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BEYOND CHEMICALS: EXPLORING GREENER OPTIONS FOR TICK CONTROL

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ABSTRACT

Ticks are obligate haematophagous ectoparasites that feed on a variety of vertebrate host animals. They transmit a wide range of disease-causing organisms and are of great medical and veterinary importance. Several characteristics of ticks confer them outstanding attributes to serve as vectors of pathogenic agents, *viz.*, the wide host range and tendency to feed on several hosts during life cycle ensures ample opportunity to acquire and transmit pathogens, hardiness and longevity enable them to survive long periods in unfavourable environmental conditions, high reproductive potential ensuring maintenance of large populations and a high frequency of host-vector contact. Conventional methods for tick control are based on the use of acaricides and insect growth regulators. The continuous emergence of ticks and tick-borne diseases (TTBs) and acaricide resistance of ticks necessitated the development of new and more effective control strategies. Better alternate options available including the exploitation of herbal resources, pheromones, vaccines, endosymbiont disruption and other biological control options are briefly reviewed.

Keywords: Ticks, Acaricide Resistance, Tick borne diseases, Semiochemicals, Phytoacaricides, Vaccines, Endosymbionts

INTRODUCTION

Ticks belong to the class of Arachnida together with spiders, scorpions, and mites. Most of the ticks of medical and veterinary importance are grouped as hard and soft ticks. Tick infestation on animals has a direct effects as well as indirect effects through disease transmission. Direct losses include reduced weight gain, damaged hides, reduced milk production, loss due to tick toxicosis and tick paralysis (Ghosh and Nagar, 2014). The impact of ticks and tick-borne diseases on the livelihood of resource poor farming communities have been ranked high (Ghosh *et al*., 2007). The significant impact of ticks and TBDs underscores the importance of tick control. Conventional methods for tick control are based on the use of acaricides and insect growth regulators. Nevertheless, the continuous emergence of ticks and tick-borne diseases (TTBs) and acaricide resistance necessitates the development of new and more effective control strategies, for which understanding of different aspects of tick biology, and their interaction with pathogens are very crucial (Galay *et al.*, 2016).

Though the mainstay of tick control measure relies on the use of chemical acaricides, serious drawbacks such as chemical pollution of the food chain and environment, apart from the worrisome selection of acaricide resistant ticks are the associated challenges to address (Oosterwijk and wikel, 2021). This review article sketches insights into the problems of acaricide resistance and its mitigation strategies. Newer tick control strategies are the development of vaccines directed against the ticks and the tick microbiota, endosymbiont disruption, use of semiochemicals, phyto acaricides and biological control agents along with the utilization of host resistance. These strategies can be integrated to device integrated pest management strategies.

ACARICIDE RESISTANCE- THE SIGNAL TO CHANGE THE TRACK

Resistance to an insecticide or acaricide can be defined as a decline in susceptibility of a parasite to the insecticide or acaricide when it is used at the advised concentration and according to all the recommendations for its use (FAO, 2004). Acaricide resistance (AR) is an inherited phenomenon. In most cases, before the introduction of a new acaricide, it is likely that genes that confer resistance are already present in the tick population at extremely low levels. Numerous variables affect the rate of establishment of a resistant allele in the population and the time it takes for the tick control to fail, these comprise the prevalence of the original mutation in the population before treatment, the frequency of acaricide treatment, the resistant allele mode of inheritance (recessive, dominant or co-dominant), the concentration gradient of the acaricide and the percentage of the entire tick population that is not exposed to the acaricide. Even though the frequency of resistant genes initially develops slowly, by the time the effectiveness of dipping or treatment starts to decline, the frequency of resistant genes is typically increasing at a rapid pace (Nolan and Schnitzerling, 1986).

In the early stage, the population has a low frequency of heterozygous resistant individuals (single allele mutations), and the rate of rise in the frequency of the resistance allele is also low. In the next, emerging phase, repeated exposure to a drug, the prevalence of heterozygous resistant individuals in the population increases. Finally, the sustained selection pressure causes an increase in the proportion of homozygous resistant individuals, which eventually dominate in the population (FAO, 2004).

Currently, chemical acaricides are used to control tick infestations. There are seven classes of commercially available pesticides: Synthetic pyrethroids, organophosphates, macrocyclic lactones, benzoylphenyl ureas, formamidines, phenylpyrazoles and isoxazolines (Rodriguez-Vivas *et al.,* 2018; Selles *et al.,* 2021). The acaricides have specific targets and unique modes of action, which affects the growth, reproduction, and survival of various tick species (Klafke *et al.,* 2017; Klafke *et al.,* 2019). The numerous ways to apply acaricides to host animals include spraying, pouring, washing, and injections (FAO, 2004). Acaricide resistance selection in ticks is mostly accelerated by improper dilution, unsuitable administration, long-term use, and excessive dosage (Aguilar-Tipacamu *et al.,* 2011; Abbas *et al.,* 2014).

In India, higher prevalence of resistant genotypes was identified in both north Indian isolates and south Indian isolates of tick species, which should be taken seriously in the wake of increasing incidence of tick-borne haemoparasitic infection. Poor management techniques and infrastructure in India contributed to the easy spread of ticks along with the favourable environment conditions that supported tick growth and survival. The liberalisation of the veterinary drug industry has made acaricides easily available to farmers. Inadequate control has resulted in incorrect dosing, increased application frequency, and a reduced rotation of acaricides. Therefore, continuous monitoring of the acaricide resistance status of all tick population is essential to curtail the spread of resistant tick population, restrict the impact of reistance and to maintain acaricide efficacy.

Mechanisms of AR development

Resistance to acaricides can develop through different pathways, which are often categorised as metabolic, target site insensitivity, and decreased acaricide penetration through the tick cuticle (Guerrero *et al.,* 2012).

i) Metabolic acaricide resistance

Metabolic resistance, which conferred resistance to numerous acaricide classes, was the most common method of acaricide resistance. The increased capacity to detoxify or sequester the acaricide was an aspect of metabolic resistance to acaricide treatment. Resistance was produced by the metabolic enzyme system in two ways, either by alteration of the catalytic centre activity which increased the rate at which the enzyme unit metabolize the acaricides or through enhanced enzyme activity, resulting in accelerated metabolism and sequestration of the acaricide (Hemingway *et al.,* 2004).

- Cytochrome P-450

The cytochrome P-450 monooxygenase enzymatic family contribute to the regulation of endogenous bioactive molecules, such as hormones; they also control the detoxifcation and metabolism of cell damaging chemicals such as pesticides, drugs, and plant toxins in arthropods (Kasai, 2004). In several arthropods, cytochrome P-450 -mediated resistance is characterized by overtranscription of gene, resulting in the insensitive to certain pyrethroids (Scharf *et al.,* 1998; Scott, 1999; David *et al.,* 2013; Liu *et al.,* 2015). Also, Cossío-Bayúgar *et al.* (2018) reported a commensurate increase in transcription of the cytochrome P-450 gene in pyrethroids-resistant population of *Rhipicephalus microplus*,

- Esterases

Carboxylesterases have a responsibility in pesticide detoxification; furthermore, the presence of mutations in their nucleotide sequence leads to the over expression of these enzymes, as reported in *Musca domestica* (Feng *et al.,* 2018). An increased carboxylesterase hydrolysis was detected in *R. microplus* resistant ticks to the organophosphate (coumaphos), which is possibly related to resistance to this pesticide (Villarino *et al.,* 2003). Gaudêncio *et al.* (2017) reported overexpression of alpha- and beta-carboxylesterases in *R microplus* resistant larvae to fluazuron.

- Glutathione S-transferase

Glutathione S-transferases are multifunctional enzymes, responsible for the detoxification and metabolism of both physiological substances and xenobiotic (Wilce and Parker, 1994). Hernandez *et al.* (2018) reported increased Glutathione S-transferases transcription in flumethrin and chlorpyrifos resistant *Haemaphysalis longicornis* tick populations.

Enzymes such as glutathione S-transferases (GST), esterases and cytochrome P450 monooxygenases could mediate detoxification of acaricides imparting metabolic resistance to the ticks. The types of enzymes implicated in metabolic resistance are reported to be frequently determined using substances called as synergists. Triphenyl phosphate (TPP), piperonyl butoxide (PBO) and diethyl maleate (DEM) are the three synergists commonly used that are considered as specific inhibitors of esterases, mono oxygenases and GSTs respectively (Guerrero *et al.* 2012). These compounds can be added to the acaricide formulation to inhibit the enzyme responsible for resistance thereby increasing the effectiveness of the formulation.

ii) Target-Site insensitivity

Target site sensitivity describes the development of resistance through the alteration of target site receptors and neuronal enzymes, resulting in acaricide ineffective binding, thus rendering tick to survive the drug treatment (Coles and Dryden *et al.,* 2014).

- Voltage channels

The Voltage-gated Na+ and K+ channels are responsible for the propagation of electrical signals and generation of action potentials in neurons (Yu and Catterall, 2003). In ticks, synganglion, fused masses of nerve tissue (Rispe *et al.,* 2022) is a key target for the existing acaricides (Roma *et al.,* 2014). Pyrethroids are broad-spectrum acaricides and their main mechanism of action is altering the function of voltage-sensitive sodium channels in nerve membranes (Sattelle and Yamamoto, 1988; Narahashi, 1996; Soderlund, 2012). Mutation mediated knockdown resistance (kdr) is the most prevalent and frequent cause of pyrethroid resistance in ticks (Castro *et al.,* 2019; Cossío-Bayúgar *et al.,* 2020). Several investigations, mostly on *Rhipicephalus spp*., reported various point mutations in the sodium channel gene associated with reduce susceptibility to pyrethroids (Stone *et al.,* 2014; Cossío-Bayúgar *et al.,* 2020; Aguilar, 2018; Klafke *et al.,* 2019; Castro *et al.,* 2021; Amrutha *et al.,* 2021a; Amrutha *et al.,* 2021b).

- Acetylcholinesterase

The enzyme acetylcholinesterase (serine hydrolase) plays a crucial role in the termination of nerve impulse transmission by breaking down the neurotransmitter acetylcholine at the synapses (Mladenović *et al.,* 2018). However, exposure of ticks to organophosphate acaricides results in cholinesterase inhibition, causing acetylcholine to accumulate at the cholinergic synapse and keep the receptors activated, leading to tick paralysis and death (Fournier, 2005; Temeyer *et al.,* 2007). The target site of organophosphate is acetylcholinesterase and resistance to organophosphate involves modification in the structure of acetylcholinesterase wherein organophosphate cannot act on the altered enzyme (Nolan, 1985). In *R. microplus,* many amino acid substitutions have been reported in all three acetylcholinesterase genes (Jyoti *et al.,* 2016; Temeyer *et al.,* 2013), however for most of these changes a direct connection with organophosphate resistance remains absent.

- Octopamine receptors

A class of acaricides known as formamidines have been suggested to have a harmful impact on tick central nervous system, by targeting octopamine tyramine receptors, as a consequence, decrease in intracellular Ca2+ and activation of K+ efflux leading to disruption of nervous transmission, ultimately resulting in death (Evans *et al.,* 1980; Nathanson *et al.,* 1985; Dudai *et al.,* 1987; Baron *et al.,* 2018). Mutations in the gene encoding the octopamine receptor may result in conformational changes in *R. microplus* that lead to resistance to amitraz (Chen *et al.,* 2007). Resistant *R. microplus* and *R. decoloratus* both reported nucleotide mutations in the octopamine tyramine receptor gene, according to Takata *et al.* (2020) and Vudriko *et al.* (2022), respectively. However, it is still unknown whether this substitution has any functional influence in amitraz resistance.

iii) Penetration resistance

Reduced penetration resistance describes the reduced access of acaricides to the internal body environment due to modifications in the tick outer layer (exoskeleton) (Schnitzerling *et al.,* 1983; Guerrero *et al.,* 2012). Penetration resistance referred to changes in the cuticle that slowed down the penetration of acaricide molecules within tick's body. Changes in cuticular composition and cuticle thickening by increased deposition of structural components such as cuticular proteins and/or epicuticular lipids were described as the two mechanisms of penetration resistance. There have been reports of two types of compositional alterations- one that is laccase-2 mediated promoting hardening of the cuticle and second mediated by ATP binding cassette transporters (ABC transporters), which were expressed in the epidermis and function as efflux pumps in eukaryotic cells, facilitating export of cuticular components to the cuticle. Furthermore, it was believed that decreasing the rate of penetration offers detoxifying enzymes more time to act, doubling their impact and producing stronger resistance phenotypes (Balabanidou *et al.,* 2018)

Monitoring Acaricide resistance

Acaricide resistance monitoring in the field study is essential for reducing resistance selection and investigating different acaricide-resistant ticks. To measure tick resistance against acaricides, the FAO (2004) recommended some specific bioassay techniques. Stone and Haydock (1962) developed the larval packet test (LPT) has been used widely for the diagnosis of resistance in field population and for the characterization of resistance mechanisms to organophosphates and synthetic pyrethroids in ticks.Although it is regarded as a highly repeatable bioassay (Jonsson *et al.,* 2007), it is constrained by the labour and time needed to acquire results (Guerrero *et al.,* 2014). Shaw (1966) developed the larval immersion test (LIT) and it is primarily used to characterise resistance mechanisms to amitraz and macrocyclic lactones (Rodriguez-Vivas *et al.,* 2006; Perez Cogollo *et al.,* 2010). Larval tarsal test (LTT) developed by Lovis *et al.* (2013), it is a highly sensitive and time-efficient in vitro test and has been used to determine resistance levels in *R. microplus*, as well as other ixodid ticks. The adult immersion test (AIT) (FAO, 2004) is probably the most extensively used bioassay technique. The AIT uses engorged female ticks which are submerged in commercial or technical acaricides (Guerrero *et al.,* 2014).

Molecular methods for acaricide resistance detection play a crucial role in monitoring and understanding the mechanisms behind resistance development in ticks and mites. Some commonly used molecular methods for acaricide resistance detection include:

1) Polymerase Chain Reaction (PCR): PCR is a fundamental technique used to amplify specific DNA sequences in the genome of ticks or mites. In the context of acaricide resistance, PCR can be employed to detect the presence of known resistance-associated genes or mutations. For example, researchers can design primers specific to genes encoding detoxification enzymes like cytochrome P450s, GSTs, or esterases that are linked to resistance.

2) Real-time PCR (qPCR): qPCR is a quantitative version of PCR that allows researchers to measure the expression levels of specific genes associated with resistance. By quantifying the mRNA levels of resistance genes, scientists can assess the extent of their upregulation in resistant acarids compared to susceptible ones.

3) DNA Sequencing: DNA sequencing is used to determine the nucleotide sequence of specific genes or genomic regions. By sequencing the target genes associated with resistance, researchers can identify mutations or genetic variations that contribute to acaricide resistance.

4) Allele-Specific PCR: This technique

is used to detect specific single nucleotide polymorphisms (SNPs) or mutations associated with resistance. Allele-specific primers are designed to specifically amplify either the wild-type or mutated allele, allowing researchers to determine the genotype of individual ticks or mites.

5) Microarray Analysis: Microarrays enable the simultaneous analysis of the expression of thousands of genes in ticks or mites. This approach can help identify overexpressed genes involved in metabolic detoxification or other resistance mechanisms.

6) Next-Generation Sequencing (NGS): NGS technologies, such as whole-genome or transcriptome sequencing, provide a comprehensive view of the genetic variations and expression profiles in acarids. NGS can aid in the discovery of novel resistance-associated genes and pathways.

7) Gene Expression Profiling: Gene expression profiling involves quantifying the expression levels of a wide range of genes in both susceptible and resistant acarids. This technique helps identify genes that are upregulated or downregulated in response to acaricide exposure, shedding light on the underlying resistance mechanisms.

8) Functional Assays: While not purely molecular, functional assays can complement molecular techniques by confirming the impact of specific genetic variations or gene expression changes on acaricide resistance. These assays involve expressing candidate genes in heterologous systems or using gene knockdown techniques in acarids to assess their role in resistance.

The research investments into mitigation strategies for tick control have been successful in spinning out various alternate options of tick control which are discussed as follows:

SEMIOCHEMICALS – A PROMISING OPTION

Semiochemicals are chemical signal vehicles of host/tick origin which are secreted into the external environment that mediate tick behaviour. Semiochemical communication in nature can be divided based on the type of behaviour they mediate and not based on the compounds that mediate behaviour. Broadly they can be divided into kairamones, allomones and pheromones. Kairomones are information bearing compounds or mixtures released by individuals of one species, detected by individuals of other species that benefit the recipient (Sonenshine, 2003). Allomones are information bearing compounds or mixtures emitted by individuals of one species that affect the behaviour of individuals of a different species for the benefit of the emitter (Sonenshine, 2003). Pheromones are the best known, intensively studied group of semiochemicals. An impressive variety of pheromones are seen in ticks including those used for food finding, arrestment, alarm, nest building and sex pheromones. Different chemicals serve as pheromones ranging from the high volatile molecules like substituted phenols namely methyl salicylate, o-nitrophenol or 2, 6-DCP to cholesteryl esters as non-volatile contact pheromones (Sonenshine, 2004). Pheromones can be classified as follows(Fig 1).

According to Sonenshine (2003, 2004, 2006), manufacture of a long-lived control device required the continuous delivery of pheromone source by a slow-release device. Arrestment pheromone impregnated device is a patented device incorporating purines from the faecal wastes of the prostriate tick, *I. scapularis* into oily droplets released from a pump sprayer was designed for delivery to vegetation. The oily droplets adhered to vegetation where *I. scapularis* quest for hosts. The arrestment pheromone components like guanine and xanthine along with acaricide, permethrin caused the ticks that encounter the droplets to cling to the contaminated surfaces where they acquire a lethal dose of acaricide (Sonenshine, 2006). Whereas 2, 6-DCP as confusants exploits the mate searching behaviour of the male by minimizing their ability to locate females as the emitting source. A sex pheromone–pesticide combination was used to confuse mate seeking male, causing them to acquire more pesticide as they wander through the pheromone and pesticide treated fur (Sonenshine, 2004, 2006). Tick decoys are micro capsules, plastic decoys, or a trap using rubber septum, hollow fibres, capillary filaments, poly ethylene or gelatine capsules or multi-layer tags made

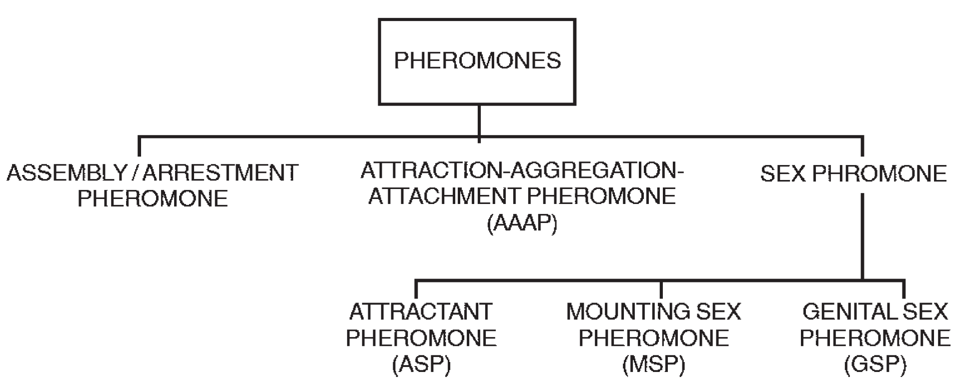

Fig.1. Types of pheromones (Sonenshine, 2004)

of natural or synthetic polymer resins served as the female mimics or the decoys. Any one of these devices was impregnated with 2, 6-DCP and propoxur. Cholesteryl oleate (MSP) was also coated on to the decoy. These decoys were attached to the hair coat of the tick infested rabbit with cement at a rate of 10 decoys per naturally attached female ticks. The males that were found in the mating posture on the decoys were 89 per cent, the remaining 11 per cent were attached to the skin of the animal adjacent to these devices. This resulted in the death of all males that were attached. The female ticks failed to engorge to repletion and most of them died. Engorged female ticks which dropped off the host failed to lay eggs (Sonenshine, 2004, 2006).

Field trials with tick lures and tick decoys consisting of acaricides and combination of semiochemicals such as 2,6-DCP, AP and carbon di oxide were performed and found to be effective (Ranju *et al*., 2013).

Semiochemical baited traps utilises semiochemicals as a bait to attract ticks to entomopathogenic fungus (*Metarhizium anisopliae)* bypassing the use of acaricides for the control of ticks (Nchu *et al*., 2008).

A recent addition to the devices using semiochemicals to bait ticks is the solar tick trap(Fig .2) , which employs

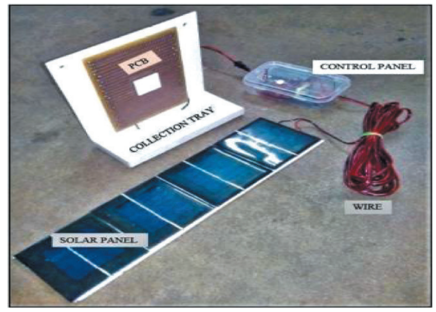

Fig .2 –Solar trap (Gowrishankar *et al*., 2021)

pheromones (AP) in the form of vapour patches to attract the ticks and kills them by electrocution in novel bamboo sticky trap (Gourishankar *et al*., 2021).

TickBot is semi-autonomous robotic device that can sweep the vegetation of host-seeking ticks in tick-infested habitats and kill them before they can attack people and/ or their pet animals. Following a guide wire and assisted by dispersal of CO_2 along its predetermined pathways, TickBot created a virtually tick-free environment within as little as 1 h following its deployment (Gaff *et al., 2015*).

Non-host derived repellents are the semiochemicals that control tick parasitism in tick resistant hosts such as beagles, that can be used on the susceptible hosts to control tick infestations. A slow-release formulation consisting of the non-host derived repellents (2-hexone and benzaldehyde) was applied in the form of collars onto the susceptible cocker spaniel breed of dogs and was found effective (Oliveira *et al*., 2017).

HOST RESISTANCE – OPTION FOR GENETIC SELECTION

The natural tick resistance of certain cattle breeds was reported to be an inherited attribute and was observed especially for purebred and crossbred Brahmin cattle. Acquired resistance was more strongly associated with a *Bos indicus* or *Bos indicus* crossbred genetic background (Johnston and Bancroft, 1918). Low resistance to *R microplus* of *Bos taurus* cattle was linked to an inflammatory response at tick attachment sites that was referred to as a non-directed pathological response to infestation, and resistant *Bos indicus* cattle were shown to exhibit a stronger T cell and CD25+ cell response at larval attachment sites (Jonsson *et al*., 2014). Resistant cattle were shown to have an earlier onset of cutaneous expression of proinflammatory chemokines and cytokines leading to an allergic contact hypersensitivity type response that resulted in basophil activation (Franzin *et al*., 2017). The development of vesicles at attachment sites, were described as blisters, that express a lymph-like exudate that traps ticks, and ticks that fed on resistant cattle were yellow in color in addition to being undersized. Hypersensitivity response at the bite site involved an influx of eosinophils and production of a serous exudate. The atypical engorgement color was subsequently shown to be due to ticks feeding on resistant hosts consuming a blood meal consisting of leukocytes rather than erythrocytes. *Rhipicephalus microplus* infestation was shown to result in mast cell degranulation in the skin of tick resistant cattle, and a histologic analysis of bovine cutaneous hypersensitivity to *Ixodes holocyclus* infestation showed an influx of basophils, eosinophils, neutrophils, and epidermal bullae formation, resulting in the trapping and killing of ticks in a serous exudate (Oosterwijk and Wikel, 2021).

Potentially underestimated as a factor in the expression of acquired tick resistance are the roles of pruritus, host grooming, and the direct effects of histamine on the feeding tick. Additional bioactive molecules, resulting from host innate and adaptive immune responses to ticks, mediate itch and pain responses by interacting with serotonin, Toll-like, protease activated, endothelin 1, and tumor necrosis factor receptors (Wikel, 2017). Infestation induced pruritus is a threat to a feeding tick and alerts the host to the presence of larvae and nymphs. Grooming, licking, and rubbing were determined to be important mechanical responses to infestation induced pruritus resulting in tick mortality (Kaufman, 1989).

Hence host resistance can be utilized to control the ticks by breeding non resistant animals with the resistant animals.

The native breeds of Kerala need to be evaluated for the tick resistant phenotypes

GENETIC MANIPULATION OF ENDOSYMBIONTS

Next generation sequencing studies have revealed that adult female ticks are frequently dominated by a single taxon with a high relative abundance, likely endosymbionts (Guizzo *et al*., 2020). Most tick endosymbionts have been located in the tick ovaries and from this organ they can access the eggs. The endosymbiont population of arthropod vectors could be exploited in different ways viz., as a chemotherapeutic target, vaccine target for the control of vectors. Expression of molecules with antiparasitic activity by genetically transformed symbiotic bacteria of disease-transmitting arthropods may serve as a powerful approach to control certain arthropod-borne diseases.

Chemotherapeutic approach: This approach exploits the endosymbionts of arthropods vectors as a chemotherapeutic target with the aim to disturb the symbiosis (Nogge, 1976).

Immunological approach: Immunization of animals with the whole killed endosymbionts or purified antigens or recombinant antigens of the endosymbionts would render them immune to tick vectors. Instead of targeting the host (vector) antigens, the endosymbionts could be targeted to disturb the symbiotic relationship between the vector and the symbiont. Following ingestion of the blood from immunized animals, these antibodies together with other components of the immune system such as complement, will destroy the symbionts inside the vector, leading either to death or to disruption of normal gut physiology of the tick and reduce growth and egg-laying ability (Willadsen, 1995).

Wolbachia cytoplasmic incompatibility (CI) based approach: Wolbachia infections in arthropods can manipulate reproduction of their hosts in a variety of ways e.g., induced parthenogenesis, male killing, parthenogenesis, and cytoplasmic incompatibility (CI). It is the phenomenon in which mating between Wolbachia infected male insect and female insect of the same species without Wolbachia infection (Unidirectional CI) and mating between insects of the same species with different Wolbachia strain infection (Bidirectional CI), result in embryonic mortality. Reciprocal mating (infected female x uninfected male) and mating between infected individuals are fully compatible (Huber *et al*., 1991).

CI is explained by two terminologies, Modification and Rescue. Modification is the process in which Wolbachia modifies the sperm of the infected male during spermatogenesis by an unknown process. The modified mature sperm is devoid of Wolbachia. If a modified sperm enters an incompatible egg (uninfected or infected with different strain), a delay in breakdown of nuclear membrane of pronuclei of sperm resulting in mitotic asynchrony and embryonic death (Huber *et al*., 1991).

Paratransgenesis

Genetic transformation of commensal or symbiotic bacteria of the arthropod vector is to alter the vector's ability to transmit pathogen, it is an alternative means of blocking the transmission of VBD's. the midgut bacteria of arthropod vectors can be engineered to express and secrete effector proteins which block the parasite invasion or kill the parasite in the midgut or haemolymph or reproductive tract. The arthropod vector that harbours the genetically transformed endosymbionts are called as Paratransgenic vector (Durvasula *et al.,* 1997). The endosymbionts of arthropod vectors can be cultured and genetically transformed to express the effector gene inside the vector in such a way the gene product kills the parasite/ pathogen that the vector transmits resulting in population of arthropod vectors refractory to the particular vector borne parasite. This strategy has shown promise in controlling the transmission of *Trypanosoma cruzi* by *Rhodinus prolixus*. The genetically transformed *Rhodococcus rhodnii* was delivered into the asymbiotic first instar nymph orally in such a manner to express an antimicrobial peptide, L-cecropin A, inside the gut lumen which conferred resistance status to the Paratransgenic (Durvasula *et al.,* 1997).

BIOLOGICAL CONTOL – THE ENTOMOPATHOGENIC ALTERNATIVES

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 biocontrol agents. Application methods can include individual inoculations or inundative releases of the natural enemies. The Bio Pesticide Manual (Copping, 2001) lists 96 commercial active ingredients based on microorganisms. Thirty-three are based on bacteria, 36 on fungi and eight on entomopathogenic nematodes.

Bacteria

Bacteria are commonly found in wild-caught ticks, but most of these bacteria are not considered pathogenic to the ticks. Nevertheless, some bacteria show pathogenicity to ticks. For example, *Proteus mirabilis* is pathogenic to *Dermacentor andersoni*. Bacteria also attack *Amblyomma hebraeum, Hyalomma marginatum* and

Rhipicephalus eversti eversti and apparently cause the blackening disease of *Boophilus decoloratus*. Bacterium *Cedecea lapagei* (Enterobacteriaceae) infects *Boophilus microplus*, this bacterium infects ticks via the genital opening and under laboratory conditions can produce up to 100% mortality. Hassanain *et al*., (1997), found that three commercial varieties of B. thuringiensis (*B. t. kurstaki, B. t. israelensis* and *B. t. thuringiensis)* produced mortality when sprayed on unfed or engorged adults of *Argas persicus* or *Hyalomma dromedarii.* The crystalline d-endotoxin of *B. thuringiensis* is produced during sporulation and disrupts insect midgut walls.

Fungi

Over 700 species of entomopathogenic fungi have been reported, but only 10 species have been or are currently being developed for the control of insects. The ability of entomopathogenic fungi to penetrate the cuticle of arthropods, the ability of a strain to kill several stages of the same pest and the relatively specific virulence of a single strain to one or a small group of pests make them good candidates as biocontrol agents. However, fungi also have some disadvantages: they are slow in killing their host, they need high humidity to germinate and sporulate, they are susceptible to UV irradiation, and some strains can potentially affect non-target arthropods (Ginsberg *et al*., 2002). *Metarhizium anisopliae* and *Bessinia bassiana* exhibited the strongest anti-tick pathogenicity. Tick eggs, in contrast to many insect eggs, are highly susceptible to fungi and up to 100% of the eggs exposed to fungi under laboratory conditions did not hatch.

Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) of the families Heterorhabditidae and Steinernematidae are known to be obligatory parasites of insects. The only free-living stage of the nematode, the third/infective juvenile (IJ), actively locates and enters the host via natural openings, and then releases symbiotic bacteria that kill the host insect within 24–72 h. The nematodes then multiply within the host cadaver and 6–18 days post infection thousands of IJs are released into the environment. The most common natural habitat of these nematodes is moist ground. The EPNs are known to be pathogenic to over 3000 insect species, whereas each strain may often be relatively specific to a small group of hosts. The injection of a single heterorhabditid nematode into a tick can cause mortality (Glazer *et al*., 2001). Tick mortality caused by EPNs seems to be due to the rapid proliferation of the nematode symbiotic bacteria within the ticks, since the nematodes do not go through their natural cycle within ticks, and most infective juveniles die shortly after entry. In vitro experiments demonstrated that tick haemolymph hinders the growth of EPNs but the reason(s) for nematode mortality within ticks is/are not yet fully understood.

Parasitoids

Most parasitoids used in the biological control of insect pests of plants belong to the order Hymenoptera. The most widespread species is *Ixodiphagus hookeri* (synonyms, *Hunterellus hookeri, I. caucurtei*) (Takasu *et al*., 2003). Nymphal ticks were parasitized while they were engorging on vertebrates and parasitoid egg development was found to be associated with ingestion of blood by its host tick. The only species that has been released for biological control of ticks is *I. hookeri.* The parasites were released as adults, in parasitized *I. scapularis* nymphs on mice. Inundative releases to control ticks in limited areas (e.g., farms, recreation areas) are potentially feasible. *Ixodiphagus* spp. parasitize only ticks, as far as is known. Therefore, non-target effects would presumably be minimal if these parasites were released for tick control.

Predators

Many tick bio-suppressors such as ants, beetles and many bird species are general predators that feed occasionally on ticks, therefore their populations do not depend on the sizes of the tick populations.

PHYTO ACARICIDES – THE POWERFUL HERBAL WAY

These compounds act by inhibiting the growth as well as development and reproduction in various ways to control the population of flies, fleas, lice, ticks, and mites of veterinary significance.

Pyrethrum

Chemically it is the mixture of several esters called pyrethrins which are extracted from the flower of *Chrysanthenum cinerariaefolium.* Pyrethrins target the sodium ion channels in the nerve cells of insects and serve as neurotoxin leading to knock down effect resulting in repeated and extended nerve firings. This hyperexcitation causes the death of the insect due to loss of motor coordination and paralysis (Marangi *et al.,* 2009).

Neem

Azadirachtin is the most biologically active principle found in the neem (*Azadirachta indica*). It is structurally similar to the insect hormones known as “ecdysones” which are responsible for metamorphosis in insects leading to antifeedant effects (Chaudery *et al.,* 2017). The important properties of neem are acting as free radical scavenger due to the rich source of antioxidant and immunomodulation.

Essential oils and plant extracts

Recently, the profound anti-tick activity of the herbal acaricide product containing Neem oil, Karanj oil, Eucalyptus oil, Rohit Gawash and Karpura against egg and adult stages of *Rhipicephalus microplus* ticks showed that treated females laid eggs very meagre in number and amongst them very few have hatched (Rao *et al.,* 2018).

The ethanolic extract of the leaves of *Jetropha curcas* at low concentrations proved to significantly inhibit the hatching of laid eggs and was considered as a possible alternative for the control of cattle ticks (Juliet *et al.,* 2012). Further studies were suggested to explore the role of flavonoids and their mechanisms in modulating the tick reproduction.

Sunil *et al.* (2013) documented the acaricidal effects of the ethanolic extract of leaves of *Casia fistula* that produced a concentration dependant mortality of adult cattle ticks. Complete blocking of hatching of laid eggs was observed at concentration above 80 mg / ml which was comparable to the effect of deltamethrin.

Studies showed that the crude extract and hexane sub-fraction of *Artemisia nilagirica,* Kerala possessed very good acaricidal activity for both adult and larval forms of *B. annulatus* comparable to deltamethrin. Phytol, eudesmol, 2, 6-dihexadecnoate, and hexadecanoic acid ethyl ester were the major compounds identified in the hexane fraction of *Artemisia nilagirica* leaves by GC-MS analysis (Udayan *et al*., 2020).

ANTI TICK VACCINES - THE IMMUNOLOGICAL WARFRONT

As the tick introduces different saliva proteins into the host, which also serve as antigens for the host to develop a successful protective immunological response called naturally acquired tick resistance also referred to as 'tick immunity', occurs after repeated tick infestations and can lead to the reduction of tick feeding success. Tick immunity is established by complex interactions of all the different mediators of the immune system as reviewed above and antigen-presenting cells, T-lymphocytes, eosinophils, mast cells, basophils, cytokines, complement, antibodies and cytokines play a central role. Tick infestation leads to IgG production against salivary gland proteins and is boosted upon re-infestation. Complement also plays a role, C3 is deposited near the tick-bite site and depletion of complement reduced tick immunity. T-cells appear to be involved in the increased cutaneous response associated with tick immunity. The cutaneous response at the tick bite site is characterized by an influx of basophils and eosinophils. Both influx and degranulation of these cells were elevated at the tick bite site in repeated tick infestations. Activation of the immune system by antigens in tick saliva is likely to create an unfavorable environment for transmitted pathogens and hence tick rejection might take place before transmission can occur.

Exposed and concealed antigens

There is a growing list of tick proteins that have been identified and evaluated as potential vaccine candidates. Two groups of possible candidate vaccine antigens are described. The first group consists of the 'exposed' antigens, which are secreted in tick saliva during attachment and feeding on a host. These antigens elicit an immune response at the tick feeding site. Exposed antigens are likely to be less immunogenic as a result of prolonged exposure to the host immune system. The second group are 'concealed' antigens that normally do not come into contact with the hosts immune system (Nuttall *et al.* 2006). Although concealed antigens do not induce an immune response upon tick infestation, they are immunogenic when prepared as extracts or recombinant proteins and inoculated artificially into a host (Nuttall *et al.* 2006). These antigens rely on vaccine-induced antibodies to be effective and repeated vaccination may be necessary to produce sufficient levels of antibodies. Subolesins, Vitellins, ferritins and other proteins involved in structural and metabolic functions, reproduction and tick protective antigens might act as potential vaccine candidates.

Bm86, a 89 kDa gut protein from the cattle tick *R. microplus* is expressed in every life stage from eggs to engorged adult tick (Willadsen, 2004). By far the only tick antigen to be commercialized as an anti-tick vaccine is Bm86. Two veterinary vaccines have been developed based on the Bm86 antigen produced in yeast: Gavac™ (Hebertech™, Havana, Cuba) and TickGard (Merck Animal Health, Madison, NJ, USA). It has been shown that these vaccines reduce tick numbers up to 74% and reduce tick fertility, combining the overall efficacy of up to 91%. In Indian veterinary Research Institute, similar research was undertaken. The protective efficacy of rBm86 against *R. (B.) microplus* (IVRI-1 line) and *H. anatolicum* (IVRI-II line) was evaluated and the results indicated moderate efficacy of commercially available rBm86 based vaccine against *R. (B.) microplus* and low efficacy against *H. anatolicum* and recommended identification of more protective antigen for development of vaccine suitable to Indian condition (Ghosh and Nagar, 2014).

CONCLUSION

Ticks and TBDs poses a significant threat to economically sustainable livestock production. Use of acaricides alone leads to development of acaricidal resistance and also leads to environmental pollution and residues in the food chains. Phyto acaricides not only possess acaricidal activities, but also have immunostimulatory properties. Thus, phytoacaricides has the potential to replace chemical acaricides for the control of ectoparasites. Use of vaccines, semiochemical impregnated devices and BCAs can play an important role in controlling the TTBDs as they do not have any residual effects. Manipulation of endosymbionts and the tick microbiomes has the potential to effectively control the TTBDs. It is evident that effective control of vectors and slowing down of emergence of acaricidal resistance cannot be accomplished by adopting only one control strategy. An integrated vector strategy which draws together a range of appropriate complementary tactics may offer the best approach for the future, allowing one tactic to mask the weaknesses of another. It is therefore essential that policy decisions should be made to adopt long-term strategies aimed at slowing the emergence of acaricidal resistance.

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