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The role of protists, nematodes and mites as natural control agents of sandfly populations

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The Phlebotomine sandflies (Diptera, Psychodidae) are hematophagous insects of immense medical and veterinary importance. Since World War II, the intensive use of chemicals to suppress and control sandfly populations resulted in development of insecticide resistance and resurgence among the sandfly populations worldwide. The use of chemicals also negatively impacted diverse non-target organisms, overall agroecosystem, crop productivity, human health etc. Due to the multiple adverse effects of the chemical compounds, more eco-friendly approaches have been evaluated. The application of entomopathogenic organisms such as nematodes, protists or mites as biocontrol agents has been vastly explored and applied in the field of forestry and agriculture. In comparison, only a little attention was given to blood-sucking insects such as sandflies. This review summarizes the findings related to the detection and use of entomopathogenic protists, nematodes, and mites in the field of biological control of sandflies. It highlights the potential of these organisms to be further explored and used for biocontrol of immature and adult stages of sandflies.

KEYWORDS

sandfly, nematode, mite, protist, entomopathogens, insect control

Introduction

The Phlebotomine sandflies (Diptera, Psychodidae) are hematophagous insects of immense medical and veterinary importance. There are approximately 1000 sandfly species, among which 98 are confirmed and/or suspected vectors of pathogens infectious to the humans and animals (1). Sandflies can transmit bacteria from the genus *Bartonella* (2), various viruses (Rhabdoviridae, Peribunyaviridae, Flaviviridae, Reoviridae, and Phenuiviridae) (3), and most importantly protozoan parasites from genus *Leishmania* - causative agent of leishmaniasis. Leishmaniasis can manifest with plethora of symptoms, being endemic in more than 100 countries worldwide, with more than 1 million reported cases and 70.000 deaths annually (4).

Due to the complexity of epidemiological and biological conditions associated with leishmaniasis transmission (5), the effective leishmaniasis control requires application of well-planned control strategies addressing multiple aspects such as effective diagnosis and

treatment of the disease, study of vectors and reservoirs, together with the development of new approaches for vector management (6).

Combating leishmaniasis proved to be extremely challenging especially in the field of sandfly vector management, which among others requires the continuous vector surveillance and implementation of different control strategies that are mostly based on insecticide use (7). Chemical strategies for the control of sandflies have gained massive popularity due to their rapid and potent effect and have been the main go to approach since the World War II (8). The emergence of sandfly insecticide resistance, as well as the immense accumulating negative effect of these chemicals on the environment and non-targeted organisms, significantly crippled the use of chemicals as the ultimate solution for sandfly and leishmaniasis control (8). These limitations strongly point toward the urgent need for more effective and novel methods that will have minimal environmental effects.

Among the environmentally friendly insect vector control strategies, biocontrol approaches have received increasing attention and popularity over the past 20 years. Biocontrol explores the potential of (micro)organisms to control the insect vector populations, and can be based on the predation, parasitism or pathobiological relationship between the biological control organism and the targeted insect hosts. As a result, numerous organisms were incriminated, and new strategies including the use of entomopathogens have emerged.

Entomopathogens are organisms that cause disease and/or death of insects (9). Several entomopathogens have been described in sandflies, including viruses, bacteria, protozoa, fungi, nematodes, and mites. Some of them are capable of killing the insect host even within a few hours, while others impact their longevity, fecundity and/or reproduction potential. The studies of entomopathogens as biological weapons for the control of sandfly population commenced early in the 19th century. These studies were very attractive, and a large number of publications have been produced targeting the identification of different pathogens. Unfortunately, under the pressure of commercial insecticides development, and their great success in controlling the sandfly populations (8), the exploration and further exploitation of entomopathogens as biological agents in sandfly control was neglected. The newly gained interest in the use of biological agents for the control of sandflies emerged after the encouraging results from the experiments involving *Wolbachia* in mosquitoes, paratransgenic approach for the control of *Trypanosoma cruzi* in triatomine bugs and development of sterile tsetse flies (10–12).

Compared to the other insects with medical and veterinary importance (or other insect pests), where investigations of biological control agents/approaches have been tested in practice and showed very promising results (11–13), the field of biological control of sandflies is still well behind. The majority of studies related to the biological control of sandflies and diseases they transmit were focused on the use of bacteria and fungi. The bacteria have been vastly explored in their role as pathogens (14), larvicides (15), for the development of paratransgenic approach (16) etc., while fungi were mainly evaluated as entomopathogens (17). In comparison to the massive amount of data generated in relation to the bacteria and fungi, other organisms such as protists,

nematodes and mites have been neglected. This review focuses on summarizing the impact, importance and potential of protists, nematodes, and mites as agents for the biological control of sandflies, and ultimately leishmaniasis.

Literature search strategy and publication selection

In total five databases including Science Direct, Scopus, PubMed, Google Scholar and Web of Science were screened for the presence of publications related to the protists, nematodes, and mites in association to different sandfly species. An intensive literature search was carried out using general terms (in English language) such as “mite”, “nematode”, and “protist” in combination with terms “sandfly”, “sand fly”, “Phlebotomine” or general sandfly genus/species names. As the initial search showed predominance of very old publications dating back to 1930-ies, that were written in native language of the corresponding author and available online only in the form of title record; the search was refined to include publications available at least in an abstract form regardless of the written language. After reading all collected publications, final filtering was performed and all studies that showed duplicate, insufficient, or not relevant data were removed.

Entomopathogenic nematodes

Nematodes are microscopic, non-segmented, elongated, colorless roundworms without appendages. Entomopathogenic nematode parasitize insects and can cause disease within an insect resulting in its death (18). Entomopathogenic nematodes described so far belong to more than 40 families, among which Steinernematidae and Heterorhabditidae have received the most attention. Members of these two families have many attributes of effective biological control agents, and have been utilized as classical, conservational, and augmentative biological control agents (19).

Entomopathogenic nematodes are highly diverse, complex, and specialized, and they work in tandem with their symbiont bacteria in causing rapid mortality within the insect host population. The life cycle of most nematodes includes an egg, four juvenile stages, and an adult stage. The third juvenile stage is the infective, or dauer stage, and is the only free-living stage. Infective juveniles locate, attack, and infect an insect host. Infective juvenile nematodes penetrate the insect’s body cavity through natural body openings, or by breaking hosts cuticle with the dorsal tooth/hook. Once inside the hosts body, the infective juveniles release bacteria that live symbiotically within their gut. The nematode-bacterium relationship is highly specific, and within Steinernematids only *Xenorhabdus* sp. bacteria co-exist, while within Heterorhabditids only *Photorhabdus* bacteria co-exist (20). Once released into the host, the bacteria multiply quickly and cause the host to die generally within 24 to 48 hours. Entomopathogenic nematodes feed on bacteria that they released, as well as on the insect host tissue. After nematodes mature, they produce a high number of new

juveniles that may undergo several life cycles within a single insect host. For the representatives of Steinernematids and Heterorhabditids it takes approximately 37 days to complete a life cycle inside an insect from egg to egg. Emergence of dauers from the host requires about 6 to 11 days for Seinernematids and 12 to 14 days for Heterorhabditids (18). When the insect host has been completely consumed, the infective juveniles (containing symbiotic bacteria), emerge from the empty shell of the host and begin the search for a new host.

Entomopathogenic nematodes are effective biocontrol organisms that have potential to infect and kill soil-dwelling larvae as well as above-ground adult forms of sandflies. The detrimental effect of nematodes such as *Tricephalobus steineri* in combination with *Procephalobus* sp. has been confirmed under laboratory conditions on *Phlebotomus papatasi* (21). This nematode declined colony productivity within 6 months from over 10,000 flies per week to less than 100 per week. Nematoda *Anandranema phlebotophaga* is a single auto generation nematode that is causing infertility in *Lutzomyia longipalpis* (22). Nematodes belonging to the genus *Anandranema* were also discovered in *Lutzomyia fischeri* but their effect on the insect was not evaluated (23). *Didilia ooglypta* was found to be a parasitic nematode of *P. papatasi* and *P. sergenti* (24). Further studies revealed that this nematode prolonged or stopped the development of larvae, reduced the longevity of adult flies, hindered the rotation of male external genitalia preventing them to mate, while adult females refused opportunities to engorge on blood (25). The nematode from the family Steinernematidae negatively affected the survival and blood feeding of *Lu. longipalpis* females (26). On the other hand, the presence of *Apbelenchoides bicaudatus* and *Coenorhabditis* sp. nematodes that were recorded in the colony of *Lu. youngi*, appeared to maintain the rearing medium in good condition (27). According to the authors these nematodes formed a useful source of protein in the food, particularly for the first instar larvae (27). The effect of *Steinernema feltiae*, *St. abbasi*, *St. carpocapsae* DD136, *St. scapterisci*, *Steinernema* sp., *Heterorhabditis bacteriophora* HP88, *Heterorhabditis* sp., and *H. indica* was evaluated under laboratory conditions on two sandfly species – *P. papatasi* and *P. sergenti* (28). It is observed that *Heterorhabditis* species were generally more virulent than *Steinernema* species, and that *P. sergenti* was relatively more susceptible than *P. papatasi* (28). Further on, the effect of *Steinernema carpocapsae* DD136, *Steinernema* sp. SII., *St. carpocapsae*, *St. abbasi* Ab, and *H. bacteriophora* HP88 were evaluated on the *P. papatasi* larvae, and demonstrated 42–94% mortality rate depending on the nematode (29). Field studies in Ethiopia showed that nematode found only in *Phlebotomus orientalis* females (0.8%) caused insects reproductive organ destruction (30). In addition to above mentioned nematodes, presence of *Mastophorus muris* was recorded in *P. ariasi*, *P. perniciosus* and *Sergentomyia minuta* larvae (31–33); *Rictularia proni* was found in *P. ariasi* larvae (34); Entomopathogenic nematode of the genus *Howardula* were detected in wild collected *Phlebotomus argentipes* and *Sergentomyia monticola* (35); Nematode belonging to Steinernematidae family were observed in the hemocoel of one specimen of *P. tobbi* (36); Natural population of *Pintomyia fischeri* was found positive for *Tylenchid* nematodes

(37); Not fully identified nematodes were detected in *Lutzomyia cayennensis braci* and *Lu. cruciata* (38, 39). The list of nematodes found in sandflies is summarized in Table 1.

Many factors can influence the successful use of nematodes as biological agents. Matching the biology and ecology of the nematode with the sandfly host is a crucial step towards successful application. It is proven that nematodes are most efficient in habitats that provide protection from environmental extremes, especially in soil (72). This characteristic makes them a good candidate for the application in control of immature stages of sandflies that dwell in soil, burrows, and different protected habitats. Nowadays, nematodes are cultured on a large scale in laboratories, and are available from many commercial suppliers, which is an additional incentive for their further exploration.

Entomopathogenic protists

Species from Microspora (Microsporidians), Sarcomastigophora (Flagellates and Rhizopods) and Apicomplexa (Gregarines and Coccidians), are referred as entomopathogenic protists (73). These protists can cause disease in insects and are of interest as agents of biological control. In the field of sandfly biological control, gregarines are the only explored entomopathogenic protists. Gregarines are monoxenous parasites and can be found in the body cavity or digestive tract of several invertebrate hosts including sandflies. The life cycle of gregarines, their taxonomy and host specificity are complex and still poorly understood.

The infective stages of gregarines are oocysts containing sporozoites. After oocysts dehiscence, sporozoites are released and they attach to the host epithelium or develop intracellularly into trophozoites. Detachment of the trophozoites from the host cell is followed by the sexual phase of the life cycle resulting in the formation of a gametocyst. Gametocyst containing gamonts undergoes multiple nuclear divisions leading to the production of gametes. Further on during sporogony, zygotes differentiate by mitoses into oocysts with sporozoites.

Ascogregarina chagasi later named *Psychodiella chagasi* is the most studied sandfly gregarine. In sandflies, the first instar larvae are infected by swallowing oocysts. The sporozoites are released from oocyst into the larval midgut where they attach to the epithelial cells and develop into trophozoites (74). In larvae, the gamonts are found mostly in the ectoperitrophic space of the intestine and in the intestinal lumen, where sexual development and oocysts production occurs. The gregarines can complete the life cycle in the larvae, and larval feces containing oocysts serve as a source of horizontal transmission. In adults, the gregarines form syzygies and gametocysts with oocysts in the body cavity. The gametocysts attach to the accessory glands of females, and the oocysts are injected into their lumen (49). This is a unique mechanism of vertical transmission supporting the hypothesis about sandflies and gregarines co-evolution (42). During oviposition, oocysts are attached to the sandfly egg chorion, which serves as a source of infection for newly hatched first instar larvae. The life cycle of most sandfly gregarines is similar to the above-described general life cycle of *As. chagasi*, with the exception

TABLE 1 Entomopathogenic nematodes, protists and mites that were found in association with sandflies.

Entomopathogen species	Sandfly host species	References
Nematodes		
<i>Tricephalobus steineri</i>	<i>Phlebotomus papatasi</i>	(21)
<i>Anandranema phlebotophaga</i>	<i>Lutzomyia longipalpis</i>	(22)
<i>Anandranema</i> genus	<i>Lutzomyia fischeri</i>	(23)
<i>Didilia ooglypta</i>	<i>Phlebotomus papatasi</i> <i>Phlebotomus sergenti</i> .	(24)
Steinernematidae family	<i>Phlebotomus tobbei</i> <i>Lutzomyia longipalpis</i>	(26, 36)
<i>Apbelenchoides bicaudatus</i>	<i>Lutzomyia youngi</i>	(27)
<i>Coenorhabditis</i> sp.	<i>Lutzomyia youngi</i>	(27)
Unidentified nematode	<i>Phlebotomus orientalis</i> <i>Lutzomyia cayennensis braci</i> <i>Lutzomyia cruciata</i>	(30, 38, 39)
<i>Mastophorus muris</i>	<i>Phlebotomus ariasi</i> , <i>Phlebotomus perniciosus</i> <i>Sergentomyia minuta</i>	(31–33)
<i>Rictularia proni</i>	<i>Phlebotomus ariasi</i>	(34)
<i>Howardula</i> genus	<i>Phlebotomus argentipes</i> <i>Sergentomyia monticola</i>	(35)
Tylenchida order	<i>Pintomyia fischeri</i>	(37)
Gregarines		
<i>Psychodiella mackiei</i>	<i>Phlebotomus argentipes</i> , <i>Phlebotomus papatasi</i>	(40, 41)
<i>Psychodiella chagasi</i>	<i>Lutzomyia longipalpis</i> <i>Lutzomyia sallesi</i> <i>Lutzomyia flaviscutellata</i> <i>Lutzomyia townsendi</i> <i>Lutzomyia sordelli</i> <i>Lutzomyia cruzi</i> <i>Lutzomyia evandroi</i>	(42–46)
<i>Psychodiella saraviae</i>	<i>Lutzomyia lichyi</i> <i>Lutzomyia schreiberi</i>	(42, 45)
<i>Psychodiella sergenti</i>	<i>Phlebotomus sergenti</i>	(47, 48)
<i>Psychodiella tobbei</i>	<i>Phlebotomus tobbei</i>	(47)
Unidentified gregarine	<i>Phlebotomus ariasi</i> <i>Lutzomyia vexatrix occidentis</i> <i>Lutzomyia shanonni</i> <i>Lutzomyia cruciata</i> <i>Lutzomyia flaviscutellata</i> <i>Lutzomyia camposi</i> <i>Lutzomyia gomezi</i> <i>Lutzomyia hartmanni</i> <i>Lutzomyia panamensis</i> <i>Lutzomyia sanguinaria</i> <i>Lutzomyia trapidoi</i> <i>Lutzomyia trinidadensis</i> <i>Lutzomyia ylephiletor</i> <i>Lutzomyia whitmani</i> <i>Lutzomyia edwardsi</i> <i>Lutzomyia firmatoii</i>	(33, 38, 49–53)

(Continued)

TABLE 1 Continued

Entomopathogen species	Sandfly host species	References
Gregarines		
	<i>Lutzomyia sallesi</i> <i>Lutzomyia fischeri</i> <i>Lutzomyia amarali</i>	
Mites		
<i>Microtrombidium hindustanicum</i>	<i>Phlebotomus papatasi</i> <i>Phlebotomus argentipes</i> <i>Phlebotomus sergenti</i> <i>Sergentomyia babu</i> <i>Sergentomyia indica</i>	(54–56)
<i>Microtrombidium nigeriense</i>	<i>Phlebotomus martini</i>	(56)
<i>Microtrombidium lewisi</i>	<i>Sergentomyia clydei</i>	(55)
<i>Microtrombidium</i> sp.	<i>Lutzomyia furcata</i>	(55)
<i>Biskratrombium coineaui</i>	<i>Phlebotomus papatasi</i>	(57, 58)
<i>Biskratrombium persicum</i>	<i>Phlebotomus alexandri</i> <i>Phlebotomus papatasi</i> <i>Sergentomyia mervynae</i>	(57, 58)
<i>Kenyatrombium macfarlanei</i>	<i>Phlebotomus martini</i>	(56)
<i>Eustigmaeus dyemkoumai</i>	<i>Phlebotomus duboscqi</i>	(59)
<i>Eustigmaeus gamma</i>	<i>Phlebotomus pius</i>	(59)
<i>Eustigmaeus gorgasi</i>	<i>Phlebotomus pius</i>	(59)
<i>Eustigmaeus lirella</i>	<i>Lutzomyia apache</i>	(60)
<i>Eustigmaeus parasiticus</i>	<i>Phlebotomus</i> sp. <i>Lutzomyia gomezi</i>	(59)
<i>Eustigmaeus johnstoni</i>	<i>Phlebotomus longicuspis</i> <i>Phlebotomus papatasi</i> <i>Phlebotomus alexandri</i> <i>Phlebotomus bergeroti</i> <i>Phlebotomus sergenti</i> <i>Sergentomyia africana</i> <i>Sergentomyia dreyfussi</i> <i>Sergentomyia magna</i> <i>Sergentomyia clydei</i> <i>Sergentomyia sintoni</i>	(61–67)
<i>Stigraeus smithi</i>	<i>Phlebotomus papatasi</i>	(61)
<i>Stigraeus sinai</i>	<i>Phlebotomus papatasi</i>	(61)
<i>Stigraeus furcatus</i>	<i>Phlebotomus alexandri</i>	(61)
<i>Stigraeus youngi</i>	<i>Phlebotomus martini</i> <i>Phlebotomus duboscqi</i> <i>Phlebotomus papatasi</i> <i>Phlebotomus sergenti</i> <i>Phlebotomus argentipes</i> <i>Sergentomyia theodori</i> <i>Sergentomyia adleri</i> <i>Sergentomyia tiberiadis</i> <i>pakistanica</i> <i>Sergentomyia clydei</i> <i>Sergentomyia schwetzi</i> <i>Sergentomyia magna</i> <i>Sergentomyia sintoni</i>	(59, 61, 68, 69)

(Continued)

TABLE 1 Continued

Entomopathogen species	Sandfly host species	References
Mites		
<i>Ledermuelleria</i> sp.	<i>Phlebotomus pius</i> <i>Lutzomyia gomezi</i> <i>Lutzomyia shannoni</i>	(38, 52)
Unidentified Uropodidae	<i>Phlebotomus aclydiferus</i>	(52)
<i>Bochartia</i> sp.	<i>Sergentomyia falax</i>	(56)
Unidentified species from <i>Eustigmaeus</i> and <i>Stigmaeus</i> genus	<i>Phlebotomus perniciosus</i>	(70)
<i>Typhlodromus evansi</i> or <i>T. primulae</i>	<i>Lutzomyia shannoni</i>	(38)
<i>Tyrophagus</i> sp. and <i>Stratiolaelaps scimitus</i>	Various sandfly colonies including: <i>Phlebotomus argentipes</i> , <i>P. sergenti</i> , <i>P. perniciosus</i> , <i>P. duboscqi</i> , <i>Lutzomyia longipalpis</i>	(71)

of *Ascogregarina mackiei* (*Psychodiella mackiei*). The development of sporozoites and trophozoites of *As. mackiei* in sandfly larvae is intracellular, and this is the only sandfly gregarine with intracellular development (40).

The host specificity studies of gregarines were conducted in detail in mosquitos, showing rather contradictory results (75, 76). Unlike extensive studies of mosquitoes, data about sandfly gregarine specificity is rather limited. Within the study of Wu and Tesh (1989) (43) seven sandfly species including *P. papatasi*, *P. argentipes*, *P. perniciosus*, *Lu. serrana*, *Lu. abonnenci*, *Lu. columbiana* and *Lu. longipalpis* were infected with oocysts of *Ascogregarina chagasi* (now *Psychodiella chagasi*). It is demonstrated that trophozoites gregarine life stages can be found in *P. papatasi*, *Lu. columbiana*, *Lu. serrana* and two strains of *Lu. longipalpis* (Brazilian and Columbian) (43). It is also shown that *As. chagasi* were able to complete their life cycle only in the Brazilian strain of *Lu. longipalpis*, while the infection rate and parasite density were higher in the Colombian strain. Infection with *As. chagasi* significantly reduced adult longevity of the Brazilian strain of *Lu. longipalpis*, but it had little effect on fecundity (43). Since *As. chagasi* demonstrated the variability in susceptibility toward different strains of *Lu. longipalpis*, it is speculated that these gregarines have strict host specificity (43).

In total, gregarines found in sandflies include 5 species from genus *Psychodiella* – *Ps. chagasi*, *Ps. mackiei*, *Ps. saraviae*, *Ps. sergenti* and *Ps. tobbi* (Table 1). *Psychodiella chagasi* was firstly described in the hemocoel and accessory glands of the *Lu. longipalpis* (44), and it was later found in *Lutzomyia sallesi*, *Lu. flaviscutellata*, *Lu. townsendi*, *Lu. sordelli*, *Lu. cruzi* and *Lu. evandroi* (42, 43, 45, 46). *Psychodiella saraviae* was described from blood-fed females of *Lutzomyia lichi* with gametocysts attached to accessory glands and oocysts in the lumen (42) and was further detected in *Lutzomyia schreiberi* (45). *Psychodiella mackiei* was described from *Phlebotomus argentipes* (40) and later was found in *P. papatasi* (41). *Psychodiella sergenti* and *Ps. tobbi* are found in laboratory reared

sandflies (*Phlebotomus sergenti* and *P. tobbi*, respectively) (47). *Psychodiella sergenti* gamonts are found in the hemocoel of *Phlebotomus sergenti* adults, and the sexual development of this gregarine occurs exclusively in blood-fed females (48). *Psychodiella sergenti* have a negative impact on its host, as gregarine infection significantly decreases the survival of various sandfly stages, but it does not impact the fecundity and mortality of blood-fed females (47, 48). Negative impact on the adult sandfly longevity was also observed in correlation with *Ps. chagasi* (43), and this species is known as common pathogen in laboratory-reared colonies of *Lu. longipalpis* (77).

Gregarines have been reported from more than 20 sandfly species but only a few of them were denominated. A number of studies recorded unidentified gregarine species from sandfly species such as *Lutzomyia vexatrix occidentis* (50), *Lutzomyia shannoni* (51), *Lu. cruciata* (38), *Lu. flaviscutellata* (49). Acephaline gregarines of one or more species were found in the hemocoel of ten species of sandflies: *Lutzomyia camposi*, *Lu. cruciata*, *Lu. gomezi*, *Lu. hartmanni*, *Lu. panamensis*, *Lu. sanguinaria*, *Lu. shannoni*, *Lu. trapidoi*, *Lu. trinidadensis*, *Lu. ylephiletor* (52). It is speculated that gregarines are not common parasites of these sandflies as only 18 from more than 6,000 females were found infected (52). Neogregarine were found in the fat body of *P. ariasi* (33), while trophozoite forms are found in the gut and/or sporocysts in the accessory glands of *Lu. whitmani*, *Lu. edwardsi*, *Lu. firmato*, *Lu. sallesi*, *Lu. fischeri* and *Lu. amarali* (53).

Other protist found in sandflies include *Adelina* sp. that was reported in *Se. minuta* and *P. perniciosus* (78, 79). The mechanisms of insect host exploitation and disruption by *Adelina* sp. is not well-characterized. Studies conducted on *Tribolium* and several other insects indicate that *Adelina* sp. can impact behavioral changes, have population regulation role and even cause death of the host insect (80). *Adelina* sp. are cosmopolitan organisms and the main transmission pathway for this coccidian parasite is via ingestion of contaminated food. Some species develop within the gut and disseminate oocysts through the host feces, while others inhabit the fat tissue (78).

The gregarine infection reduces the resistance of insects, and negatively affects their development and reproduction (81). The studies of sandfly gregarines are unfortunately very rudimentary, and the potential of these protists as agents of biological control of sandflies is underestimated.

Entomopathogenic mites

Mites are ectoparasites, and they can be phoretic, parasitic or both. Mites attach to the insect exoskeleton, which is harmful to the host, and if they are parasitic, they depend on their insect host for sustenance, maturation, and multiplication. Mites can parasitize on different developmental forms of sandflies, and some are known to affect larvae, while others can be found on adults.

Up to the present moment a total of 15 mite families have been associated with sandflies and they include Ascidae, Acaridae, Cheyletidae, Microtrombididae, Microdispidae, Trombididae, Erythraeidae, Hydrachnidia, Stigmaeidae, Parasitidae, Phytoseiidae,

Uropodidae, Tenuipalpidae, Pterygosomatidae, and Oribatulidae (61). Among them, Microtrombidiidae, Trombidiidae, and Erythraeidae, which are terrestrial parasitengone mites, constitute a large group of natural enemies and in their larval, deutonymphal and/or adult stage are ectoparasites on various arthropod groups including sandflies.

The larva of the *Biskratrombium coineaui* are ectoparasites of adult *P. paptasi*, while *B. persicum* larvae are ectoparasites on the adults of *P. alexandri*, *P. papatasi* and *Sergentomyia mervynae* (57, 58). Sandfly hosts of *Microtrombidium hindustanicum* are *P. papatasi*, *P. argentipes*, *P. serganti*, *Sergentomyia babu* and *Se. indica* (54, 55). *Phlebotomus martini* is a host for *Microtrombidium nigeriense* and *Kenyatrombium macfarlanei* (55, 56), *Sergentomyia clydei* is a host of *Microtrombidium lewisi*, while *Microtrombidium* spp. has been associated with *Lutzomyia furcata* (55, 56).

Numerous species of the family Stigmaeidae including genus *Eustigmaeus* and *Stigmaeus* are sandfly parasites. From genus *Eustigmaeus* species such as *Eustigmaeus dyemkoumai*, *E. gamma*, *E. gorgasi*, *E. johnstoni*, *E. lirella* and *E. parasiticus* are most frequently found in association with sandflies. *Eustigmaeus dyemkoumai* is ectoparasite of *Phlebotomus duboscqi* (59); *E. gamma* and *E. gorgasi* are parasiting on *P. pius* (59); *E. lirella* on *Lutzomyia apache* (60) and *E. parasiticus* on *Phlebotomus* sp. and *Lutzomyia gomezi* (59). *Eustigmaeus johnstoni* is by far found in most sandfly species including *Phlebotomus longicuspis*, *P. papatasi*, *P. alexandri*, *P. bergeroti*, *P. sergenti*, *Sergentomyia africana*, *Se. dreyfussi*, *Se. magna*, *Se. clydei*, and *Se. sintoni* (61–67). Species in the genus *Stigmaeus* including *St. smithi* and *St. sinai* are found on *P. papatasi*, *Stigmaeus furcatus* was found on *P. alexandri* (61), while *Stigmaeus youngi* has been associated with *P. martini*, *P. duboscqi*, *P. papatasi*, *P. sergenti*, *P. argentipes*, *Sergentomyia theodori*, *Se. adleri*, *Se. tiberiadis pakistanica*, *Se. clydei*, *Se. schwetzi*, *Se. magna* and *Se. sintoni* (59, 61, 68, 69). Species of the genus *Ledermuelleria*, also belonging to family Stigmaeidae, have been found on *Phlebotomus pius*, *Lutzomyia gomezi*, and *Lu. shannoni*, while not fully identified members of the Uropodidae were recorded on *Phlebotomus aclydiferus* (38, 52). *Bochartia* sp. has been recorded on *Sergentomyia falax* (56). Unidentified species from *Eustigmaeus* and *Stigmaeus* genus were found on *P. perniciosus* (70). An adult female of a Phytoseiidae, most likely being *Typhlodromus evansi* or *T. primulae*, was found on a *Lu. shannoni* (38). The research of mites in the field of sandflies was mostly focused on purely reporting the presence of mites (Table 1). Although relatively high number of mite species has been detected in association with sandflies, most of them are parasitic with very limited number of predatory species (38, 71, 82). Their relation and potential impact on sandflies were only briefly explored within the study of Dinesh et al., 2014 (82) showing good potential to be used as agents of biological control. This study evaluated the predatory ability of mites and spiders living in the same dwellings as sandflies. The mites and spiders were collected together with sandflies directly from the field and evaluated under laboratory conditions (82). Nymph and adult forms of mites were attacking and penetrating the larval body, damaging, and eating away its content leading to the death of larvae. The mites caused a decline in sandfly population within 15 days, with only 5% of the larvae being able to reach adult

stage. On the contrary, spiders were found more efficient in predating adult forms of sandflies (82). The exact species of these predator mites and spider has not been determined, but it is demonstrated that they have potential to be used as biological agents of sandfly control (82). Most recently, *Tyrophagus* sp. and *Stratiolaelaps scimitus*, were recorded among the different species of laboratory reared sandflies (71). These two mite species impacted the productivity of various sandfly colonies leading to significant colony declines (overall colony growth fell drastically by 30.9%–31.6% depending on the sandfly species). It has been shown that mites from the *Tyrophagus* genus can contribute to the sandfly colony decrease by competing for the food source with sandfly larvae, and by secreting metabolites that stop larval development (83). On the contrary, *Stratiolaelaps scimitus* are predatory mites that feed on fungus gnats, thrips pupae and other small insects in the soil (84), and their impact on sandfly colonies needs to be investigated to a greater extent (71). Mites are generally commonly observed within sandfly colonies, and if they are present in high numbers without any control mechanisms in place, they can lead to sandfly colony decimation (71, 82). The general presence of mites even more discourages scientists from colonizing sandflies under laboratory conditions which hinders the progress of further studies. The use of predatory mites for the control of insect populations has a long history especially in the agricultural settings (85, 86). Several species of predatory mites dwelling in soil showed success in insect control (84), and these species might be considered for the control of immature forms of sandflies.

Conclusions

Since World War II, the intensive use of chemicals for insect control resulted in development of insecticide resistance/resurgence among the insect populations worldwide. The chemical overuse negatively impacted non-target organisms, overall agroecosystem, crop productivity, human health etc. Due to the multiple adverse effects of insecticides, more eco-friendly approaches that are safe and effective have been developed and tested. The application of entomopathogenic organisms such as nematodes, protists or mites as biocontrol agents has been vastly explored and applied in the field of forestry and agriculture. In comparison, only a little attention was given to blood-sucking insects such as sandflies.

Findings herein reported demonstrated that the majority of studies in the field of sandfly and leishmaniasis control are mainly focused on pure detection and identification of entomopathogenic organisms (Table 1), while the deeper understanding of their interaction with sandflies and overall impact on sandfly population is lacking. Considering the complexity of the sandfly life cycle, and the fact that they have below and above the ground developmental stages, the application of different biological agents for their control might be beneficial. The exploration of predatory and pathogenic organisms, along with the development of paratransgenic approach and the application of green chemistry must be prioritized.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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