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Mini- Review Article

A mini-review of *Bacillus thuringiensis* application to control important economic and zoonotic parasites

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Abstract

Bacillus thuringiensis (*Bt*) is a gram-positive and ubiquitous bacterium, isolated from various habitats, materials, and organisms. Over 100 varieties and ~60,000 strains of this bacterium have been identified. The produced toxins from this bacterium are specific to invertebrates with various modes of action. More reports are available regarding toxins indicating potential for biological control against agricultural pests and human and animal parasites. Therefore, an excellent and promising prospect for their use in the future as unique alternatives for current synthetic pesticides can be imagined. In this mini-review, we only reviewed the findings of the previous studies about the biological activity of *Bt* products on important medical and veterinary parasites.

***Keywords**: Bacillus thuringiensis*, Biocontrol, Toxin, Zoonotic, Parasites

Introduction

Bacillus thuringiensis (*Bt*) is a gram-positive, spore-forming, entomopathogenic, and ubiquitous bacterium. This bacterium has been isolated from various habitats, materials, and organisms. To now, over 100 *Bt* subspecies (such as *Bt kurstaki* (*Btk*), *Bt israelensis* (*Bti*), *Bt morrisoni* (*Btm*), *Bt osvaldocruzi* (*Bto*), *Bt thuringiensis* (*Btt*), *Bt aizawai*, *Bt tenebrionis*, *Bt tolworthi*), and several strains for each subspecies have been identified. Therefore, each subspecies produces one or more specific toxins (proteins). Based on the last findings, ~952 toxin genes, encoding different toxins, and ~60,000 strains have been identified. The produced toxins are highly biodegradable, fast-acting, specifically toxic to invertebrates, non-toxic to vertebrates or beneficial invertebrates, easily applicable and cheap. More reports are available concerning their application for biological control against the most important agricultural pests and some important human and animal parasites. Most toxins are produced during the sporulation phase, known as δ-endotoxins. *Bt* strains can also synthesize other toxins during the vegetative growth phase, including: 1) Vegetative Insecticidal Proteins (VIP), and 2) Secreted Insecticidal Protein (SIP). Except for these principle toxins, other toxins are also synthesized by *Bt* strains (Palma et al., 2014; Dunstand-Guzmán et al., 2015; Salehi-Jouzani et al., 2017). The classification of *Bt* toxins is as follows:

A. δ-endotoxins

δ-endotoxins produced by *Bt* strains are functionally two types: Crystal (Cry) and Cytolytic (Cyt) toxins (Fig. 1). These toxins are environmentally safe with specific modes of action. δ-endotoxins are produced during the sporulation phase. Once ingested by a specific invertebrate, these toxins are solubilized in the digestive system; the released protoxins are then proteolytically activated by proteases and bind to specific receptors located on the cell membrane, leading to osmotic lysis, cell disruption, and parasite death (Salehi-Jouzani et al., 2017; Carvalho et al., 2021).

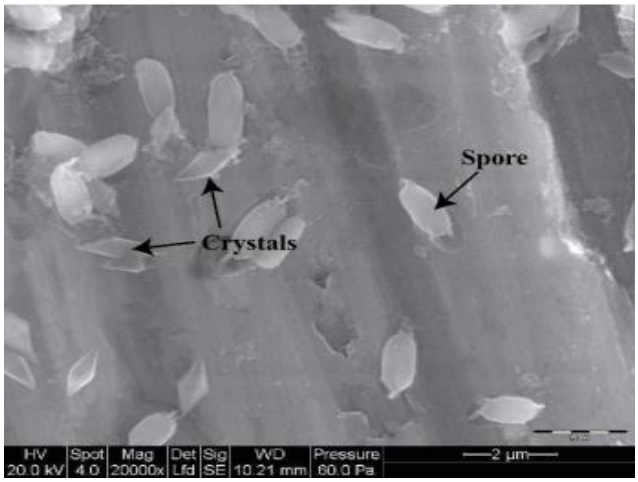

Fig.1. Spores, Crystal proteins (toxins) of *Bt* H29.3 strain (Palma et al., 2014).

A.1: **Cry toxins**

Currently, Cry toxins constitute the largest group of toxins. Based on the amino acid sequence similarities, 74 Cry toxin families (Cry1–Cry74) with 770 different Cry genes have been specified. Cry toxins are structurally classified into two families: 1) Three domain, 2) Non-three domain Cry toxins (Salehi-Jouzani et al., 2017).

A.1.1: **Three domain Cry toxins** (the major Family)

Bt three domain Cry toxins display toxic activity against different orders of Arthropoda and nematodes. Three different models have been proposed for the mode of action for these toxins: "classical", "sequential binding", and "signaling pathway" models (Palma et al., 2014).

A.1.2: **Non three domain Cry toxins**

Several families of unrelated toxins belong to these toxins, including: ETX_MTX2 family, Toxin_10 family, and aerolysin family. A similar mode of action for three domain Cry toxins is also attributed to these Cry toxins (Palma et al., 2014).

A.2: **Cyt toxins**

Cyt toxins have been primarily classified into three different families (Cyt1, Cyt2, and Cyt3). These toxins constitute a more minor, distinct group of toxins exhibiting a general cytolytic activity mostly against some mosquitoes and black flies. Additionally, some Cyt toxins have toxicity on mammalian cancer cells. Another interesting characterization of Cyt toxins is their potential to synergize the parasitic activity of other Cry or VIP toxins and to decrease possible resistance to some Cry toxins. Two different modes of action have been proposed for Cyt toxins: 1) a pore-formation mode, and 2) a less specific detergent action mode (Palma et al., 2014; Salehi-Jouzani et al., 2021).

B. **Secreted toxins**

B.1: **VIP toxins**

In the vegetative growth phase, some *Bt* strains produce toxins secreting into the culture. Studies have demonstrated that these toxins also have biotoxicity on various insects. These toxins are two types: 1) "Vegetative Insecticidal Proteins (VIP)" 2) "Secreted Insecticidal Protein (SIP)". To now, ~138 different VIP genes have been identified encoding VIP toxins being categorized into four families (VIP1-VIP4; Salehi-Jouzani et al., 2017).

B.2: **SIP toxins**

Data regarding these toxins are rare. Only for SIP1Aa1, it has been proposed that its toxicity may be caused by pore-forming, but its mode of action is unclear (Palma et al., 2014).

C. Other toxins

Due to little investigation, data regarding accessory toxins of *Bt* strains are also rare but efforts are being made to discover novel toxins of this group along with their modes of action. Some toxins related to this group are: A 41.9-kDa protein, Sphaericolysins, Alveolysins, ß-exotoxins, S-layer proteins, Thuringiensin, Enhancin-Like proteins, P19 and P20 Helper proteins. Evidence of the

toxicity of some of these toxins on some invertebrates is available (Salehi-Jouzani et al., 2017).

Generally, the parasitic activities of *Bt* products have been characterized since 1901 (Kondo et al., 2002). Approximately, 30 years ago, some novel strains were found to be toxic to a wide variety of parasite species belonging to Arthropoda, Protozoa, and Helminths (Xu et al., 2004). In this mini-review study, we intend to review the most important findings of research regarding the toxicities of *Bt* toxins on parasites that are important from a health and economic point of view:

1. Arthropoda

1.1. Mosquitos

Compared to other parasites, most studies related to *Bt* parasitic activity have been conducted on mosquitoes. Overall, the laboratory and field trials have indicated that *Bti* has considerable activity on various mosquitoes. Some of the most important findings resulting from the mosquitocidal activities of *Bt* strains are as follows:

In assessing long-time exposure of continuously generated larvae of *Aedes aegypti* to *Bti* toxins no resistance has been found in the biological parameters (Carvalho et al., 2021). 30 serial generation of *Ae. aegypti* exposed long-term to *Bti* did not show any altered susceptibility to this subspecies or its other individual toxins (Cry11Aa and Cry4Ba). For each larval generation, *Bti* exposure resulted in a mean of 74% mortality (Carvalho et al., 2018). In the infection of L_3 larvae of *Aedes caspius* with *Bt*, an initially significant increase and then regulation to normal level in phenoloxidase activity and nitric oxide level has been recorded. Also, a significant elevation of cellular apoptosis, leading to cytological damage in the gastric caeca, and finally, the significantly smaller body sizes of larvae before death, has been reported (Ahmed, 2013). In *Anopheles coluzzii* L_3 larvae subjected to LC_{70} doses of *Bti*, the resultant was a reduced longevity in adults. In the exposed females, time to death was shortened by 2.58-times, longer wings were found 12%, and no difference in the egg-laying rate was found between the treatments (Gowelo et al., 2020). Under laboratory and semi-field conditions, in which two commercial products of *Bti* were evaluated on *An. stephensi* larvae, although in the lab the susceptibility was found for both VectoBac-WDG (Water-Dispersible Granule formulation) and Mousticide WP, it was insignificantly higher to the latter. In a semi-field trial, VectoBac-WDG was more active (Naz et al., 2014). In Iran, five doses of a *Bt* formulation (Bioflash®) were applied to laboratory and semi-field bioassays on *An. stephensi*. The manufactured dose ($0.017g0.1m^{-2}$) indicated the highest mortality rate by two assays. Under the semi-field conditions, the application of only Bioflash® accompanied by very low efficiency (21%). These findings have been attributed to some important eco-biological factors (Hosseinpour et al., 2019). Different concentrations of *Bti* AM65-52-WDG formulation (VectoBac®) against *Anopheles* larvae and pupae, showed that 100% of larvae and 98.5% of pupae perished with all doses of WDG, even at 0.2 $kgha^{-1}$ showed highly mosquitocidal activity and higher concentrations remained a slightly long time residual effect, by three days (Dambach et al., 2014). To investigate the oxidative stress and apoptotic signs suggested pathogenicity biomarkers for the insecticidal activity of *Bt* on *Culex pipiens*, the results showed an increase in the rats of lipid peroxidation and protein oxidation biomarkers. Although, the infected larvae and adult mosquitoes showed significantly higher levels of both lipid peroxidation and protein oxidation, the infected larvae indicated significantly higher oxidative stress and apoptosis signs (Ahmed, 2013). Surprisingly, similar results were found for the alteration of oxidative stresses in *Btk*-infected larvae and adults of *Ae. caspius* (Ahmed, 2012). Bioassays on *Culex quinquefasciatus* and *An. albopictus* using *Bt* S2160-1 and *Bti* indicated that *Bt* S2160-1 and *Bti* displayed high toxicity to larvae of two species with LC_{50} = 5.668 and LC_{50} = 21.113 $ngml^{-1}$, respectively (Zhang et al., 2012). In Zomba, the lower effective doses of granular *Bti* to cause 100% mortality for *Culex* and *Anopheles* larvae were determined as 47.73 and$103.41gha^{-1}$,

respectively. This difference was attributed to physiological and behavioral differences in these two mosquitoes. In addition, it was found that when *Culex* and *Anopheles* larvae were subjected to a similar dose of liquid *Bti* (0.001mlL^{-1}), *Culex* larvae were slightly, but insignificantly, more susceptible than *Anopheles* ones (Dylo et al., 2014). Larvicidal bioassay of *Bt* isolates removed from different habitats in Saudi Arabia against *Cx. pipiens*, indicated that three isolates had approximately similar activity to the reference *Bti* (*Bti*-H14), while seven ones had 1.6-5.4 times more activity than *Bti*-H14. 23 isolates (Ahmed et al., 2017). Surprisingly, close to these results were found for the larvicidal bioassay of *Bt* isolates removed from different habitats in Saudi Arabia against *An. gambiae* (El-Kkersh et al., 2016). In a dose-selecting study, *Cx pipiens* L_4 larvae were exposed to spore/δ-endotoxins mixtures of *Bt* SY49-1 strain (at 50, 100, 250 and 500 µgmL^{-1}), the 500 µgmL^{-1} mixture resulted in 100% mortality for the treated larvae (Azizoglu et al., 2017). In India, the river treatment using *Bt* (at 10 PPM) against *Simulium himalayense* resulted in 15-22% larval reduction (Singh and Tripathi, 2003).

1.2. Mites

Although studies on the miticidal activity of *Bt* toxins are rarer compared with other parasites, all of the studies, to date, on the most important veterinary and medical mites were compiled here:

In an *in vitro* acaricidal activity of *Bt* GP532 on *Psoroptes cuniculi*, the reported histopathologic alterations were intercellular dilatation in the basal membrane, membrane dissociation of the peritrophic matrix, and morphological variations in the intestinal columnar cells (Dunstand-Guzmán et al., 2015). In another clinical bioactivity of *Bt* GP532 on rabbits naturally infested with *P. cuniculi*, GP532 toxin was applied by aspersion in both pinnae, with reapplication after seven days. GP532 application decreased infection, as early as three days post-treatment. At days 14 and 30 PT, reduced infection in the left and right pinnae was considerable (Dunstand-Guzman, 2017). Using a dose of 100 µgmL^{-1}, pathogenicity caused by all Bacillus-like isolates on *Varroa destructor* has been revealed. Nine isolates were highly pathogenic, of which the best were related to EA49.1 (100% mortality), EA11.3 (93%), EA26.1 (90%), and EA3 (86.7%), respectively (Alquisira-Ramírez et al., 2014). Under optimal conditions by feeding treatments (at doses from 0 to 100 mgg^{-1}), *Bt tenebrionis* Cry3A on *Acarus siro*, *Tyrophagus putrescentiae*, *Dermatophagoides farinae*, and *Lepidoglyphus destructor*, prevented the population growth of all species insignificantly, after 21 days. *D. farinae* showed a lower population density than others. Doses <10mgg^{-1} showed no remarkable suppressive effects on growth rates. *A. siro* growth rate was higher in doses <10mgg^{-1} compared to the control. Near 78% of removed bacteria from dead *Tyrophagus similis* showed some pathogenic activity against mites. *Bacillus* spp. spores prolonged the development of *Dermatophagoides pteronyssinus* tritonymphs. *Bt maize* and *Btk* prolonged the development time of *A. siro*, resulting in the reduction of the mean female lifespan, and remarkably decreased survivorship of larvae and nymphs (Erban et al., 2009). Finally, Ahmed et al. (2016) reported the acaricidal activities of *Bti* (81.22%) and *Bt tenebrionis* (90.91%) on *T. putrescentiae* (Ahmed et al., 2016).

1.3. Ticks

Like mites, studies on the acaricidal activity of *Bt* toxins against the most important veterinary and medical ticks do not have a long history and are rare. From the early studies, all of which are reviewed in the following:

It has been reported that spraying mixtures of *Btk* (at 1 mgmL-1), *Bti* (at 2.5 mgmL^{-1}) and *Btt* (at 5 mgmL^{-1}) against *Argas persicus* engorged females caused 100, 100, and 93.3% mortality after five days, respectively. But, about *Hyalomma dromedarii*, none of the *Bt* strains induced 100% mortality, even at a dose of 10 mgmL^{-1} (Fernández-Ruvalcaba et al., 2010). By spraying a commercial product of *Btk* (Dipel 2X at 20 gL^{-1}), in *in vitro* assay all of *H. dromedarii* individuals were found dead, but in *in vivo* assay some of the females were engorged. Also, the treated eggs were unaffected (El-Kelesh and El-Refaii, 2006). Zhioua et al.

(1999) have, also, mentioned 96% mortality for *Ixodes scapularis* engorged larvae using *Btk* strains with a dose of 10^8 sporesmL^{-1}, and LC_{50} = 10^7 sporesmL^{-1} (Zhioua et al., 1999). By immersion assay, the mortality rates caused by four native *Bt* strains (GP123, GP138, GP139, GP140) on *Rhipicephalus microplus* adult females have been determined. All strains have shown higher mortality rates compared with controls, but similar to each other. GP138 strain has indicated the most pathogenic and faster effect than others. In the analysis of the acaricidal activity of the strains on *R. microplus* egg laying and hatching, GP138, GP139, and GP140 have shown similar inhibitory effects (Fernández-Ruvalcaba et al., 2010). To determine the virulence of *Bt* GP543 S-layer toxin against engorged *R. microplus*, the lowest (50 µgmL^{-1}) and highest (300 µgmL^{-1}) doses have caused more than 50% and 75% mortality, respectively. Some of the survived ticks could not oviposit, or laid egg masses that had lowered weight. The highest inhibition of oviposition was related to 200 µgmL^{-1}. All doses, eventually, gave rise to higher than 85% inhibition of hatching (Lormendez et al., 2019).

Contrary to the positive acaricidal activity of *Bt* strains against different acarin, the mode of action of acaricidal *Bt* toxins is unknown, yet. However, based on the enzymes (trypsin, alkaline phosphatase, and some aminopeptidases) present in the digestive tract of the studied acarin, it is postulated that the histopathologic effects of *Bt* toxins in their digestive tract may be due to the activation of *Bt* protoxins, like Cry1A, inducing pore-like alterations in brush border membrane vesicles (Dunstand-Guzmán et al., 2015; Salehi-Jouzani et al., 2021)

1.4. Lise

Bt WB3S16 strain has displayed high toxicity against *Bovicola ovis*, resulted in general paralysis and finally death. But *Bt* spores did not have toxicity per se to *B. ovis*, however, they caused septicemia after germinating in the gut of the louse. This strain contains Cry1A and Cry2A toxins, showing highly toxic to *B. ovis*, and even the toxicity of Cry1A is significantly higher (Hill, 1998). Although, all sheep wool removed *Bt* strains have shown good toxicity when screened on *B. ovis* adults, 9 of which (*Btk* HD-263, *Btk* HD-266, *Btk* WB3S-10, *Btk* WB3S-11, *Btk* WB3S-13, *Btk* WB3S-16, *Btk* WB3S-17, *Btk* WB3S-18, and *Btt* HD-120) have shown high toxicity. *Btk* WBS3-16 was recorded as the most toxic strain, causing 97% mortality by 72 h, LT_{50} = 50±2 h, LC_{50} = 0.13 mg (Drummond et al., 1992). In louse feeding bioassay using 96 isolates of *Bt*, out of five isolates showed 44–66% mortality for *B. ovis*. Only JLF 17.22.7 isolate, as a highly pathogenic isolate, indicated 85% mortality (Gough et al., 2002).

1.5. Flies

In an ultrastructural investigation to characterize cytological activities and elucidate the mode of action of *Bt tolworthi* on *Lucilia cuprina*, cytopathological effects were found in the nervous system, midgut epithelial cells, Malpighian tubules, muscles, and fat. Invasion by bacteria toward haemocoel was also observed. *Bt tolworthi* can also produce thuringiensin. Sublethal effects of this β-exotoxin interfere with larval ecdysis. These observed cytopathological and other abnormalities may be due to thuringiensin and/or other exotoxins (Cooper et al., 1999). In a bioassay using 96 *Bt* isolates, out of 18 isolates caused higher than 70% mortality for *L. cuprina* larvae. Out of 6 isolates caused higher than 90% mortality. Mortalities increased insignificantly between pupation and emergence of the adult fly (Gough et al., 2002). In bioactivity against adult *Haematobia irritans exigua*, none of 96 *Bt* isolates showed high activity against adult flies. Only JLF 2.5.6 isolate resulted in 37% mortality; the rest caused less than 25% mortalities. In light of larvae, out of 67 isolates caused higher than 70% mortality, while 44 of which caused higher than 90% mortality. Mortalities increased insignificantly between pupation and emergence of the adult fly (Gough et al., 2002). To understand if a spray formulation of *Beauveria bassiana* would improve the larvicide potential of *Bti* against *Musca domestica* larvae and adults emergence, it was recorded that larval mortalities were 11% for *B. bassiana* alone, 41% for 250 mgkg^{-1} *Bti* alone, and 42% for 500 mgkg^{-1}

Bti alone. While, larval mortalities were 45 and 52% for 250 and 500 $mgkg^{-1}$ doses of *Bti+B. bassiana* treatments, respectively. In the inhibition of the adult emergence, *Bti* treatments were more effective than Larvadex, and even more effective than Larvadex+*B. bassiana* (Mwamburi et al., 2009).

2. Protozoa

One of the important and novel toxins, that displayed killing abilities, permitting it to be applied to control and treat protozoan parasites of humans and animals, is the toxin produced by the PS81F strain (Rosas-García, 2009). It has been demonstrated that Cry1A protoxins can induce potent cellular and humoral immunity. By inoculation of the Cry toxins of 8 *Bt* strains into the mice infected with *Plasmodium berghei* and *Plasmodium chabaudi*, the mean life of the infected mice was determined to be ~8.5 days. In contrast, for those treated with Cry toxins from 5 strains, the mean life was extended to 13.5-15 days. The protection caused by Cry1Ac against two protozoa was induced based on dropped parasitemia, prolonged the survivorship, regulation of pro- and anti-inflammatory cytokines, and raised levels of specific antibodies. Also, in mice immunized with only *Negleria fowleri* lysates or with Cry1Ac, strong protections to *N. fowleri* were found, so that the survivorship of mice increased to 100% by *N. fowleri* lysates + Cry1Ac. In comparison, it decreased to 60% for lysates alone (similarly 60% for only Cry1Ac immunized mice) (Zhaohui et al., 2004). To investigate the activity of *Btt*-H14 active toxin on *Leishmania major*, this active protein was bioassayed against *L. major* promastigotes suspension. As the results, the cytopathological investigation of the treated promastigotes revealed that the pathological changes passed through degenerative changes that started with shortening, swelling of promastigotes, followed by sluggish movement and augmented swelling along with granulation of cytoplasm. The promastigotes changed from spindle shape to spheroid passing through berry shaped cells. Then, the cells died, followed complete lyses, flagella loss, and granulation and segmentation of the cytoplasmic proteins with an enormous increase in size (El-Sadawy et al., 2008). In Japan, by evaluating the anti-trichomonad activity of toxins from 816 *Bt* strains on *Trichomonas vaginalis*, it showed that 10 strains restricted the growth of protozoa to 6-100%. Furthermore, B622 and B626 strains indicated apparent anti-trichomonas activity (Kondo et al., 2002).

3. Helminths

Anti-nematode actions of *Bt* toxins resemble the same insecticidal modes, therefore, nematodes must ingest Cry toxins, to be intoxicated (Sinott et al., 2013). Several families of Cry toxins from decades of *Bt* strains (Cry5, Cry6, Cry12, Cry13, Cry14, Cry21, and Cry55) have been found for nematicidal effects on various free-living and parasitic nematodes. In addition, these Cry toxins synergistically affect nematodes, as well as, a few other *Bt* products (such as exotoxins, proteases, lipases, chitinase) show nematicidal activities (Iatsenko et al., 2014; Salehi-Jouzani et al., 2021). Recently, the toxicity of three *Bt* Cry toxins (Cry5B, Cry14A, and Cry21A), against *Caenorhabditis elegans* and the free-living stages of some parasitic nematodes has been recorded. To test if *C. elegans* specific glycolipid/glycoprotein receptor (carbohydrate moieties) for Cry5B is the same in hookworms, Cry5B (with and without galactose) was fed to *Ancylostoma ceylanicum* adults. Like *C. elegans*, galactose inhibited Cry5B toxicity in *A. ceylanicum* (Cappello et al., 2006; Hu et al., 2012). Similarly, *in vitro* feeding Cry5B to adults and larvae of *A. ceylanicum* resulted in significantly impaired motility, decreased egg laying, and morphologic alterations of hookworms. On the other hand, *in vivo* oral feeding of Cry5B to infected hamsters resulted in significant therapeutic consequences, including improved growth, increased blood hemoglobin levels, considerable reduction of fecal egg excretion, and intestinal worm population (Sinott et al., 2013). In *in vitro* test of IBaCC containing Cry5B on *Ascaris suum* L_4 larvae, the morphopathologic observations demonstrated an evident intoxication of *A. suum* larvae. Because in other treatments no intoxication was found. In *in vivo*, Th2-immune

deficient mice treated with Cry5B-IbaCC showed a 92.5% intestinal worm population reduction. These findings confirmed that Cry5B acts directly against parasites and its activity is independent of the host immune system. Also in pig, a single dose of Cry5B-IbaCC resulted in a 96% reduction of *A. suum* L_4. In naturally Parascaris-infected foals treated with Cry5B-IbaCC, EPG=0 one week post-treatment was, also, reported (Urban-Jr et al., 2021). The nematicidal effects of *Bti*, *Btk*, *Bto*, and *Btm* were assayed in free-living larval stages of *Haemonhus contortus*. A spore+crystal preparation of each strain was added to the feces of naturally infected sheep. A significant dose-dependent reduction of the larval development was found only by *Bti*, *Bto*, and *Btk* preparations (Sinott et al., 2013). The lethal percentages of 70 and 25 kDa purified fractions of soluble toxins produced by *Bt* IB-16 strain against *H. contortus* histiotropic L_4 have shown toxicity as 67.1% and 17.3%, respectively (Vazquez-Pineda et al., 2010). By adding to naturally *H. contortus* egg-infected sheep feces and following up coproculture, the larvicidal activities of *Bti* Cry11Aa and its *E. coli* expressing recombinant have revealed the larval reductions of 62 and 81%, respectively. Also, by orally administration to naturally infected lambs, the mentioned toxins have shown reductions of ~79 and ~90%, respectively (Stori-de-Lara et al., 2016). In an *in vitro* assay, all stages of *Strongiloides stercoralis*, by manifesting motility impairment and decreased viability, were susceptible to Cry5B. Cry5B showed strong potential as an effective anthelmintic for the treatment and transmission control of human strongyloidiasis. *Strongiloides papillosus* free-living stages have, also, indicated high susceptibility to *Bt* DB27 strain (Iatsenko et al., 2014). In naturally infected with Trichostrongylides cattle feces incubated with spore suspensions of *Bto*, *Bti*, *Btk* or *Bacillus circulans* (*Bcir*), all the *Bacillus* varieties indicated 60% or more larvicidal activities. The larvicidal activities (%) of *Bto*, *Btk*, *Bcir*, and *Bti*, were 66, 89, 90, and 94%, respectively. Based on *in vitro* larvicidal activity of all *Bacillus* varieties on each identified gastrointestinal nematode, the highest mortality rate related to *Trichostrongylus* spp. (90.3%), followed by *Ostertagia* spp. (87%) and *Haemonchus* spp. (85.7%). Finally, about tapeworms, in an *in vitro* bioassay by 37 *Bt* strains on *Centrocestus formosanus* metacercariae and cercariae, only six strains showed high mortality. Of which, GP308, GP526, GP543, and IB-16 strains exhibited the highest mortality, respectively, on metacercariae. While, MEI, GP543, and GP426 strains showed the highest toxicity, respectively, on cercariae (Mendoza-Estrada et al., 2016).

Conclusion

In overall, the findings of the researchers and also the extraordinary potential of *Bt* have drawn a promising future for indigenous and recombinant products and also biosynthesis of different nanoparticles of *Bt*. All these predictions seem quite reasonable. In the future, more effective subspecies and strains will be discovered, making these unique compounds as a definitive alternative to other synthetic and non-synthetic pesticides.

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Not applicable.

Ethical approval

Not applicable.

Conflicts of interest

The author declares no conflict of interest.

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