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Kluyvera ascorbata: A Plant Growth-Promoting Bacteria (PGPB) to Manage *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae)

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Abstract – **The aim of this study was to evaluate the effects of different concentrations of the endophytic isolate EN4 of the plant growth-promoting bacteria (PGPB) *Kluyvera ascorbata* in the development of *Plutella xylostella*, and the influence of the bacteria on the biological characteristics of the pest in different stages of vegetative growth of cabbage. Different concentrations of PGPB were sprayed on kale leaves before they were fed to caterpillars in the laboratory. After consumption, we evaluated the biological characteristics of the insects. Overall, the results demonstrated that the EN4 isolate had a negative effect on *P. xylostella* because the viability (larval and pupal) was dramatically reduced in treatments containing the bacteria, which led to a significant reduction in the insect population and less damage to the plants.**

Keywords – **Diamondback Moth, Biological Control.**

I. Introduction

The diamondback moth, *Plutella xylostella* (L., 1758) (Lepidoptera:Plutellidae), is a major pest of Brassicaceae. *P. xylostella* causes damage to commercial plantations in several producing regions of the world, reducing the production and invalidating some growing areas, especially during hot and dry seasons, which promote better conditions for population increase [5]. Its control primarily uses insecticides that, supposedly, bring quick and convenient results. However, besides presenting a risk of contamination to the environment, animals, and humans, the continuous and inadequate use of these products can lead to selection of insecticide-resistant populations, making this method ineffective.

In many regions, insecticides are applied up to 3 times per week, and yet, the damage caused by insects is not satisfactorily reduced [4]. [1] released a report on activities related to the 2010 Program for the Analysis of Pesticide Residues in Food. Kale and cabbage are among the tested crops with high use of unauthorized products and residues above the authorized limit. To minimize insecticide-resistance problems, it is necessary to reduce the number of insecticide applications using information about economic injury level [3] and change the insecticide mode of action.

To minimize insecticide-resistance problems, it is necessary to reduce the number of insecticide applications using information about economic injury level [3] and change the insecticide mode of action. In addition, integration of alternative techniques such as the use of resistant varieties, pheromones [8][11], insecticidal plants [23], crop rotation, removal of crop residues [5], sprinkler irrigation to reduce the number of larvae on plants [12], use of biological control agents [6], and use of bacteria that promote plant growth [2] can reduce the population of the pest and minimize damage.

The induced-resistance of plants can be caused by insect injuries and microorganism infection. These factors trigger certain metabolic processes in plants, leading to the production of chemical compounds that are often involved in plant resistance to pests [15]. These compounds are generically called secondary substances and are not related to primary plant functions such as photosynthesis, respiration, and plant growth [13]. Among the insect specialists of Brassicaceae, secondary chemicals such as glucosinolates incite feeding and stimulate oviposition [17].

Among the microorganisms that act as mediators of induced-resistance are plant growth-promoting bacteria (PGPB), which are found naturally in the soil and colonize epiphytic or endophytic plants. PGPBs provide positive effects to plants, including further development of plants, resistance to arthropods and disease, and adaptation to environmental stresses [7][10][18][22]. The PGPBs act on biochemical pathways that are related to plant secondary metabolites, and they promote changes in some of these substances that act as elicitors that induce defense responses in plants, resulting in the expression of induced systemic resistance [24]. When applied directly to insects, PGPBs have an entomopathogenic effect [19].

This work aimed to evaluate the effects of different concentrations of the EN4 isolate of the PGPB *Kluyvera ascorbate* in the development of *P. xylostella*.

II. Materials and Methods

The experiment was conducted in the Laboratory of Biology and Insect Rearing (LBIR), Department of Crop Protection, Sao Paulo State University—Faculty of Agriculture and Veterinary Science (Unesp), Jaboticabal, Sao Paulo, Brazil.

P. xylostella larvae were obtained from a stock rearing from the LBIR and fed on kale leaves cv. Manteiga, according to procedures recommended by [21].

The endophytic isolate EN4 of the PGPB *K. ascorbata* was obtained from the collection of the Laboratory of Phytobacteriology, Department of Agronomy, University Federal Rural of Pernambuco-UFRPE, Recife, PE.

The bacteria were inoculated into Petri dishes containing Nutrient Yeast Dextrose Agar (NYDA) culture medium [14]. After 36–48 h of incubation at 30°C, bacterial suspensions were prepared in sterile distilled water at 9 ×

10^8, 9×10^9, 9×10^{10}, 9×10^{11}, and 9×10^{12} CFU/mL. The adhesive spreader Tween 20® was added to the suspensions at a concentration of 0.05%, and suspensions were quantified in a spectrophotometer Biospectro SP-220. These concentrations were obtained by applying the formula $y = e^{(6.702 - 9.041\alpha + 11.159x^2)}$, where "x" corresponds to CFU/mL and "y" corresponds to the relative absorbance (optical density) observed in the spectrophotometer.

The suspensions of bacteria were sprayed on kale leaf disks "Manteiga" that were 8 cm in diameter, with application of 1 mL per disk. In the control, we applied only distilled water and 0.05% Tween 20®. The spraying was performed with a gun for airbrush painting, coupled to a compressor (Schulz, MS Model 2.3) which was operated at a laminar flow of 25 lbf/pol^2.

After drying at environmental temperature, the disks were individualized in Petri dishes and kept on a disk of filter paper moistened with distilled water. Twelve second instar larvae of *P. xylostella* were placed on each leaf disk. The dishes were wrapped with polyvinyl chloride (PVC) plastic film and maintained in a room at a temperature of 25 ± 1°C, 70 ± 10% relative humidity, and 12 h photophase. Each treatment contained 5 replicates with 12 larvae per replicate. The pupae were individually placed in ELISA® plates until adult emergence. The following biological characteristics were evaluated: duration and viability of larval and pupal stages, pupal weight, and sex ratio of adults. The assessments were performed every 24 h.

To determine the response of *P. xylostella* to the consumption of leaves sprayed with different concentrations of EN4 isolates, the results weresubjected to regression analysis. The analysis was conducted using SAS Proc REG [16] considering the larval and pupal duration and viability, weight of pupae, and sex ratio as dependent variables (y) as a function of the concentrations of bacteria as independent variables (x). The curve model was selected on the basis of the significance of the coefficient ($P < 0.05$) and its contribution to a better biological representation.

III. Results and Discussion

The concentration of 9×10^8 CFU/mL was found to promote the longest duration of larval period (Fig. 1A), with an increase of 0.52 days compared to the control. On the other hand, 9×10^{11} CFU/mL reduced the larval stage by 0.76 days. The other concentrations tested resulted in larval periods that were similar to that of the control.

The pupal period (Fig. 1B) was higher in the concentrations of 9×10^8, 9×10^9, and 9×10^{11} CFU/mL than in the control, with increases of 0.15, 0.43, and 0.14 days, respectively; the pupal period was reduced by 0.91 and 1.81 days with 9×10^{10} and 9×10^{12} CFU/mL, respectively. The pupal weight (Fig. 2C) decreased by 0.32 mg with 9×10^9 CFU/mL compared to that in the control, and the pupal weight increased by 0.30, 0.33, 0.77, and 0.28 mg for the concentrations of 9×10^8, 9×10^{10}, 9×10^{11}, and 9×10^{12} CFU/mL, respectively.

The ratio of the number of males to the number of females (Fig. 1D), as evidenced by values less than 0.5, was higher in the treatments compared to the control.

The larval and pupal viabilities (Figs. 1E and 1F) were negatively affected by all concentrations tested, with 9×10^{10} CFU/mL being the most harmful (33.33 and 23.17%, respectively), with a reduction of 40.01 and 27.57% compared to control. The number of adults obtained was not sufficient for a fertility study.

The results showed that the isolate EN4 had a negative effect on diamondback moths because the viability in larval and pupal stages was drastically reduced in the treatments containing the bacteria. [20], studying several isolates of PGPB, demonstrated that EN4 stood out because it reduced larval viability of *P. xylostela* by 80% at a concentration of 9×10^{10} CFU/mL.

The sex ratio showed values below 0.5, indicating a probable reduction in the number of insects in subsequent generations, which leads to a significant reduction in the population, and thus, less damage to the plants.

Moreover, a low concentration of EN4 increased the duration of the larval and pupal stages, whichcontributed to a lower number of annual generations of the pest.

While most biological control agents act against a restricted group of pests, PGPBs act against a broad spectrum of insects and pathogens. These advantages, along with the great period of activity demonstrated in this work and the fact that PGPBs do not cause risks to the environment, promote resistance in insect pests, and rely on the farmer's technical knowledge to use this technique to manage diamondback moth, makes this control strategy a good choice for pest control use in brassicas.

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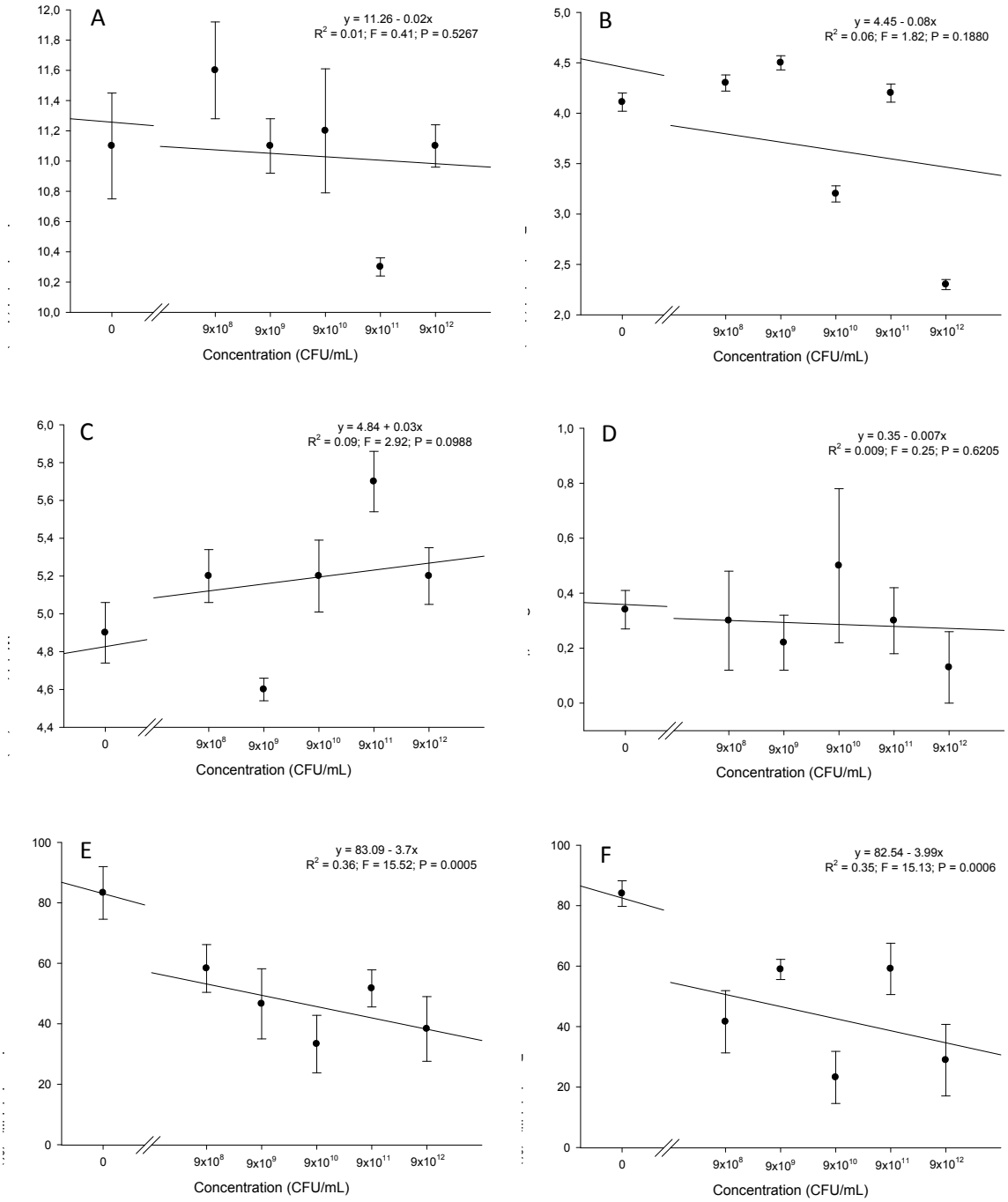

Fig.1. Regression curves for the biological characteristics of *Plutella xylostella* fed with kale leaves ("Manteiga") treated and untreated with EN4.