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Status of bacterial biocontrol agents against cattle tick *Rhipicephalus* (*B.*) *microplus* (Acarina: Ixodidiae): Review

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Abstract
Currently, the control methods against ticks are focusing on the use of insecticides that lead to high cost and adverse effect to the environment. Thus user-friendly and eco-friendly approach of biological control by using entomopathogens *viz.* fungi, bacteria, viruses, and nematodes, provides an alternative way. Within the bacterial group, the microorganisms most widely used worldwide in the control of several insect pests with the highest success, are the bacterium, *Bacillus thuringiensis* Berliner (Bacillales: Bacillaceae). The use of *B.thuringiensis* is increasing rapidly because it is highly specific, significantly lowering the damage to other organisms compared to use of chemical insecticides, and because it is self-perpetuating and is therefore accepted as an environmentally friendly alternative. However very scanty studies are available on bacterial pathogens against ticks. Thus an attempt is made through this review article to highlight the work undertaken on possible ways and role of bacteria in the control of ticks. The sole purpose of the present review is to compile the pertaining literature and to create the interest of researchers in this area.

Keywords: Bacterial bio-control, *Bacillus thuringiensis, Rhipicephalus* (*B.*) *microplus*

Introduction

Concept of Integrated Tick Management: In the current era where a large section of the human population is showing their interest towards organic food and willing to have rid-off form food containing traces of hazardous chemicals. On the other hand the requisite quantity of production of food grains cannot be achieved without control of pests. In such situation the solution which will maintain equilibrium without much damaging to pests and will minimize the use of hazardous chemical pesticides but will not affect on the quantity of production is necessary. Such solution is nothing but the use of bio-control agents and biological control. Same thing is true for control of animal pests and for which necessary thing is the development of bio-control agents. Worldwide research on biological control, bio-control agents and integrated management is in full swing against the crop pests particularly belonging to lepidoptera. However the pace of development of bio-control agents against animal pests is slow and needs to be accelerated. Therefore to highlight the current research undertaken globally, it is presented in the form of present review, which will help to encourage young researchers from India to make efforts in this area of immense importance. The bio-control agents developed against crop pests mainly belongs to bacteria, viruses, fungus, helminthes etc. All these are easy to be used on the lepidospterous pests. However use of bio-control agents against dipteran pests and acarina pests is quite difficult because they need to be applied either on animal body, animal shed or their breeding places. Till then with all difficulties fungal, bacterial and herbal biopesticides and biocontrol agents are used with efficiency and many research article and review article can be quoted as a reference [1]. Present study also witnessed the effect of bacteria belonging to *Bacillus* genus and toxins of *B. thuringiensis* var *kurstaki* on the *Rhipiciphalus microplus* ticks and these bio-control agents exercised their effect by quadruple mode i.e., a) acaricidal b) oviposition deterrent, c) ovicidal, and d) ovicidal effect perpetuated from parent female tick to eggs and resulted in ovicidal action.

In the literature a very scanty work on use of bacteria against acarina pests i. e. ticks and mites of livestock is being conducted or if conducted, not used on the field. Hence such work is needed to be geared up and one study has been conducted by the author. The said study succeeded in highlighting the importance of five bacteria as bio-control agents against adult and egg stage of tropical cattle tick *Rhipicephalus* (*Boophilus) microplus* (Acari: Ixodidae). Incorporating the results of this study present review paper has been prepared with specific objective to boost the research on this topic. According to Samish *et al*. [2]. biological control is becoming an increasingly attractive approach to tick management because of: (1) increasing concerns about environmental safety and human health (e.g. the gradual decrease in use of chemical insecticides in several countries is stimulating the growing market of 'organic' food); (2) the increasing costs of chemical control; and (3) the increasing resistance of ticks to pesticides. Classical biological control includes the recognition, evaluation and importation of a natural enemy from elsewhere, the conservation of local natural enemies and the augmentation of the bio-control agents. Further possibility of IPM against ticks was explored by Martinez *et al*. (2013) [3] and Singh *et al*. (2016) [4].

Chronology of Development of Bt: Singh and Mathew (2015) [5] in a research article on "The Effect of *Bacillus thuringiensis* and Bt Transgenics on Parasitoids during Biological Control" narrated the global status of *Bacillus thuringiensis which* is presented here in the chronological order. The purpose behind mentioning the chronology of development of *Bacillus thuringiensisis* is to understand how from 1915 to 2015 it acquired the global status. Chronology is also highlighting the importance of these bacteria in biological control:

1. 1915: These parasporal inclusions are formed by different insecticidal crystal proteins (ICP) or δ-endotoxins. Though, the existence of parasporal inclusions in Bt was first noted in 1915 [6].
2. 1950: Protein composition was not delineated until the 1950s [7].
3. 1959: Discovery of *Bacillus thuringiensis* crystal toxicity
4. 1977: Bt subspecies can synthesize more than one inclusion, which may contain different ICPs. These crystals have variously shaped depending on their ICP composition. A partial correlation between crystal morphology, ICP composition, and bioactivity against target insects has been established [8, 9].
5. 1981-1987: Bt is a member of the Bc (*Bacillus cereus)* group of Gram positive, spore-forming soil bacteria. During the sporulation process, it produces one or more characteristic crystalline proteinaceous inclusions adjacent to the endospore, which have been found to be toxic for invertebrates, primarily insect species in the orders *Coleoptera*, *Diptera* and *Lepidoptera*, distinguishing it from *Bacillus cereus* [10]
6. 1984-1991: Once ingested by the target larva, the parasporal crystalline ICP is dissociated to the protoxin form in the midgut, and the protoxin is then activated to a biologically active holotoxin by the proteolytic enzymes and specifically the alkaline environment of the gut [11, 12]. Shortly afterwards, the gut becomes paralysed and the larva ceases to feed. Pore or ion channel formation occurs after the binding of the toxin to the receptor and the subsequent failure of trans-membrane electric potential. This results in colloid-osmotic lysis of the cells [13], which causes vegetative cells of Bt and the pre-existing microorganisms in the gut to proliferate in the haemocoel causing septicaemia, and may thus contribute to the mortality of the insect larva.
7. 1992-1993: This is the leading biopesticide used in commercial agriculture, forest management and mosquito control. *Bacillus thuringiensis* is also a key source of genes for transgenic expressions to provide pest resistance in plants [14, 15]
8. 1997: proved as one of the most widely used entomopathogenic microorganism among many
9. 1997: Bt has attained a wide commercial use against major lepidopteran pests and has emerged as the most successful microbial pesticide having great potential in IPM programmes [16].
10. 1998: These δ-endotoxins, encoded by the C*ry* and C*yt* genes, have molecular weights between 14-160 kDa and can be visualized under light microscopy as inclusion bodies [17].
11. 1998: Bt crops offer great promise in controlling lepidopteran pests. A decrease in synthetic insecticide use in Bt transgenic crops could increase beneficial arthropod diversity and abundance. Among the spray formulations, Bt var. *kurstaki* (Btk) HD-1-based products are widely used in many crop ecosystems against over 100 insect species worldwide including [18].
12. 1998: The first *Bt* microbial product registration in the U.S. was in 1961 and by 1998, there were approximately 180 products registered in the U.S. Environmental Protection Agency [19].
13. 1999: The success and extensive use of *Bt* microbial pesticides worldwide can be attributed to their high specificity against target insect species while greatly limiting the negative impacts to beneficial and non-target organisms, and lack of environmental persistence of Bt toxins [20-22]. (WHO/IPCS, 1999; Betz *et al*,2000; Federici and Siegel, 2008)
14. 2000: Microbial Bt formulations applied orally or to the host are generally non-toxic against parasitoids, because most hymenopterans lack receptors in their midgut necessary for binding of Cry toxins. However, some laboratory studies using Bt sprays have reported adverse effects [23].
15. 2006: The Bt toxins in formulations and those expressed by transgenic plants that are commercially grown have a narrow range of activity, and no direct negative effects have been reported on natural enemies belonging to other orders than the one targeted by a specific Bt toxin [24].
16. 2007: Approximately 276 registered *Bt* microbial formulations in China [25].
17. 2012: have reported at least 120 microbial products in the European Union
18. 2014: Developing suitable methods of pest control in accordance of the philosophy and methodology of modern integrated pest management (IPM) programme is a daunting task in an increasingly environmentally conscious world of ours [26].
19. 2014: The use of microorganisms has assumed a prominent position among the options that seek to control insect pests without the use of chemicals and with high specific toxicity applied in agro ecosystems
20. 2015: For the biological control of insect pests, *Bacillus thuringiensis* (Bt) has emerged as the oldest and one of the most widely used entomopathogenic microorganism [27].

Chronology of Bacterial use against the Dipteran pests

1. 1965: The first reported *Bacillus sphericus* strain active

against mosquito larvae was isolated from moribund mosquito larvae [28].
2. 1973: The identification of the strain SSII-I in India and first suggested that like *B. thuringiensis* against Lepidoptera, B. *sphuericus* acts by toxemia rather than septicemia
3. 1977-1978: The use of microorganisms as a source of biological compounds for insect pest control started after the discovery of the highly insecticidal bacteria *Bacillus thuringiensis*. The discovery of the strain *B. thuring*iensis serovar *israelensis* [29, 30]. (de Barjac, 1978; Goldberg and Margalit, 1977) made possible efficient microbiological control of Diptera Nematocera vectors of diseases, such as mosquitoes (Culicidae) and black flies (Simuliidae).

Chronology of Bacterial use against the tick pests

1. The possibility of using bacteria probably started from 1997 with the work of Hassanain *et al*. (1997) [31] who evaluated the activity of three subspecies of *B.thuringiensis* (*kurstaki*, *israeliensis,* and *thuringiensis*), spraying spore/crystal mixtures on the soft tick *Argas persicus* and the hard tick *Hyalomma dromedarii*.
2. Samish and Rehacek (1999) [32] mentioned 100% mortality using mixtures of *B. thuringiensis* spores and blood fed to *Ornithodoros erraticus* through an artificial membrane.
3. Zhioua *et al*. (1999) [33] evaluated *B. thuringiensis kurstaki* strain against engorged larvae of *Ixodes scapularis*, achieving 96% mortality with a dose of 10^8spores/ml.
4. Brum *et al*. (1991) [34] used Enterobacteriaceae as a microbial pathogen against the hard tick *Boophillus microplus* and also found that the microbe has produced a genital infection and/or the death of engorged females.
5. Ostfeld *et al*. (2006) [35] in a review article on 'Controlling Ticks and Tick-borne Zoonoses with Biological and Chemical Agents' described the possible future of natural enemies of ticks include insectivorous birds, parasitoid wasps, nematodes, *Bacillus thuringiensis* bacteria, and deuteromycete fungi. According to review, although several bacterial species are pathogenic to ticks, the usefulness of bacteria as biocontrol agents is poorly studied. Further they opined that *Bacillus thuringiensis*, which is used as a bio-control agent for many insects, is pathogenic to ticks, but apparently must be ingested to be effective [32, 33]. Because ticks tend to ingest only host blood, inducing ticks to ingest these bacteria seems impractical, and the prospects for *B. thuringiensis* as a biocontrol agent seem poor. Recent surveys of microbes naturally infecting blacklegged ticks and American dog ticks [36, 37]. Reveal a rich flora including spore-forming and crystal-forming bacteria that, if found to be entomopathogenic, could be developed as potential biocontrol agents.
6. Fernández-Ruvalcaba *et al.* (2010) [42] stated that in Integrated Pest Management (IPM) the use of *B. thuringiensis* is increasing rapidly because it is highly specific, significantly lowering the damage to other organisms compared to use of chemical insecticides, and also because it is biodegradable and is therefore accepted as an environmentally friendly alternative. In addition, *B. thuringiensis* has no adverse effects on humans. *B. thuringiensis* products can be combined with other pest control techniques and it is an essential component in Integrated Pest Management (IPM).
7. Martinez *et al.* (2013) [3] studied an Integrated Pest Management of ticks with the aim to use natural products to gradually reduce the use of conventional chemicals. They opined that use of the entomophatogenic fungi, *Metarhizum anosopliae* and *Bacillus thuringiensis,* and extracts from plants that have shown biocidal or biostatic activity that can be used for the control of livestock pests of economic importance like the *R. (Boophilus) microplus* tick.
8. Singh *et al.* (2016) [4] during the presentation in an international conference on the topic "Prospects for biological control of cattle fever ticks by natural enemies along the Texas-Mexico border" expressed that, for the control of cattle ticks, candidate methods include ants, predatory mites, chickens, parasitoid wasp, *Bacillus thuringiensis,* entomopathogenic nematodes and oxpeckers.

Acaricidal properties of the Entomopathogenic bacteria

About the Bacteria: Martinez *et al.* (2013) [3] in a research article entitled as "Evaluation of natural origin products for the control of *Rhipicephalus* (*Boophilus) microplus* (Acari: Ixodidae) on cattle artificially infested" described the nature and characteristics of *Bacillus thuringiensis* which are described here. The characteristics of *Bacillus thuringiensis* described are: is a gram-positive bacterium, aerobic strict and its life cycle has two phases: vegetative growth, when the bacteria duplicate by bipartition and the sporulation. *B. thuringiensis*, is considerated an ubiquitous bacteria since it has been isolated from several parts of the world and from diverse systems, like soil, water, plants leafs, insects bodies, spider webs, and others.

Possibility of *Bacillus thuringiensis* for control of ticks: In the beginning several workers (Samish and Rehacek, 1999; Zhioua *et al*. 1999) [32, 33] expressed their doubt about the usefulness of *Bacillus thuringiensis* bacteria as bio-control agents because of two reasons, one as a) ticks being hematophagous ingesting only host blood and b) to occur tikicidal activity of bacteria, like insects, bacteria must be ingested in the gut. This problem was addressed by the research of (Habeeb and El-hag, 2008) [38] who have shown first time that *B. thuringiensis* toxins are lethal to hemoplast cells of *H. dromedarii* ticks*.* Hemocytes are the circulating cells of arthropods which are as much as 50-60% of the hemolymph, or the circulating fluid content in ticks (Sonenshine, 1993) [39] were functional equivalent to mammalian immune cells. Once immune system is destroyed ticks will not survive. It is a general principle which is well documented by (Giradin *et al*., 2002; Estrada-Pena *et al*., 2004) [40, 41]. That, innate immune system is one of the most important factors in the ability of metazoan organisms to survive when challenged by microbes. The innate immune system comprises cell-mediated and soluble components and is initiated through recognition of Pathogen Associated Molecule Patterns (PAMP). In the light of this principle, the work of Habeeb and El-hag [38] created a ray of hope and their work has also thrown the light on probable mechanism of action on hemoplast cells. They have undertaken the study by intra-hemocoelic injections of *Bacillus thuringiensis* serovar *thuringiensis* H14 –endotoxin (43-kDa Cry4Ba toxin) on the hemocytes which provided the spores access to the more favorable environment of the hemocoel, where they germinate and reproduce. According to their work mechanism of killing was extensive cell lysis leading to septicemia and death of ticks. They also reported that tick mortality was only in injected groups while the orally exposed ticks were not affected. This may be due to inactivation of *B. t. Cry4Ba* 43-kDa protein by midgut proteases which may not give the toxic protein any chance to interact with hemocytes, while injection of toxic proteins allows it to bind directly with hemocytes. The work of Fernández-Ruvalcaba *et al*. (2010) [42]. showed another ray of hope in which they concluded that some *B. thuringiensis* strains had a toxic effect on *R. microplus* using the adult immersion assay. The *R.microplus* acaraside-resistant strain could be controlled with pathogenic *B. thuringiensis* Strains and indicated that immersion trials are effective to control *R.microplus* and mode of action as other than ingestion, probably by means of the spiracles or genital pore. Zhioua *et al*. (1999) [33] also reported the route of infection as spiracles or genital pore.

The many earlier studies also witness the use of bacteria for control of ticks.

1. Acaricidal effect of *B.thuringiensis* varies according to the dose, time of application, and insect species. The toxic effect was most marked during physiologically critical stages such as molting, pupation, or metamorphosis [43-49]. Even if mortality is not produced, the surviving insects may succumb at any later stage due to retarded development or failure to accomplish pupation, or emergence [50-54].

2. Use of bacterial species with high efficacy against adults, immature stages, and eggs of different species of mites [55, 47, 48].
3. Carlberg and Lindstrom (1987) [56] have reported that *Bacillus* strains produce two main types of toxins: delta endotoxins, which are mainly, used for the control of various Lepidoptera, and beta exotoxins, which are mainly used for the control of various Diptera.
4. Brum *et al*. (1991) [34] used agents from Enterobacteriaceae family as a microbial pathogen against the hard tick *Boophillus microplus*. They also found that the microbe produced a genital infection and/or the death of engorged females.
5. Hassanain *et al*. (1997) [31] first time evaluated the activity of three subspecies of *B.thuringiensis* (*kurstaki*, *israeliensis*, and *thuringiensis*), spraying spore/crystal mixtures on the soft tick *Argas persicus* and the hard tick *Hyalomma dromedarii*. He reported that *B. thuringiensis kurstaki* produced 100% mortality against *A. persicus* engorged females after five days at a dose of 1 mg/ml. *B. thuringiensis israelensis* caused 100% mortality at a dose of 2.5 mg/ml, and *B.thuringiensis thuringiensis* at a 5 mg/ml dose induced 93.3% mortality. With *H. dromedarii,* none of the *B. thuringiensis* strains produced 100% mortality, even at doses as high as 10 mg/ml.
6. Samish and Rehacek (1999) [32] mentioned 100% mortality using mixtures of *B. thuringiensis* spores and blood to feed *Ornithodoros erraticus* through an artificial membrane
7. Zhioua *et al.* (1999) [33] evaluated a *B.thuringiensis kurstaki* strain against engorged larvae of *Ixodes scapularis*, achieving 96% mortality with a dose of 10^8 spores/ml. it was shown that *B.thuringiensis kurstaki* spores (10^6/ml) were toxic to engorged *I. scapularis* larvae. However, an LC_{50} has been reported with 10^7spores.
8. Casique-Arroyo *et al*. (2007) [57] reported that *B. thuringiensis* strains have chitinolytic activities and hence can be used in mite control.
9. Ostfeld *et al*. [35] reported the use of *B. thuringiensis* for cattle tick control
10. Fernández-Ruvalcaba *et al*. (2010) [42] The four selected *B. thuringiensis* strains GP123, GP138, GP139, and GP140 produced 62.5, 81.25, 64.58, and 77.08% mortality, respectively, by the fifth day. These data indicated that the GP138 strain was the most pathogenic. Analysis of the effect of *B. thuringiensis* strains on *R. microplus* with the immersion aassay led us to infer that the *B. thuringiensis* strains can affect *R. microplus* through approaches other than ingestion, probably by means of the spiracles or genital pore.
11. Martinez *et al*. (2013) [3] reported high mortality of adult *R. microplus* females in the presence of *B. thuringiensis kurstaki* strains.
12. Dunstand-Guzmán *et al*. (2015) [58] reported the high efficacy of *Bacillus thuringiensis* protein extracts on the mite *Psoroptes cuniculi* and observed that acaricidal effect through histological damage.

Action Mechanism: The ultrastruture and characteristics of hemocytes of ixodidae tick, *Hyalomma dromedarii*, after treatment with *Bacillus thuringiensis* serovar *thuringiensis* H14 -endotoxin were studied by Habeeb and El-Hag (2008) [38]. To evaluate the effect of *B. thuringiensis* 43-kDa toxins elicit a toxic response in the hemocoel, intra-hemocoelic injections of 43-kDa Cry4Ba toxin on the hemocytes (ultra structure and characteristics) and survival of *Hyalomma dromedarii* engorged female was studied. After study author reported that, of 43-kDa Cry4Ba toxin was highly toxic within short term (48h) to *Hyalomma dromedarii* engorged female. The result indicated that the complete growth was arrested and death in a dose-dependent manner. On receiving 10µl of 157µg per ml soluble *B. t.* toxin/tick a rapid paralysis, followed by hemocytic disruption and death was occurred. This investigation revealed that a severe damage in the cells membrane and granulocytes of the hemolymph after injection with -endotoxin. *Bacillus thuringiensis* var. *thuringiensis* H14 -endotoxin, this toxin destroys the granular cell and renders it abnormal. In short toxin kills the tick by causing a malfunction of the cellular immune system of the tick. The study also suggested that *Bacillus thuringiensis* var. *thuringiensis* -endotoxins targets are not only the gut but also are haemocoel.

Summarizing the observations of many studies conducted by Hassanain *et al*. (1997), Zhioua *et al*. (1999), Fernandez *et al.*, (2006a), Martinez *et al*. (2013), Solanke (2018) [3, 31, 33, 59, 60] it can be concluded that *Rhipicephalus* (*Boophilus) microplus* can be effectively controlled through use of bacteria as biological control agents. Three sub species of *B. thuringiensis* (*kurstaki*, *israeliensis* and *thuringiensis*) against effectively works against tick species as *Argas persicus* and *Hyalomma dromedarii, Ixodes scapularis, Rhipicephalus (Boophilus) microplus)* species (hard ticks).

Conclusions

An experiment was conducted [60] to develop the effective bio-acaricides which will be alternative to chemical acaricides against an important pest of cattle *Rhipicephalus (B) microplus* in which five bacteria tested, which were *Bacillus* *thuringiensis var israelensis*, *Bacillus weihenstephanensis* var WSBC, *Bacillus weihenstephanensis* var KBAB4, and *Bacillus sphaericus* which were available in the Department of veterinary Parasitology as a lyophilized powder containing 2x 10^{10}, 1.3x 10^{10}, 1.3x 10^{10} and 1.5x 10^{10} spores per gram of powder. One bacteria namely *Bacillus thurinngiensis var kurstaki* and its toxin was produced from market. For judging the effects of bacteria on *Rhipicephalus (Boophilus) microplus* ticks for criteria's were employed namely a) mortality of adult ticks and eggs, b) reduction in egg laying capacity, c) hatchability of eggs laid by treated female ticks and, d) hatchability of treated eggs by following standard procedure in the laboratory. These bacteria were found very efficacious and shown efficacy as adulticidal, reduced the significant egg laying capacity of female ticks, reduced the hatchability of eggs laid by treated females and reduced the hatchability of the treated eggs. While causing mortality in the adults, bacteria might have probably entered through either genital opening or cuticle. Thus the present experimentation helped to draw the conclusions that the only ingestion of bacterial toxin is not the sole mode of causing activity but, it can been other routes also. As spores of bacteria can enter through genital opening, spiracles or through cuticle and followed by their entry in haemocoel and producing damage to tick body. The effect of bacteria on adult ticks was comparatively less than the eggs. Eggs were found more susceptible to the action of bacteria. Adult females treated with different bacteria, it resulted in significant reduction in egg laying capacity. Looking into potent activity of all types of bacteria they can be very well inducted in the integrated tick management program. Hence in the integrated tick management programmes particularly against ticks bacterial bio-control agents can be very well inducted along with other managemental practices. Further research in the different geographic areas is the need of hr.

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