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Bioinsecticide activity of *Bacillus thuringiensis* isolates on tomato borer, *Tuta absoluta* (Meyrick) and their molecular identification

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ABSTRACT

Twelve (12) bacterial isolates were isolated from dead larvae of *Tuta absoluta* (4th instar) from tomato cultivated fields at Fayoum Governorate, Egypt. All isolates were preliminarily identified as members of the genus *Bacillus* based on morphological and biochemical characteristics. According to the results of the pathogenicity of *Bacillus* isolates against different larval instars of *T. absoluta,* the 12 isolates revealed varying efficiencies and the isolates B1, B2, B3 and B4 showed high mortality of 93.3, 90, 86.7 and 80% on day 7, respectively, on the 4th instar larvae. Also, protecto (*Bacillus thuringiensis* subsp. *kurstaki*) recorded the highest mortality when the 4th instar larvae were treated with 2 g / 2 liter of water (96.7%) on the 5th day post treatment. Isolate B12 recorded the lowest percentage mortality of the 4th instar larvae (13.3%). In addition, there was a significant decrease in egg hatching percentage reaching 44.12% compared with the control, meanwhile, the adult emergence decreased after treatment and only 38 and 30 adults emerged from the cages containing tomato plants infested by eggs with B1 and protecto compared with the control which gave 253 adults that emerged. Further, genetic identification of 12 isolates was performed using randomly amplified poylmorphic DNA (RAPD) markers to determine their genetic diversity pattern. Different random primers were used for RAPD amplification, which generated a total of 52 fragments; of these 42 were polymorphic and 10 monomorphic. The primers OPA02, OPA04, and OPA07 produced 100% polymorphic fragments, whereas primers OPA1, OPA3, OPA05, OPA06, OPA08 and OPA09 produced 1, 3, 1, 2, 1 and 2 monomorphic fragments, respectively. When the RAPD banding pattern data was subjected to dendrogram construction, the 4 isolates fell into two separate clusters, clusters I cluster II, which includes 1 and 3 *B. thuringiensis* isolates, respectively. The RAPD technique was shown to be effective in differentiating closely related isolates and applied to confirm the identification of *Bacillus* isolates by API system which was used to reveal the phylogenetic relationships between the isolates.