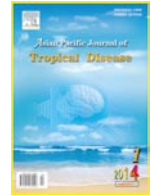




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Mites and spiders act as biological control agent to sand flies

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PEER REVIEW

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Comments

The manuscript is well written and provides information relevant to the vector control. I really encourage the publication of these results.

Details on Page S466

ABSTRACT

Objective: To find out natural biological control agents of sand flies vector of kala azar in Bihar, India.

Methods: Sand flies collected from the field using CDC light trap installing overnight to the collection site scrutinized for *Phlebotomus argentipes*, the established vector of visceral leishmaniasis. Blood fed adult females were confined in the insectary for its development of life cycle. During developmental stages 2nd to 4th instars larvae were examined closely by using compound microscope for mite infestation. Adult spider residing along with sand flies collected in trap were kept in cage along with sand flies and their activities were watched closely and recorded by video and picture.

Results: Mites were found predated 2nd to 4th instars larvae only under the laboratory conditions and lowering down the population of sand flies up to basal level within 15 d after infestation. One specific spider was found eating blood fed female sand flies kept inside the cage ($n=50$) attacking on lower part of thoracic region to kill the sand fly and ate desired soft part.

Conclusions: Both predators, mites and spiders are acting as biological control agents to larvae and adults of sand flies respectively resulting variable density of vectors due to variable association with these predators and also cause lowering the transmission of the disease as hidden natural controlling agent of sand flies. The extensive study will be of immense help in controlling sand flies without use of environmental pollutant *i.e.* chemical insecticide.

KEYWORDS

Sand fly, *Phlebotomus argentipes*, Mites, Spider, Predator, Diptera

1. Introduction

Visceral leishmaniasis (VL) is a major health problem in Bihar. It has alone captured almost 50% out of total burden of VL in the Indian sub-continent. Being a border state and located nearer to the border area of neighbour countries, Bihar seems to be the "hot spot" of VL. VL has a huge social and economic impact due to lost educational potential, reduced economic productivity due to missed days of work for adults, especially the family breadwinner and stigma. Therefore, VL not only occurs in the context

of poverty, but through their adverse social impact they may also promote poverty. VL leads to a loss of about 400 000 DALYs (Disability adjusted life years) every year in this region. This amounts to a loss of approximately USD 140 million annually (calculated at a loss of about USD 350 per DALY lost which is average yearly income in the endemic countries of the region estimated in 2008[1]. Since VL mainly affects the border areas of India, Bagladesh, Nepal, it was realized that the elimination has to be started in all the affected countries of the India subcontinent. As a result, a Tripartite MoU was signed by the three affected

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countries under the objective of reducing the incidence of kala-azar and post kala-azar dermal leishmaniasis to less than one per 10000 populations at the district level[2]. The cyclic reappearance of the disease in epidemic form after 15 years may be due to some another hidden factors like biological control agents in nature for sand flies like mites and spiders. VL is a vector borne parasitic disease caused by the protozoan parasite *Leishmania donovani* and transmitted by the established vector *Phlebotomus argentipes* (*P. argentipes*) (Diptera : Psychodidae) in nature in India. *P. argentipes* is endophilic and endophagic in nature and sometimes peridomestic. It is an opportunistic feeder. It prefers cattle shed more than the human dwellings to spend the life. Its life cycle is holometabolous including eggs, larvae (four instars), pupae and adult. The total life span of *P. argentipes* is approximately 30 d under favourable conditions at temperature 25–27 °C and relative humidity (RH) >72%. Sand flies are vectors of some other pathogens like bacteria and viruses. However, worldwide sand flies include vectors of different leishmaniasis. Some other parasites like fungi, nematodes and mites were found associated with sand flies as endo and ecto-parasites. Some of them have killing effect to sand flies. Phlebotomine sand flies spend most of their life time in dark and damp places. They lead their developmental stages in forest leaf litter, tree buttresses, caves, rodent burrows, cracks and crevices. The prevailing conditions in such places are also conducive to the development of many entomopathogens. Thus sand flies may be imminently suitable for biological control. It is exceptionally difficult to find the immature stages in nature. There are some reports on the preliminary laboratory studies on pathogens of Phlebotomine sand flies. The transmission of VL is continuing since more than century in Bihar. The density of sand flies has direct effect against environmental and ecological conditions. There might be some biological control agent in the particular region which is able to control the sand flies density below critical density due to which further transmission of the disease is restricted in natural disease occurrence cycle. The choice of insecticide is dichlorodiphenyltrichloroethane to control further transmission of kala-azar by killing sand flies. It is developing resistance in certain parts of Bihar[3]. It indicates that there is certain biological control agent in the nature which is playing important role in breaking down the transmission of the disease. The capacity of *Pimeliaphilus plumifer* mites were evaluated as biological control agent of Tritominae bugs and found very effective in 2007[4]. Mites and spiders were found controlling sand flies under laboratory conditions collected from field.

2. Materials and methods

Sand flies were collected using CDC (Centres for Disease Control and Prevention) light traps by fitting overnight inside the cattle sheds and human dwellings from 18:00 p.m. to 6:00 a.m. and morning indoor resting collection of sand flies was conducted using flashlight and aspirator from 6:00 a.m. to

8:00 a.m. The attention was made to collect spiders coming with sand flies in the rearing pot and watched their activities after releasing inside the Baraud cage (18×12×12 inches) having glass fitted at top. The whole process of predation was viewed directly and video graphed simultaneously. Sand flies were scrutinized in the laboratory either dead or alive after four hours. Live female sand flies were confined inside rearing pots having mites on their body. Sterilized larval food (mixture of rabbit faeces and sand) was provided inside the rearing Hilton pot, which has plaster of Paris in the base. The pot was kept on lint cloth inside a tray to maintain the humidity. The temperature was maintained up to 25–27 °C with RH 72%–90% inside the insectary. The development of larvae and their association with mites were closely examined under dissecting microscope to see the whole procedure of predation.

3. Results

The live predation of larvae by mites was observed under microscope attacking in mass and penetrating inside the larval body by scraping and eating the entire internal body parts (Figure 1). Bunch of nymphs and adults ($n=30-50$) were found attacking the whole body of the larvae of sand flies from 2nd instar to 4th instar. Mites did not prefer the first instars and pupae. Mites initially scratched the exoskeleton of the larvae and entered inside the body followed by damaging whole internal organs resulting to the death of larvae. Many scars were observed on the body of larvae. The live scene was visualized under dissecting microscope and mounted the dead larvae along with mite inside the body in Canada balsam and photographed. These have infested all 50 rearing pots having 30–50 sand fly larvae each and brought down the adult emergence up to basal level within 15 d. Only 5% of the larvae could be able to reach up to adult stage. The result had shown the significant control of larval number. This may act as one of the control measures of sand fly larvae.



Figure 1. Mites predated larvae (inside the body).

The predation to adult sand fly by spider was observed live inside the cage topped with glass (18×12×12 inches) and 50 mL glass test tube, while releasing wild sand flies and spider together (Figure 2). First of all, the spider was found attacking on the lower part of the thorax of the female resulting immediate death of the sand fly. Then it started eating them holding between feet and bringing up to the mouth. The experiment was repeated in 50 mL glass test tube having 10 blood fed sand flies and similar spider. The predation procedure was found similar. All procedures were video graphed for documentation. Morphologically dissimilar

another type of spider was kept together with similar sand flies population but predation was not observed even during three repeated experiments. It proved that particular spider (unidentified) is acting as biological control agent to adult sand flies in nature and controlling their population to break down the further transmission of the disease.

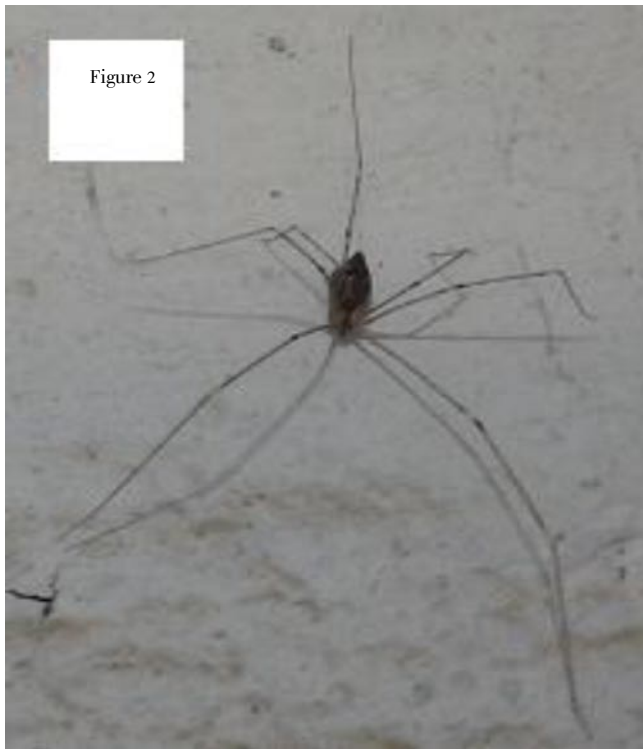


Figure 2. Spider attacking to sand fly.

4. Discussion

A major association of mites (*Acari* sp.) to sand flies was observed by Lewis and Macfarlane. Mites of 14 families, particularly Stigmaeidae with 16 genera and 21 species were found associated with 39 species of Phlebotominae. However, infestation rate was found 0.1%–9.0% and attack rate up to 43% indicated by scars on the body surface. The manifestation of the mites were found located on the coxae, ventral abdomen and pronotum of the bugs. The infestation of mites also reduced the molting rate in nymphs and longevity in adults^[4]. Mites were also reported from other Dipterans. A mite may be both phoretic and parasitic. In old world *Sergentomyia* sp. feeds on reptiles and *Phlebotomus* sp. on mammals. Most stigmaeids, in contrast to trombidids, were found on *Phlebotomus* sp. It proves that mostly stigmaeids are coming from mammal dwellings. *Phlebotomus papatasi* (*P. papatasi*) and *P. argentipes* are mainly domestic sand flies. Mites usually attach to the abdominal pleura of the adult sand flies where as many as 12–16 often observed. The melanised scars left by the mites mouth parts reveal even higher infestations. The significance of the mite lies in the harm making massive lesions up to 0.08 mm long. Two apparent features are associated between certain taxa of sand flies and mites with the great variation in rates and intensity of the infestation. Mites are very small and may easily miss which must often

higher than recorded. It is easily being detached as is found as collected in test tubes. However, some are found strongly attached with the host body. In India, 9% of the sand flies were found infested. In Saudi Arabia, 23 mites were found on one larva and 40 scars on others. In Cyprus, 43% of *P. papatasi* larvae had scars due to mites. A mite infested *P. papatasi* was observed during a study on sand flies of one of the southern provinces of Iran, near to the Persian Gulf. Several scars resulting from mite attachment were found on abdominal tergites of this female sand fly. The mites were identified as *Eustigmaeus johnstoni* in 2013^[5]. Mite's infestation was also reported in phlebotomine sand flies in southern Sinai, Egypt and Spain. *P. papatasi*, the vector of cutaneous leishmaniasis in the Mediterranean basin and USSR breeds in rodent burrows which provide suitable habitats for long term viability of many entomopathogens. However, in the present study mites were found attached with larvae of *P. argentipes* ($n= 30-50$) except first instars larvae and pupae and controlling the population up to basal level. The nymphs of mites were found predated the larvae of sand flies eating from outside of the skeleton to inside the muscular part and diminishing the population of sand fly larvae very low while making culture in the rearing pots. These might be acting as biological control agent to larvae of sand flies in nature. Spiders, are the most common ubiquitous animals on land, constitute an essential portion of the predatory arthropods in several ecosystems^[6]. With the current concern surrounding indiscriminate use of insecticides to control insect pests of crops leading to unnecessary pollution of environment and disturbance in natural balance of predators, use of biological control alone or along with chemical controls takes on greater significance. Spiders are voracious predators of insects. They are well adapted to certain habitats because of their ability to withstand periods of low food availability and also to take advantage of periods of prey abundance. There is increasing evidence that polyphagous predators to which spiders belong play an important role in the regulation of the number of insects preventing their mass occurrence. *Argiope anasuja* is a suitable biocontrol agent for the winter vegetable pest *Phyllotreta cruciferae* and have shown potentials of pest suppression^[7]. A particular species of spider (unidentified) was found attacking the blood fed sand flies on thorax in a cage/test tube to death, after getting paralyzed then eaten the whole soft body parts. The action was observed in the cage with glass top after collecting sand flies along with spider through CDC light trap. It has been suggested to install CDC trap away from the wall to prevent trapping of spider crawling on the wall which may damage sand flies inside the collection pot in 2008^[8]. It proved that the particular spider (unidentified) can be used as biological control agent of adult blood fed female sand flies which generally occur in nature. Spiders have a wide insect host range and thus can act as biological control agents of insect pests in agro-ecosystems also. Spider fauna in the major Iranian cotton fields were explored which are capable of controlling cotton pests. Effects of the spider on major cotton insect pests were studied under laboratory conditions

[(28±2) C°, (65±5)% RH and photoperiod of 12:12 (L:D) h]. Five spider species [*Thanatus formicinus* (Clerck), *Oxyopes salticus* (Hentz), *Cheiracanthium erraticum* (Walckenaer), *Philodromus cespitum* (Walckenaer), *Thyene imperialis* (Rossi)] were evaluated on four cotton pests (*Bemisia tabaci*, *Aphis gossypii*, *Empoasca decipiens* and *Nezara viridula*). The maximum predation in *Thyene imperialis* and *Oxyopes salticus* and *Thanatus formicinus* were also found suitable for reduction of suitable for reduction cotton pests in Tehran in 2008^[9]. Some researchers investigated dominant spider species cotton field in 2008^[10] and some in citrus orchards in Iran 2008^[11]. Both such mites and spiders may act as biological control agent to larvae and adult stages of *P. argentipes* respectively if released in mass after culture.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The leishmaniasis is an important disease in the Americas and all studies related to this parasitic disease deserves to be published. Moreover, all the ways to control parasites/vectors without the use of insecticides is very important for public health, since these agents are bioaccumulative. I really encourage the publication of this manuscript.

Research frontiers

The research is very interesting from an epidemiological standpoint. The methodology used was appropriate.

Related reports

The vector control is the main way to minimize the incidence of neglected diseases, since the pharmaceutical industries have little interest in studying these diseases that affect poor regions. Biological data vectors are always important as it can help in vector control programs. The results presented in this manuscript are really important since it can be used as a tool for vector control programs as an alternative non-toxic for the control of leishmaniasis.

Innovations & breakthroughs

The paper studies the action of applied parasites and

predators of the larvae of the vectors of leishmaniasis. These data are innovative, as it demonstrates the use of such organisms as alternative to control these vectors and, thus, the control of leishmaniasis. I emphasize again that these data are important for public health.

Applications

This study is directly applicable in control of leishmaniasis. It demonstrates an alternate non-toxic biological control method to minimise the burden of the disease.

Peer review

The manuscript is well written and provides information relevant to the vector control. I really encourage the publication of these results.

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