* L.) es la plaga que causa los mayores perjuicios en plantaciones de brásicas, pudiendo llegar al 100% en algunos casos. El método de control más utilizado sigue siendo el químico, pero el uso sucesivo y indiscriminado de insecticidas en el control de esta plaga ha contribuido a la evolución de la resistencia a la mayor parte de los insecticidas comercialmente disponibles, con lo que el desarrollo de métodos alternativos se ha vuelto indispensable para el control exitoso de esta plaga. El objetivo de este estudio fue seleccionar rizobacterias que actúen en el control efectivo de la Palomilla dorso diamante en el cultivo del brócoli. El delineamiento utilizado fue de bloques al azar, donde se probaron tres aislados bacterianos: 1 - *Kluyvera ascorbata* (EN4); 2 - *Bacillus subtllis* (R14); 3 - *Bacillus cereus* (C210) y 4 – Control (agua destilada más dispersante adesivo), con 5 repeticiones, en parcelas de 20 m^2 (4,0 x 5,0 m). Se evaluaron la viabilidad larval (VL) y pupal (VP), duración larval (DL) y pupal (DP), peso pupal y mortalidad total (MT). El aislado bacteriano de *Kluyvera ascorbata* (EN4) fue el que presentó los mejores resultados para viabilidad larval y pupal, duración larval y pupal, además de la mortalidad total. Los aislados C210, EN4 y R14 pueden ser indicados para su uso en el manejo de la traza de las crucíferas en cultivos de brócoli.

Palabras clave: *Brassica oleraceae* var. *itálica*; los entomopatógenos; control biológico.

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INTRODUCTION

The Brassicacea plant family encloses about 350 genera and 3000 species, distributed throughout the world, majorly in temperate and tropical climates (Mendes, 2016). Within this family, the *Brassica* genus stands out by their socioeconomic importance. In tropical countries, *Brassica* species can be produced throughout the year, and have fast growth, high commercial and nutritional value (Kano *et al*., 2010).

Cabbage, cauliflower and broccoli are among the most cultivated and consumed *Brassica* species in Brazil (Bernardes *et al*., 2016). Broccoli (*Brassica oleracea* var. *italica*) is mainly grown in regions with mild temperatures (South and Southeast of Brazil), having most of its production destined for *in natura* consumption (Lalla *et al*., 2010; Filgueira, 2013; Carvalho *et al*., 2016).

The main factor of reduction of the production of these brassicas in Brazil and over the world has been the occurrence of the diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), that can cause losses of up to 100% in the crop. The importance of this pest is due to its high reproductive potential and short life cycle, with a high number of generations per year (Thuler *et al*., 2009). In addition, it has a cosmopolitan distribution, a voracious feeding behaviour and is hosted by many commercial plants (Renwick *et al*., 2006). This pest causes serious damage, depreciates the product, interferes with the growth of the plant and may lead to the death or total loss of the crop (Carvalho, 2008).

Its presence in Brazil is regularly reported and the chemical control is often the option chosen for managing it. According to Delen and Tosun (2004), chemical control has been the most widely used method in pest control because it is easy, provides quick and, most of the times, effective results, even though it increases production cost, changes the environmental balance and can be harmful to human health.

The successive use of insecticides to control diamondback moth has contributed to the evolution of resistance to most insecticides commercially available (De Bortoli *et al*., 2013). In 1989, this insect had proven resistance to 50 products insecticides (Georghiou and Lagunes-Tejada, 1991), while Maia (2005) reported that 504 species of insects presented resistance to pyrethroids, carbamates and phosphorous insecticides. Situations like that rise the need for alternative methods to control the diamondback moth.
The use of alternative methods to control this pest involves practices of integrated pest management, such as biological control, which can be performed in a straightforward way of releasing entomopathogenic microorganisms in the environment to control the pest; and in an indirect way using biopesticides containing parts or secretions of organisms (Federici *et al*. 2010).

According to Cardoso *et al*. (2010) and Filgueira (2013) to control Lepidoptera insect species, one should preferably use the *Bacillus thuringienses* biological control, which is a bacterium with exclusive action on caterpillars. The active ingredient spinosad is a secondary metabolite of aerobic fermentation of the actinomycete *Sacchapolyspora spiniosa*, which has moderate selectivity to the cruciferous moth parasitoids (Villas Boas *et al*., 2004). Also, among these microorganisms, highlight plant growth promoting rhizobacteria, which comprise a group with great potential as biological control agents (Thuler *et al*., 2006). In this context, the objective of this study was to select isolates of rhizobacterias that act in effective control of the diamondback moth in broccoli.

MATERIAL AND METHODS

Experimental area

The study was conducted in an experimental area from the Federal Institute of Triangulo Mineiro (IFTM), campus Uberaba, Minas Gerais, Brazil, between August 2010 and July 2012. The area is located at the latitude 19°39'44" South and longitude 47°57'58" West were the average altitude is 795 m.

Type of soil

The soil of the experimental area was characterized as a Red Latosol Dystrophic (Embrapa, 2013), medium texture, relief soft wavy. The layer of 0-0.2 m presents: 210 g kg^{-1} of clay, 710 g kg^{-1} of sand and 80 g kg^{-1} of silt, pH $CaCl_2$ 5.5; 76 mg dm^{-3} of P (resin); 2 $mmol_c$ dm^{-3} of K^+; 22 $mmol_c$ dm^{-3} of Ca^{2+}; 10 $mmol_c$ dm^{-3} of Mg^{2+}; 17 $mmol_c$ dm^{-3} of H+Al and 19 g dm^{-3} of soil organic matter.

Region's climate

The climate of the region is classified as Aw, tropical, hot, according to the classification of Köppen, having hot and rainy summer and cold and dry winter. In the region, the annual averages of rainfall, temperature and relative humidity of the air are 1.600 mm, 22.6 ºC and 68%, respectively (Uberaba, 2009).

Experimental Design

The experimental design was in randomized blocks, with three bacterial isolates: 1 - *Kluyvera ascorbata*

(EN4); 2 - *Bacillus subtllis* (R14); 3 - *Bacillus cereus* (C210) and 4 - Control (distilled water + spreader sticker), all conceded by the Laboratory of Phytobacteriology (Federal Rural University of Pernambuco), in broccoli plants (BR-068® Syngenta), with 5 repetitions in 20 m^2 plots (4 x 5 m). This broccoli hybrid features open canopy plants, with few leaves, uniformity of maturity and medium cycle (85 to 90 days in winter).

Soil Preparing and Fertilization

The soil was conventionally prepared with deep plowing, harrowing, then mechanically entourage and fertilized five days before transplanting the seedlings. The planting beds were mechanically prepared. During the preparation of the furrows, before transplanting the seedlings, manure was applied at an equivalent dose of 20 t ha^{-1}. Mineral fertilizer was applied during planting according to the Committee of Soil Fertility of the State of Minas Gerais (1999) at an equivalent dose of 100 kg ha^{-1} of P_2O_5, 100 kg ha^{-1} of K_2O and 50 kg ha^{-1} of N, 2 kg ha^{-1} of boric acid (17.5% of B). Nitrogen (50 kg ha^{-1} $application^{-1}$) was also applied at 30 and 45 days after planting.

Seed Inoculation

The inoculation was done by seed immersion in a suspension of each isolate [9x10^8 CFU mL^{-1}, in distilled water + spreader sticker (0.05%)]. The concentration of suspensions was calculated by the equation: $y = e^{(6.702-9{,}041x+11{,}159x^2)}$, using the values of absorbance of 0.77 nm (spectrophotometer). A control containing distilled water + spreader sticker (0.05%) was included among the treatments.

After drying for 12 h, the seeds were sown in polystirene trays with 128 cells, containing plant substrate made of pine bark and coconut fiber. Trays were kept in glasshouse. When the seedlings developed their fourth to fifth leaves (26 days after sowing), they were transplanted to the planting beds.

Fertilization

Fertilizer doses for Brassica crops were based on soil analysis and recommendations of the Soil Fertility Commission of the State of Minas Gerais (1999). The recommended nitrogen (N), phosphorus (P), and potassium (K) doses for the crops were: 150 kg ha^{-1} N (urea), 100 kg ha^{-1} P_2O_5, and 100 kg ha^{-1} K_2O. Nitrogen and potassium doses were split-applied: at planting, 30 days after planting, and 45 days after planting. In addition, 1 g of boric acid (17.5% B) was applied per pit.

Bioassay

The bioassay was performed 45 days after transplanting the seedlings to the planting beds. Leaf disks (8 cm diameter) were placed over filter paper lightly moistened with distilled water in 9 cm Petri dishes.

In each Petri dish, 10 second instar *P. xylostella* larvae were placed on a leaf disk. The Petri dishes were closed with plastic film, to keep moisture and prevent the larvae escape (Thuler *et al*. 2007). The insects were obtained from the creation-stock of the Laboratory of Entomology (IFTM) and kept according to Barros and Vendramim (1999).

After three days, the following biological characteristics of the insect were daily evaluated: larval viability (LV), pupal viability (PV), larval stage duration (LD), pupal stage duration (PD), pupal weight (PW) and total mortality (TM) - sum of mortality in all stages.

When the insects reached the pupal stage, they were weighted and transferred to ELISA® plates for individualization and observation of the emergence. After emerging, the sex of the adult was observed, and the sex ratio was calculated.

Statistical Analysis

The data were subjected to Deviance test for the generalized models at 5% probability, where the modelling was done using the probability distribution of the response variable. The total mortality, larval and pupal viability follow binomial distribution, the larval and pupal duration follow Poisson distribution and the variable pupal weight follows a Gaussian distribution. For the cases where the effect of the treatments was significant, the generalized Tukey test was used to compare the averages. The R Core Team software was used to carry out the analyses.

RESULTS AND DISCUSSION

Larval, pupal viability and pupal duration

The biological parameters of diamondback moth showed significant differences between the treatments tested, indicating that the use of the rhizobacterias R14, EN4 and C210, altered the biological cycle of the insect. In Figure 1, from a linear function supported by the Deviance test, it is possible to observe a significant difference between the treatments.

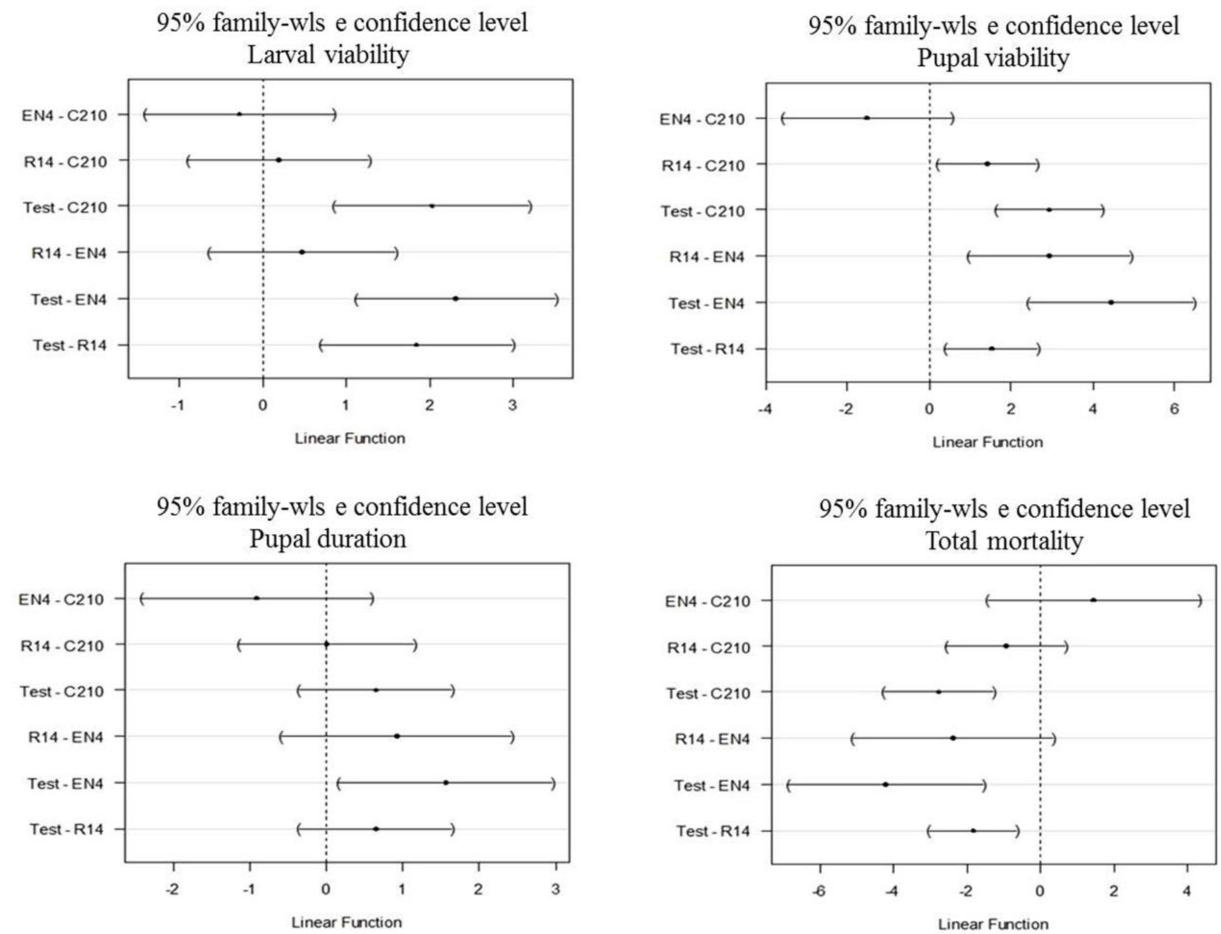

Figure 1. Generalized Tukey test for the biological characteristics of *Plutella xylostella* fed on leaves of broccoli plants inoculated and non-inoculated with isolates of rhizobacteria.

The values observed for pupal duration, and pupal and larval viability (Figure 2) were reduced and similar among the rhizobacteria evaluated, however they differed significantly from the control ($p<0.05$), proving that the treatments were efficient to the control the insect at these stages. This characteristic is important at the field level, because with the reduction of larval and pupal viability, the number of individuals reaching the maturity will be reduced, as well as the next generation of the insect in the field. Also, with a lower number of caterpillars, there are less damages and losses caused to crops, consequently, as highlighted by Maroneze and Gallegos (2009).

The lowest diamondback moth viability (larval and pupal stage) was caused by the isolate EN4 (26%), when compared to R14 (36%), C210 (38%) and control (65%), which suggests that the use of this EN4 rhizobacterium can be more effective in controlling diamondback moth. The influence of this rhizobacterium (EN4) on post-embryonic viability suggests that there is a cumulative effect of reductions in larval and pupal viability, resulting in a greater increase in total mortality. Similar results were observed by Crialesi *et al.* (2017), who showed that EN4 generally caused low larval and pupal viability, as well as reduction in pupal duration.

Evaluating the effect of plant growth promoting rhizobacteria on the development of *P. xylostella* on cabbage, Thuler *et al*. (2006) observed that post-embryonic stages viabilities were affected by isolates of *K. ascorbata* (EN4) and of *Alcaligenes piechaudii* (EN5), reducing by 80 and 50%, respectively, the viability of the such stages. In this study, the authors also highlighted that *Bacillus thuringiensis* pv. *kurstaki* (HPF14), *Bacillus amyloliquefaciens* (PEP81) and *Bacillus megaterium* pv. *cerealis* (RAB7) isolates also showed potential for use as an insect control strategy.

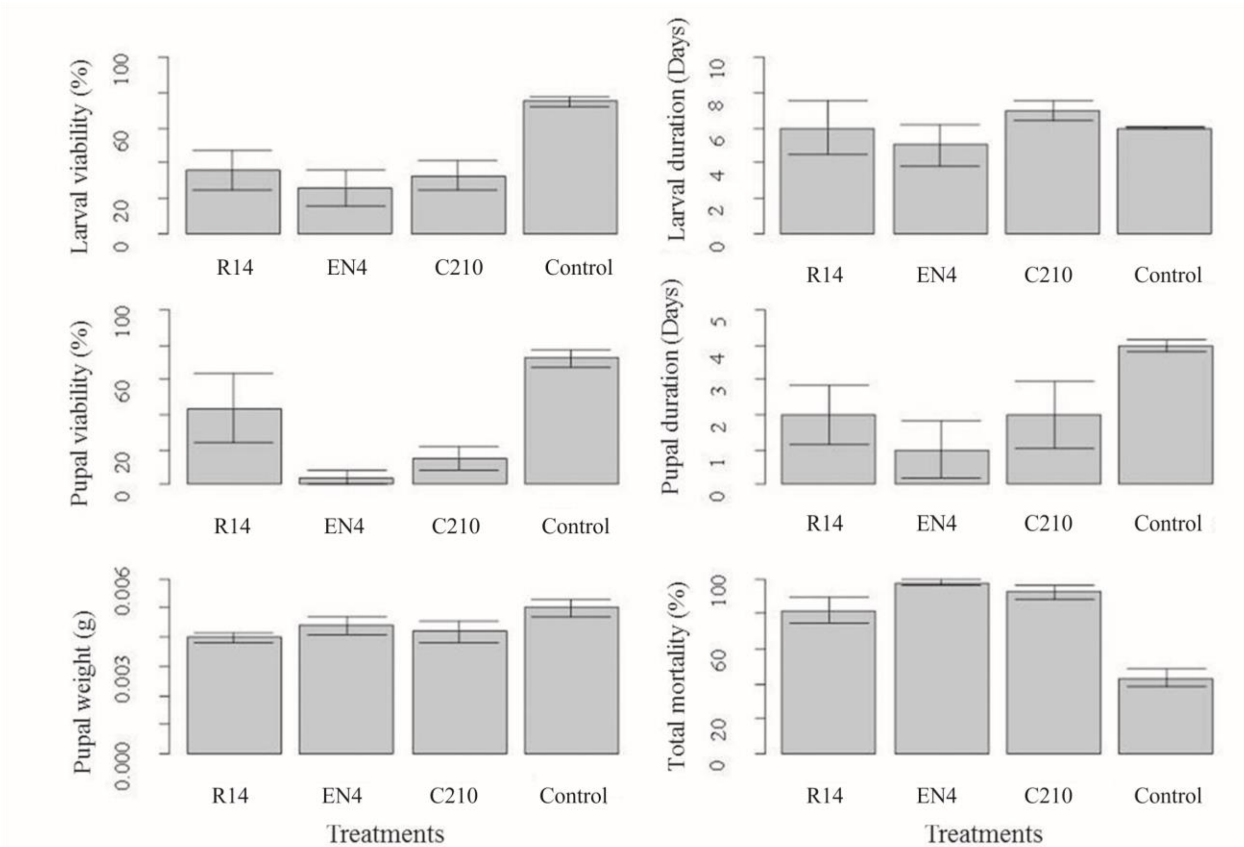

Figure 2. Means and standard error for biological characteristics of *Plutella xylostella* fed on leaves of broccoli plants inoculated and non-inoculated with isolates of rhizobacteria.

Larval duration and pupal weight

The larval stage duration was not affected by the rhizobacteria isolates. According to Hernandez and Vendramim (1997), a putative increase on this stage duration may be related to a small amount of food intake due to one or several factors, which decreased the feeding time on the leaf treated with rhizobacteria or yet as a result of any nutritional imbalance.

The pupal weight was not affected by the rhizobacteria either, suggesting that the caterpillars that were viable continued feeding normally, regardless of the treatments with rhizobacteria. This behaviour can be positive, because it shows that the insect doesn't avoid the treated plants, thus it will be affected by the rhizobacteria. Thuler *et al*. (2009) when evaluating diamondback moth resistance to glucosinolates, observed that the weight of the pupa is highly correlated with other parameters like the viability and duration of the larval and pupal stages.

Mortality rates

All the rhizobacteria evaluated caused high corrected mortality rates of diamondback moth, being 98% for EN4, 92.5% for C210 and 82% for R14, with no differences among them ($p<0.05$), but significantly higher than the control (43.2%). These results point out a direct influence of the rhizobacteria treatments on the diamondback moth biology. The raise of the diamondback moth mortality due to seed treatment with rhizobacteria isolates will lead to reductions on its population over the course of several generations of this insect.

Other studies found similar results when evaluating the effects of rhizobacteria treatments on control of insects. Medeiros *et al*. (2005) observed high larval mortality (LM) and low pupal viability (PV) of diamondback moth on cabbage when *Enterobacter cloacae* (ENF14), *Alcaligenes piechaudii* (EN5) or *K. ascorbata* (EN4) isolates were applied. The isolates *Enterobacter cloacae* (ENF14), *Alcaligenes piechaudii* (EN5) or *K. ascorbata* (EN4) were also observed to have control action against diamondback moth (Silva *et al*. 1999). In field experiments, the cucumber seed inoculation with a suspension of the *Bacillus pumillus* (INR-7) and *Serratia marcescens* (90-166) isolates was more efficient than insecticides to reduce the population of *Diabrotica undecimpunctata* (Barber) and *Acalymma vittatum*

(F.) (Coleoptera: Chrysomelidae) (Zehnder *et al*. 1997).

Our results are supported by other previous studies and contribute to improve the knowledge about the use of rhizobacteria as a biological option to control insects in agriculture. This procedure can be easily incorporated to any strategy of broccoli cropping, and has the potential to reduce the use of chemical insecticides, environmental disturbances and human exposition to hazardous products, and increase cropping profits.

CONCLUSIONS

All rhizobacteria isolates evaluated caused low larval and pupal viability, decreased larval and pupal stage duration, and resulted in a high total mortality.

These isolates can be indicated as biological control agents in a management strategy to control diamondback moth in broccoli.

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