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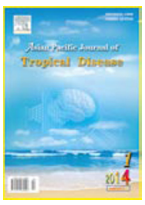

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Mites and spiders act as biological control agent to sand flies

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PEER REVIEW

Peer reviewer

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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 scrutitinized 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 predating 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, *Phlebotoms 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 10 000 populations at the district level[2]. The cyclic repearance 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 predating 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