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Native *Bt* strains efficacy against cotton aphid *Aphis gossypii* Glover

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Abstract

The microbial pesticide market is dominated by *Bacillus thuringiensis* (*Bt*) but still a significant number of insect pests that cause immense losses on crop production are not sensitive to the commercially available *Bt* toxins in spite of the variability of Cry proteins. In the present study three native *Bt* strains (VKK-ENT1, VKK-ENT2, VKK-ENT3) were isolated from the soil and evaluated their efficacy against the cotton aphids in three different forms of feeding assays *i.e.* pre-solubilized, solubilized and trypsinized form. In pre-solubilized form the mortality ranged from 23.33 to 46.66%. In solubilized (30 to 53.30%) and trypsinized form (13.33 to 36.66%) mortality was found. Further studies are required on biochemical, physiological and molecular characterization of these *Bt* isolates.

Keywords: *Bacillus thuringiensis*, Cry toxins, Cotton aphids

Introduction

Insect pests cost billions of dollars in the form of crop losses and insecticides usage every year globally. The indiscriminate use of chemical insecticides for the last six decades has led to several problems such as insecticide resistant populations, major outbreaks of secondary pests, pest resurgence, besides environmental pollution and increase in human health problem. Resistance is a critical area of pest control and is indeed a global phenomenon. In India, insects alone are reported to cause crop losses worth US \$ 15000 million annually (Dhaliwal *et al.*, 2010) [6]. More than 500 species of insects and related arthropods have evolved resistance to one or more insecticides globally (Whalon *et al.*, 2013) [17]. Safety and environmental issues surrounding the use of chemical insecticides has led to the emphasis on development of alternative control measures such as bio-pesticides.

The bio-pesticides are living organisms, which are pathogenic for the agricultural pests. Bio-pesticides or biological pesticides include bio-insecticides/microbial insecticides, bio-fungicides (*Trichoderma*) and bio-herbicides (*Phytophthora*). These bio-insecticides contain microbial or biochemical agents produced by microorganisms. The benefits of microbial control agents are efficiency, safety for humans and other non-target organisms, reduction of pesticide residues in food, conservation of other natural enemies (Lacey and Sigel, 2000) [8]. Bio-pesticides represent only 2.5% of total pesticide market but their share is expected to increase to about 4.2%, or more than \$1 billion, of which 90% of all bio-pesticide products are based on *Bacillus thuringiensis* (*Bt*). The reason behind success of *Bt* as bio-pesticide is its quick and sustained larvicidal activity and can be applied using standard equipment with negligible effects on beneficial insects and non-target organisms. Moreover, it is the most prospective for the production point of view, amenable to genetic engineering and above all compatible with integrated pest management.

Bt is widely distributed in the environment. The ingested Cry protein is activated by insect gut proteases, bind to and get inserted into the microvilli brush-border membranes in the midgut of susceptible insects leading to disruption of osmotic balance, lysis of epithelial cells, starvation and ultimate death of insect (Schnepf *et al.*, 1998) [13]. Pathogenicity and specificity are determined by the functional cry gene types that an isolate possess. Recently, Crickmore *et al.* (2014) [4] described the schematic overview of the current nomenclature system used by the *Bt* toxin nomenclature committee for δ - endotoxins (Cry and Cyt) and secretable toxins (Vip and Sip). The numbers for the proteins change depending on the percentage of amino acid similarity. Till date, more than 700 cry gene sequences coding for cry proteins are reported (Van Frankenhuyzen, 2009) [9] and large plasmids are the usual location for these genes.

Cotton is affected by a severe pest complex. Aslam *et al.* (2004) [2] reported that boll worm & sucking pest complex cause about 20-40% yield losses in Pakistan. Cotton aphids reduce leaf area by 58% and shoot biomass by 45% and in addition,

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virus transmission can lessen the cotton yield up to 30-40% (Sarwar *et al.*, 2014) [12]. Though cotton aphid is reported as an intermittent and secondary pest, it is often cited that it has the potential to be a serious pest in any season (Akey & Bulter, 1989) [1]. It is still necessary to search for more toxins, since a significant number of pests remain to be uncontrolled with the available Cry proteins and to provide alternatives to overcome the problem of insect resistance especially with regard to the expression of *Bt* genes encoding insecticidal proteins in transgenic plants.

Material and Methods

Bt strains isolation

Soil samples were collected from different locations from fields of Division of Entomology, IARI, New Delhi. Sodium acetate selection procedure was used for Isolation of *Bt* strains developed by Travers *et al.* (1987) [14] and modified by Carozzi *et al.* (1991) [3]. The isolated strains were separated based on the morphology studies. After that Gram staining and phase contrast microscopy was done to identify the spores and crystals. From all the *Bt* strains spore crystal complex was prepared and used for further studies.

Aphids source and rearing

Initially aphids were collected from the fields of Indian Agricultural Research Institute, New Delhi. Aphids were reared on cotton twigs by changing the twigs every alternate

days under controlled conditions of 18 ± 2 °C, $70 \pm 10\%$ RH, and 16:8 L:D in the biochemical oxygen demand (BOD) incubator.

Bioassays

For conducting of bioassays an artificial diet was standardized. Bioassays were performed in three different forms *i.e.* pre-solubilized form, solubilized form, trypsinized form by diet incorporation method at single concentration (10 $\mu\text{g g}^{-1}$ of diet) on the basis of total protein concentration. Mortality data was recorded and per cent mortality was calculated on 4th day of bioassay.

Results

None of *Bt* strains isolated from the soil *viz.*, VKK-ENT1, VKK-ENT2, VKK-ENT3 attained more than 50% mortality in pre-solubilized and trypsinized form. In pre-solubilized form mortality ranged from 23.33 to 46.66%. But in case of VKK-ENT1 attained only 23.33% mortality. In solubilized form mortality ranged from the 30 to 53.30% mortality. However in trypsinized form all the soil isolated strains were attained nominal mortality. The mortality ranged from 13.33 to 36.66%. The strain VKK-ENT2 showed 13.33% mortality. In all the three forms the strain VKK-ENT1 was showed very less efficacy against adults of cotton aphids (Table.1 and Fig. 1).

Table 1: Toxicity of *Bt* strains against cotton aphids

S. No	<i>Bt</i> Strains ID	Corrected per cent mortality*		
		Pre-solubilized	Solubilized	Trypsinized
<i>Bt</i> strains isolated from soil				
1	VKK -ENT1	23.33	30.00	36.66
2	VKK -ENT2	40.00	53.30	13.33
3	VKK -ENT3	46.66	36.66	26.66

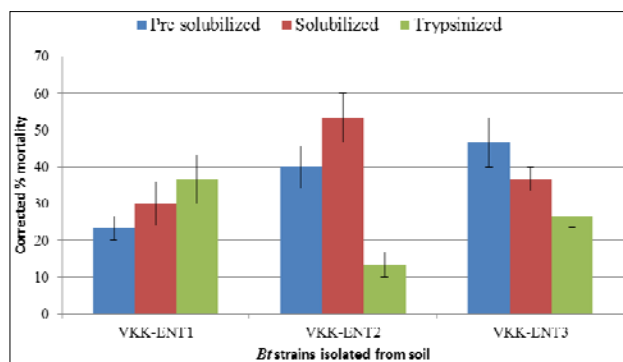


Fig 1: Efficacy of *Bacillus thuringiensis* strains isolated from soil in pre-solubilised, solubilised and trypsinized form against adults of *Aphis gossypii*

Discussion and Conclusion

Bt can be present naturally in many different habitats such as soil, ware houses, insect cadavers, grains, agricultural lands, olive tree related habitats, phyllosphere, and aquatic environments (Kaur and Singh, 2000; Saravanan and Gujar, 2006) [7, 11]. The microbial market is dominated by *Bacillus thuringiensis*, still a significant number of insect pests that cause immense losses on crop production are not sensitive to the commercially available *Bt* toxins in spite of the variability of Cry proteins. The present studies determine reserves of *B. thuringiensis* isolates in soil.

In our studies three *Bt* strains were isolated from the soil and efficacy was tested against adults of cotton aphids. Studies on

Bt toxicity against Hemipterans are rare due to the fact that rearing protocols for Hemipterans in laboratory have not been completely established. Merely a few Cry proteins have been found to be weakly to moderately active against Hemipterans in artificial diet feeding assays against potato aphid (Walters and English, 1995) [16] and pea aphid (Porcar *et al.*, 2009). Interestingly, variable toxicity was observed in the *Bt* strains isolated from soil. These results agrees with earlier hypothesis (Cristotoletti *et al.*, 2003; Li *et al.*, 2011) [5, 9] to certain extent but the lowest efficacy of trypsinized form of protein yet to be elucidate. Further studies are required on biochemical, physiological and molecular characterization of these *Bt* isolates.

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