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Entomopathogenic fungi as potential tickicidal agent-future perspective

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Abstract
Ticks, the obligate blood feeding parasites have been capturing the attention of mankind for the last several decades because of their medical and veterinary importance. They have substantial impact over the economy and health of the world population. Though chemical acaricide have played a major role in tick management, its injudicious usage has led to undeniable consequences which is a matter of great concern. To curb their impact, biological control using entomopathogenic fungi (EPF) has been manipulated in many countries over the last few decades. However, it still remains to be an unstudied area in developing countries like India. The present review intends to do a small-scale comparison of the efficacy of tickicidal activity of fungal genera like *Metarhizium*, *Beauveria*, *Verticillium*, *Paecilomyces*. The concentration at which these fungal strains are effective against the different developmental stages of ticks, their percentage of mortality and the time taken have been tabulated. Among which, the Conidia-oil formulations of *Metarhizium* and *Beauveria* are found to be the most effective and could be categorised as the most potent pathogenic strains against tick population. Besides effectiveness, their negligible non-target effect also makes them more eco-friendly when compared to chemical acaricides. With an increasing resurgence of tick-borne zoonoses, this review aims to outline the need to develop and to implement entomopathogenic fungi as potential tickicidal agent in near future.

Keywords: entomopathogenic fungi, *Metarhizium*, *Beauveria*, *Verticillium*, *Paecilomyces*, fungal-oil formulations, tick-borne zoonoses

Introduction

Ticks are among the most significant blood-sucking arthropods, in terms of effect on animals and human beings. These ectoparasitic hematophagous group belongs to the order Acari, suborder Ixodida which is wide in distribution. Although they are recognized widely as a pest, they are best known for their notorious vector nature (Prakasan & Ramani, 2007) [41]. Through vectoring serious pathogens, they act as agents of Bovine anaplasmosis, Babesiosis, Theileriosis, Ehrlichiosis and many others (Gayle & Ringdahl, 2001) [13] which results great economic losses in livestock around the world (Maniania *et al.*, 2007) [26]. When comes to public health, there were 250000 human cases of Lyme disease reported in United States from 2000 to 2010 (Dantas-Torres *et al.*, 2012) [9], as well as 50000 cases per year in Europe (Piesman & Eisen, 2008) [38].

In India, the first case of tick borne zoonoses, Kyasanur Forest Disease was reported late in1957 from Shimoga district of Karnataka (Work & Trapido, 1957) [57]. Chakraborty *et al.*, in 2019[6] have retrospectively analysed an estimated 9,594 cases of KFD within 16 districts of Southern India, during the period from 1957 to 2017. Other tick borne zoonoses like Tick typhus (Padbidri *et al*., 1984) [36], CCHF which are associated with high mortality rates also have turned up in the country (Mourya *et al*., 2014) [33].

While considering the livestock in developing countries like India, Ticks and Tick-borne Diseases are ranked high in terms of their impact on poor- farming communities (Ghosh *et al*., 2006) [14], with an estimate of US$ 498.7 million per annum for the management of TTBDs (Minjauw & McLeod, 2003) [31]. It causes poor quality of livestock products which influences the economic growth of the country.

Controlling these tiny organisms were started parallelly with its dispersion over the globe. As time evolved, control strategies progressed from the use of Arsenious oxide solutions (Wilson., 1996) [56] in Zimbabwe to different chemicals like organophosphate, cypermethrin, etc. However, the use of these chemical acaricides for controlling ticks have been a concern from the time of its adoption. Undesirable side effects of acaricides like environmental pollution (Shanmuganath *et al.*, 2021) [48], pesticide residue in meat and milk and resistance development in target species (Onofre *et al*., 2001) [35] demanded an immediate shift from the conventional use of chemicals. Predators, parasitoids, parasites and pathogens are known to influence tick population among which fungi are considered to be the most important natural enemies of ticks (Chandler *et al*., 2000) [7].

Therefore, unmitigated studies about the utility of fungal groups in controlling tick population should be included in the priority list of young researchers in India. In this review, articles up to recent years were referred. The present review aims at the compilation of the potency of different fungal strains to quantify and summarize their effect on the ticks. This paper also intended to bring the attention of scientist to the field of alternative control strategy using entomopathogenic fungi, which is imperative in present days.

Biocontrol of ticks using entomopathogenic fungi

As the dangers of the long-term use of acaricides are clearly visible, and importance of alternative strategies are becoming major area of thrust, there has been a resurgence of interest in employing fungal pathogen against insect pests (Samson *et al*., 2013) [46]. The pioneer work of Agostino

Bassi with *Beauveria bassiana* in silkworms in 1834 (Glare & Milner, 1991) [17] proved that fungi could cause infectious diseases in insects. From 1880s through the early 1900s, the spectacular epizootics caused by entomopathogenic fungi led to studies about their potential use for pest control (Roberts & Hajek, 1992) [44]. This can be a critical advantage for convenient field applications. The greater efficiency in killing host, great variability in virulent isolates, which can be used for killing large pest range and safety for the environment and humans (Alves, 1998: Zimmermann, 2007) [3, 60] has made EF a good biocontrol agent.

Unlike other pathogens such as viruses and bacteria, fungi need not to be ingested to kill the host (Jyoti & Singh, 2017) [49], since it employs infection through insect cuticle. Comprehensive accounts of the infection processes, pathology, and epizootiology of entomopathogenic fungi were given by Tanada and Kaya (1993) [52]. They usually infect their hosts through specialized spores that attach to, germinate on, and penetrate the integument. The penetrating fungus multiplies within the hemocoel and soft tissues of the host, and death occurs usually within 3 to 10 days after infection by water loss, nutrient deprivation, gross mechanical damage and the action of toxins. Under favourable conditions, the fungus sporulates extensively on the cadaver to facilitate further infections in the host population and thus continue the disease cycle. A broad review (Chandler *et al.*, 2000) [7] reported that 58 species of fungi, either in nature or in laboratory experiments, infected at least 73 species of Acari. Important genera of entomopathogenic fungi, *Beauveria*, *Metarhizium*, *Paecilomyces*, and *Verticillium* are found to be effective in the area of tick control (Chandler *et al*., 2000) [7]. They belong to the class Deuteromycetes (Scholte *et al*., 2004) [47] which have global distributions and can be mass-produced readily (Chandler *et al*., 2000) [7]. The EF has greater ovicidal capacity than its adulticidal activity and they can be considered as potential means for reducing tick population by killing egg masses (Greeshma & Narladkar, 2018) [54].

Method of use of fungi

In most of the studies, fungal conidia in aqueous suspensions containing Tween 80 used to treat different stages of ticks including egg, larva, nymph, adult (Fernandes, 2012) [11]. Since the environmental conditions like temperature, humidity influence the fungal activity (Ibrahim *et al*., 1999: Ment *et al*., 2010) [19, 28], use of vegetable and mineral oil in the fungal formulation have found to protect conidia from desiccation and provide synergic effect against target pests (Alves, 1998) [3]. Conidia-oil formulation of *Metarhizium anisopliae*, *Beauveria bassiana* (Kaaya & Hassan, 2000) [20] and conidia-polymerized cellulose gel (Reis *et al.*, 2008) [42] formulation of the same are found to be a promising method in controlling *Rhipicephalus (Boophilus) microplus*, *R.sanguineus*, *R. appendiculatus*, *Amblyomma variegatum*, *Anocentor nitens* in the field. Study conducted by Kaaya and Hassan about control of *R. appendiculatus* and *A. vereigatum* by *M. anisopliae* and *B. bassiana* in 2000 revealed that mortality rate of larvae was higher (100%) than nymphs (80-95%) or adults (78-80%) when the conidia-oil formulation was used. Study conducted by Kaaya and Hassan (2000) [20] also found significant reduction in the population of *R. appendiculatus* on grazing cattle after spraying conidia of *M. Anisopliae* or *B. bassiana* on grass in pastures seeded with larvae of *R. appendiculatus*. It was also noted that, a system with pheromones and carbon dioxide delivery in the field could attract ticks to a localized fungus-treated spot in the vegetation (Maranga *et al*., 2003: Maniania *et al*., 2007) [27, 26].

Studies have been conducted using combinations of chemical acaricides and EF in order to find out the compatibility and synergism between them. Studies of Paião *et al*., (2001) [37] and Bahiense *et al.,* (2006) [5] have evaluated the positive effect of combining chemical acaricides and EF on *Rhipicephalus (Boophilus) microplus* mortality. Inundative and augmentative releases were the main methods employed for the introduction of entomopathogens, including fungi, into the ecosystem (Maniania *et al*., 2007) [26]. It is also confirmed that the fungal powder that kept away from the sunlight, at room temperature, in a dry condition can hold its activity for more than 4 years, by Greeshma in 2018 [54].

Isolation of fungi

Isolation of EF from soil was done with the *Galleria mellonella* L. bait method in a study conducted by Alcala-Gomez in Mexico (2017) [2]. The EF used in most of the studies was isolated from ticks itself. In a study conducted in Iran, the strain 685, 689 of EF *M. Anisopliae* was collected from *Ixodes ricinus* from the UK. Most of the studies on fungi were based on isolation from the cadavers of insects or soil (Weeks *et al.,* 2020) [55]. Methods with susceptible insect host and selective media have been utilized for the isolation of entomopathogenic fungi from soil (Zimmermann, 2007) [60], and plants (Chase *et al.,* 1986) [8]. The use of insect bait was a very sensitive detection method in which larvae of *Galleria mellonella* were used and entomopathogenic fungi could be selectively isolated (Keller *et al*., 2003) [22]. Baiting soil samples with larvae of *G. mellonella* was a widely applied tool to screen for the indigenous species of entomopathogenic fungi (Keller *et al*., 2003: Meyling & Eilenberg, 2006) [22, 30]

Effectiveness of entomopathogenic fungi on ticks

The efficacies of fungal strains on tick populations are tabulated (Table 1). By studying the obtained data about the efficacy of fungal pathogen on tick mortality, the genera *Metarhizium* and *Beauveria* are found to be the most effective in controlling ticks. *B. bassiana* and *M. anisopliae* were found to be capable of inducing high mortality, decreased fecundity and egg hatchability (Kaaya *et al*., 1996) [21]. While it is most desirable to use tick control methods that are completely free of chemical acaricides, occasionally it may be necessary to use limited amounts of acaricides in combination or alternately with other control measures.

This will help to reduce the cost of acaricides, environmental pollution and the rate of development of resistance (Kaaya *et al*., 1996) [21]. Each fungus has a host range of more than of approximately 700 insect species (Goettel *et al.,* 2000) [18] and in nature they exist as an unspecialized insect pathogen whose activity may be locally intense on particular species when environmental conditions are favourable.

Table 1: Mortality in different stages of tick populations with the application of fungal strains: studies from different countries

Tick species	Fungal species/ strain	Concentration of fungal suspension conidia/ml	% of mortality (≥80)	Number of days after infection	Stage of life	Place of study	Reference
H.annatolicum	- *M.anisopliae*					Iran	[53] (Tavassoli, 2008).
	(715C)	10^4	88.2	18	L		
H. punctata	- M. anisopliae					Iran	
	(689)	10^4	80.4	4	L		
B. annulatus	- *M.anisopliae*					Iran	[39] (Pirali, 2007)
	(IRAN37C)	10^7	100	14	ENF		
	(DEMI001)	10^7	100	14	ENF		
	(DEMI001)	10^7	100	30	ENF		
	-*B. bassiana*						
	(IRAN403C)	10^7	80	14	ENF		
	(IRAN403)	10^7	96.5	7	L		
	(DEBI004)	10^7	100	30	ENF		
R.(B.) annulatus	-*V.lecanii*	5×10^8	100	7	L	Egypt	[1] (Aboelhadid *et al.*,2018)
	-*B. bassiana*	2×10^8	100	5	L		

Table 1: Cont

R.(B.) micropus	-*M. anisopliae* (M.aAT04)	10^7	95	14	ENF	China	[43] (Ren *et al.*, 2012)
		10^8	100	14	ENF		
		10^9	100	14	ENF		
	- *B. bassiana* (B.a AT03)	10^7	90	14	ENF		
		10^8	100	14	ENF		
		10^9	100	14	ENF		
	- B.a AT13)	10^7	85	14	ENF		
		10^8	100	14	ENF		
		10^9	100	14	ENF		
	(B.a AT01)	10^7	85	14	ENF		
		10^8	100	14	ENF		
		10^9	100	14	ENF		
R. microplus	-*M. anisopliae* (LCMS04)	10^7	90.4	5	L	Brazil	[29] (Mesquita *et al.*, 2020)
		10^8	94.6	5	L		
	(LCMS06)	10^7	86.9	5	L		
		10^8	90.6	5	L		
	(LCMS02)	10^7	86.3	5	L		
		10^8	93.3	5	L		

Table 1: Cont

R.microplus	- *M. anisopliae*	10^7	90.4	5	L	Brazil	[29] (Mesquita *et al.*,2020)
	(LCMS04)	10^8	94.6	5	L		
	(LCMS06)	10^7	86.9	5	L		
		10^8	90.6	5	L		
	(LCMS02)	10^7	86.3	5	L		
		10^8	93.3	5	L		
H.longicornis	- *M. anisopliae*	10^8	100	14	N	Korea	[24] (Lee *et al.*,2019)
	(JEF-214)	10^8	90	11	N		
	(JEF-279)	10^8	80	14	N		
	-*C.fumosorosea* (JEF229)	10^8	100	3	N		
			90	2	N		
B.annulatus	- *M. anisopliae*	10^7	100	6	L	Israel	[15] (Gindin *et al.*, 2002)
	(7) (43)	10^7	90.6	6	L		
	-*M. flavoviridae*	10^7	98.09	6	L		
	(11) (5)	10^7	97.46	6	L		
	-*M.anisopliae* (7)	10^7	90	7	ENF		

Table 1: Cont.

H. excavatum	-*M. anisopliae* (7) (43) (108)	2.5×10^3	100	7	UNL	Israel	[15] (Gindin *et al.*, 2002)
		2.5×10^3	95	7	UNL		
		2.5×10^3	100	7	UNL		
	-*M. flavoviridae* (5)	2.5×10^3	82	7	UNL		
R. sanguineus	- *M. anisopliae* (7) (108)	2.5×10^3	92	7	UNL		
		10^7	100	21	ENF		
	-B.bassiana (Nab)	2.5×10^3	95	7	UNL		

		10^7	94	21	ENF		
R.sanguineus	*-B.bassiana*	10^8	95	21	N	Florida	[23] (Kirkland *et al.*, 2004)
	-M. anisopliae	10^8	91	21	N		
A.maculatum	*-B.bassiana*	10^8	98	28	A	USA	[23] (Kirkland *et al.*, 2004)
		10^8	95	28	N		
	-M. anisopliae	10^8	88	21	N		
		10^8	99	28	N		

Table 1: Cont.

R. (B.)annulatus	*-M. anisopliae*					Iran	40] (Pirali-Kheirabadi *et al.*, 2007)
	(DEMI001)	10^7	100	12	ENF		
	(IRAN437C)	10^7	96	12	ENF		
	-B. bassiana (IRAN403)	10^7	90	12	ENF	Spain	
O. erraticus	*- B.bassiana* (Bb2157)	10^8	90	28	A		[58] (Zabalgogeazcoa *et al.*, 2008)
H.anatolicum	*-B.bassiana*						[51]
	(B.bAT01)	10^8	100	21	ENF	China	(Sun *et al.*, 2011)
	(B.bAT17)	10^8	100	21	ENF		
	(B.bAT14)	10^8	90	21	ENF		
	-M. anisopliae (M.aAT26)	10^8	93.3	21	ENF		

Table 1: Cont.

R.microplus	*-M.anisopliae*					Mexico	[4] (Ángel-Sahagún *et al.*, 2010)
	(Ma14)	10^8	100	14	L		
	(Ma10	10^8	100	14	L		
	-I. fumosorosea	10^8	94	14	L		

ENF- Engorged Female
UNF- Unfed female
A- Adult
UNL- Unfed larvae
L- Larvae
N-Nymph

Metarhizium

It is one of the most common entomopathogenic fungi, with a worldwide distribution (Scholte *et al*., 2004) [47]. It has a large host range, including arachnids and five orders of insects comprising 200 species (Goettel *et al.*,2000) [18]. Several studies from different countries have revealed the biological performance of various *M. anisopliae* strains with high virulence under laboratory conditions (Sun *et al*., 2011) [51]. In a study conducted by Stafford and Allen during 2014 [50], the high insecticidal and acaricidal activity of *M. anisopliae* has been confirmed. *Metarhizium* isolates were found to be effective against engorged females of several tick populations including Rhipicephalus (*Boophilus) annulatus*, *R. (B.) microplus*, *A. persicus*, *R. sanguineus*, *Hyalomma anatolicum*, (Pirali., *et al*., 2007: Pirali-Kheirabadi *et al*., 2007: Sun *et al*., 2011: Ren *et al*., 2012) [39, 40, 51, 43]

The engorged females were found to be more prone to the fungal attack than an unfed adult. Most of the strains of *M. anisopliae* have found to cause more than 50% mortality of different stages of ticks. Only those strains which caused mortality ≥80% were selected for tabulating. The strain 689 resulted in 80.4% mortality of larvae of *Haemaphysalis punctata*, within the 4th day of infection when applied in a concentration of 10^4Conidia/ml. The same strain took 16 days to cause more than 80% mortality when used in a concentration of 10^3Conidia/ml (Tavassoli, 2008) [53]. It indicates that there is a dose-dependent criterion in the efficacy of fungal infection. In a study conducted by Gindin in 2002[15], it was found that *M. anisopliae* was much pathogenic to different life stages of *R.*(*B) annulatus*, *H. Excavatum* and *R. sanguineus* than genus *Beauvaria*, *Paeciliomyces*, and *Verticillium*. In a recent study conducted by Mesquita *et al.*, 2020 [29], three isolates of this genus resulted in more than 90% mortality of larval stages of *R. microplus* after 5 days of infection.

For cultivation, its conidia require an RH of at least 92% to germinate (Ferron, 1981) [12] and those stored in dry condition showed high germination rate about 96-80%, than, when stored in paraffin oil (93-73%) (Morley *et al*., 1996) [32]. At a high temperature & low humidity fungus will not work. However, *M. Anisopliae* was found to work at 60-70% humidity where the temperature ranges between 20-30°C (Kaaya & Hassan., 2000) [20]. On supporting this, Greeshma & Narladkar in 2018 [54] have stipulated that storage conditions are more critical to spore survival and virulence than the substrate upon which conidia are produced.

Ginsberg *et al.*, (2002) [16] have studied the non-target effect of *M. anisopliae* in which it was found that concentration ≥ 10^8 Conidia/ml of the same affected other insect groups including beetle and cricket, in laboratory condition. As they do not provide an accurate assessment of the true risk in nature, it also has to be evaluated for better use of these groups of the organism as a potential control method.

Beauveria

Beauveria is one of the most frequently isolated entomogenous fungal genera and has a cosmopolitan distribution. *Beauveria bassiana* is the most pervasive species of the genus found and isolated from a vast variety of insects of different orders (MacLeod,1954) [25]. The infection of these genera does not appear to be temperature-dependent, but the conidia cannot withstand high temperature (Ferron, 1981) [12]. Among the conidial stage and blastoconidial stage, the latter was found to be much more pathogenic (Ferron, 1981) [12]. Investigations demonstrated that beauvericin, toxin produced by *B. bassiana* has insecticidal, antibiotic, cytotoxic, and ionophoric properties (Zimmermann, 2007) [60]. It was found to be more effective towards dog tick *R. sanguineus*, and *H. longicornis* when used in oil formulation (Dominic *et al*., 2019) [10]. The articles studied show a high pathogenicity of this genus against different life stages of tick population but a little less than that of *Metarhizium*. It was also found that this fungal genus works in a dose-dependent manner.

In a study conducted in Iran, it was recorded that the *Beauveria* with a concentration of 10^7 conidia/ml resulted in 96% of death of *R.*(*B) annulatus* larvae (Pirali *et al*., 2007) [39], which was quite different from the result obtained from Egypt, where a concentration of $2{\times}10^8$ of the fungi was needed for killing the same species within 5 days of post-infection (PI) (Aboelhadid *et al*., 2018) [1]. This showed the regional difference in fungal activity. Study of Ren *et al.*, in 2012[43] showed that three strains of *B. bassiana* effectively caused mortality of engorged females of *R. (B). microplus*, whereas only one strain of *M. anisopliae* caused the same. This species was also found to be effective against both adult and nymphal stage of *Amblyomma maculatum* causing 98 and 95 % of mortality respectively with a concentration of 10^8 conidia/ml.

Other genera

Among entomopathogenic fungi examined against different tick species, the most pathogenic were *Beauveria bassiana* and *Metarhizium anisopliae*. They caused high mortality to various stages of *B. microplus* (Onofre *et al*., 2001) [35], *R. appendiculatus* (Mwangi *et al*., 1995) [34], *R. sanguineus* (Samish *et al*., 2001) [45], and *Ixodes scapularis* (Zhioua *et al*., 1997) [59]. Even though genera *Verticellium, Lecanillum*, *Paeciliomyces*, and *Aspergillus* were included in a list of high pathogenic fungi against insect population, its effectiveness was far less against ticks, than that of *Metarhizium* and *Beauveria* (Pirali-Kheirabadi *et al*., 2007: Ángelo-Sahagún *et al*., 2010: Sun *et al*., 2011) [40, 4, 51]. *Isaria fumosorosea* caused 94% mortality of *R. microplus* larvae in 14 days (Angelo-Sahagun *et al*., 2010) [4]. Fungi from the genera *Beauveria*, *Paecilomyces* and *Verticillium* were far less pathogenic than *M. anisopliae* (Gindin *et al*., 2002) [15].

In 2018, Greeshma & Narladkar [54] have submitted first report on the use of *Verticillium lecanii* against tropical cattle tick, *R.(B) microplus*. In this report, they stated the cidal capacity of two strains of *V. lecanii* on the adult *R.(B) Microplus* and the effect of fungus on egg hatchability of this species. In a study conducted by Aboelhadid (2018) [1] in Egypt, *V. Lecani* showed a mortality of only 60-72% when applied with high concentration of $\geq 5{\times}10^8$ Conidia/ml on *R. annulatus* adult ticks. But it was also found that application of *V. lecanii* with a concentration $1{\times}10^8$ conidia/ml in oil formulation on engorged female of *R. microplus* resulted in a mortality of 97.6 % (Angel-Sahagun *et al.*, 2010) [4].

Conclusion

In India, even though the negative impact of chemical acaricides has been much discussed, the fungal control is being the least studied area to the date. The need for non-chemical control of ticks is likely to increase in the near future because of the unimaginable side effects of synthetic acaricides. Most of the studies and field trials in foreign countries have demonstrated that fungal strains are effective against all life stages of ticks. Despite scientific advancement, only fewer studies have so far been conducted in India about controlling ticks using EF. As geography, and climatic factors like temperature, humidity, solar radiation, are found to affect the performance of EF, a detailed study about these pathogens of acarine group is a critical one in a country like India. Studies about the non-target effect and synergic effect of these groups are also an imperative one. Although the study of fungal pathogens of Acari is considered as a minor stream of invertebrate pathology, they have been proved in most of the countries to be significant natural enemies of Acari, and there are some good opportunities to use them for effective biocontrol in India.

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