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Impact of climate change on filarial vector, *Culex quinquefasciatus* and control using bacterial pesticide, spinosad

Nareshkumar Arjunan^{1*}, Murugan Kadarkari², Madhiyazhagan Pari², Nataraj Thiyagarajan², Shobana Kumar²¹Department of Zoology, Periyar University, Salem-636011, TN, India²Department of Zoology, School of Life Sciences Bharathiar University Coimbatore-641 046, India

PEER REVIEW

Peer reviewer

Dr. P. Thiyagarajan, Assistant Professor
Bharathidasan University,
Tiruchirappalli, India.
Tel: +91-9944842511
E-mail: rajanphd2004@yahoo.com

Comments

The present study is valuable indicating the impact of increase in temperature *i.e.*, the raise in global warming on mosquitoes which leads to epidemics of vector borne diseases. The study also pinpoints the importance of bio-insecticides (spinosad) which are environmentally safer, target specific and bio-degradable.

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ABSTRACT

Objective: To show the effect of temperature on the biology of *Culex quinquefasciatus* and also to show the effect of the bacterial pesticide, spinosad on developmental stages of the filarial vector.

Methods: A laboratory colony of mosquito larvae was used for the larvicidal activity of temperature and spinosad. Twenty-five numbers of first, second, third, fourth instar larvae were introduced into the 500 mL glass beaker containing 250 mL of de-chlorinated water with desired temperatures (16 °C, 20 °C, 24 °C, 28 °C, 32 °C, 36 °C), similarly spinosad, at different concentrations. The development was observed for every 24 h.

Results: The results showed that the rise in temperature acts as a growth inhibiting factor for mosquitoes. And no development was found in the temperature below 16 °C and above 36 °C. The hatchability was increased as the temperature was increased up to 32 °C, after which eclosion rates dropped gradually.

Conclusions: 32 °C was obtained as the maximum sustainable temperature and after which the developmental rate was gradually reduced. The optimal temperature for development was lower than the temperatures at which development was quickest. The bacterial pesticide spinosad showed that it is an effective mosquito control agent and can be used for further integrated pest management programmes.

KEYWORDS

Culex quinquefasciatus, Spinosad, Larval toxicity, Pupal toxicity

1. Introduction

Mosquitoes are common flying insects in the family Culicidae that are found around the world. There are about 3 500 species. The females of most mosquito species suck blood (hematophagy) from other animals, which has made them the deadliest disease vector known, killing millions of people over thousands of years and continuing to kill millions per year by the spread of infectious diseases. Disease organisms transmitted by mosquitoes include West

Nile virus, Saint Louis encephalitis virus, Eastern equine encephalomyelitis virus, Everglades virus, Highlands J virus, La Crosse Encephalitis virus in the United States; dengue fever, yellow fever, Ilheus virus, and malaria in the American tropics; Rift Valley fever, *Wuchereria bancrofti*, Japanese Encephalitis, dengue fever, yellow fever, chikungunya and malaria in Africa and Asia; and Murray Valley encephalitis in Australia. Insect-transmitted disease remains a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 200 million

*Corresponding author: Nareshkumar Arjunan, Department of Zoology, Periyar University, Salem-636011, TN, India.

Tel: +91-9787412379

E-mail: nareshphd@gmail.com

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people annually. India reports more than 1000 deaths from malaria, 2000 confirmed cases of Chikungunya virus and 1000 deaths from Japanese encephalitis^[1–3]. Although mosquito-borne diseases represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk and no effective vaccines are available^[4].

Culex is a genus of mosquito, and is important in that several species serve as vectors of important diseases, such as West Nile virus, filariasis, Japanese encephalitis, St. Louis encephalitis and avian malaria. *Culex quinquefasciatus* Say (*Cx. quinquefasciatus*) (Diptera: Culicidae) is the principle vectors of human lymphatic filariasis estimated to afflict about 120 million people worldwide^[5].

Temperature has been ascribed a primary role in the ecology of aquatic insects. Human activities should not change water temperatures beyond natural seasonal fluctuations. To do so could disrupt aquatic ecosystems, which leads to development of vectors and vector born diseases. The rates of metabolic processes in mosquitoes are dependent on various environmental conditions such as temperature and hydrology^[6,7]. In general, mosquito density tends to increase with increasing temperature, giving rise to a concern regarding potential increase in mosquito related diseases, given a scenario of global warming. Management of these disease vectors using synthetic chemicals has failed because of insecticide resistance, vector resurgence and environmental pollution. Consequently, an intensive effort has been made to find alternative methods of control^[8].

Spinosad is a mixture of tetracyclic macrolide neurotoxins, spinosad A and D, produced during the fermentation of the soil actinomycete, *Sacharopolyspora spinosa*. As such, it may be considered as a bioinsecticides^[9]. The insecticidal properties of *Sacharopolyspora spinosa* metabolites were first detected in a qualitative mosquito bioassay, during routine screening of soil sample for biologically active compound in the early 1890s^[10]. In the present study, an attempt has been made to evaluate the effect of temperature and spinosad on the filarial vector, *Cx. quinquefasciatus*.

2. Materials and methods

2.1. Collection of eggs

The eggs of *Cx. quinquefasciatus* were collected from local (in and around Coimbatore, India) different breeding habitats with the help of a 'O' type brush. The eggs were then brought to the laboratory and transferred to 18×13×4 cm size enamel trays containing 500 mL water and kept for larval hatching. They were hatched and reared, and have been still maintained from many generations in the laboratory. The eggs and larvae obtained from this stock were used for

different experiments.

2.2. Maintenance of larvae

The larvae reared in plastic cups. They were daily provided with commercial fish food^[11]. Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through foreign mosquitoes.

2.3. Maintenance of pupae and adult

The pupae were collected from culture trays and were transferred to glass beakers containing 500 mL of water with help of a sucker. The glass beaker containing pupae was then kept in 90×90×90 cm size mosquito cage for adult emergence. The cage was made up of wooden frames and covered with polythene sheets on four side (two laterals, one back and other one upper) and the front part was covered with a muslin cloth. The bottom of the cage was fitted with strong cardboard. The freshly emerged adults were maintained (27±2) °C, 75%–85% Relative Humidity, under 14L:10D photoperiod cycles. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding.

2.4. Blood feeding of adult *Cx. quinquefasciatus* and egg laying

The adult female mosquitoes were allowed to feed on the blood of rabbit (shaved on the dorsal side) for two days, to ensure adequate blood feeding for five days. After blood feeding, ovitraps were placed inside the cage for the adults to lay eggs.

2.5. Preparation of spinosad

Success of spinosad was purchased from Kalpatharu pesticide Limited, Coimbatore, Tamil Nadu, India. Spinosad 2.50% copolymer of ethylene oxide and propylene oxide 0.17%, ammonium salt of naphthalene sulphonic acid 0.11%, polyalkyl siloxane 1.00%, propylene glycol 4.14%, polysaccharide gum 0.15%, Hydrated magnesium aluminum silicate 0.92% and water 9%. Total 100% w/w, active specifically against insects. Required quantity of spinosad was thoroughly mixed with distilled water to prepare various concentrations, ranging from 0.001 to 0.008 mg/L.

2.6. Temperature effect on *Cx. quinquefasciatus* larvae

A laboratory colony of mosquito larvae was used for the

larvicidal activity of temperature. Twenty–five numbers of first, second, third, fourth instar larvae were introduced into the 500 mL glass beaker containing 250 mL of de–chlorinated water with desired temperatures (16 °C, 20 °C, 24 °C, 28 °C, 32 °C, 36 °C). Larval food was given for the test larvae. At each tested temperature, 2 trials were made and each trial consisted of three replicates. The development was observed for every 24 h.

2.7. Larval and pupal toxicity test of spinosad

A laboratory colony of mosquito larvae and pupae was used for the larvicidal and pupicidal activity. Twenty–five numbers of first, second, third and fourth instar larvae and pupae were introduced into the 500 mL glass beaker containing 249 mL of de–chlorinated water and 1 mL of desired concentrations of spinosad was added separately. Larval food was given for the test larvae. At each tested concentration, 2 to 5 trials will be made and each trial consisted of three replicates. Mixing 1 mL of acetone with 249 mL of de–chlorinated water set up the control. In the plant extracts, the larvae exposed to de–chlorinated water without acetone served as control. The control mortalities will be corrected by using Abbott’s formula[12].

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$$

LC₅₀, LC₉₀, regression equation and 95% confidence limit of lower confidence of limit and upper confidence limit were calculated from toxicity data by using probit analysis[13].

3. Results

The effect of temperature on the biology of *Cx. quinquefasciatus* is shown in Table 1. The larval durations were highly altered as the temperature range varies. At 16 °C, the larval durations were 3.9, 3.8, 3.5 and 3.4 d from 1st instar to 4th instar respectively. At 20 °C, the larval durations were 3.1, 3.0, 2.7 and 2.6 d from 1st instar to 4th instar respectively. At 24 °C the larval durations were 2.2, 2.1, 2.0 and 2.4 d from 1st instar to 4th instar respectively. At 28 °C,

the larval durations were 1.2, 1.4, 1.4 and 1.8 d from 1st instar to 4th instar respectively. At 32 °C, the larval durations were 0.6, 0.7, 0.6 and 0.8 d from 1st instar to 4th instar respectively. At 36 °C, the larval durations were 2.3, 2.1, 2.2 and 2.0 d from 1st instar to 4th instar respectively.

Table 1

Effect of temperature on the biology of *Cx. quinquefasciatus*.

Treatment/ Temperature (°C)	Larval duration (days)			
	I instar	II instar	III instar	IV instar
Control	1.2 ^b	1.3 ^b	1.4 ^b	1.9 ^b
16 °C	3.9 ^e	3.8 ^e	3.5 ^e	3.4 ^d
20 °C	3.1 ^d	3.0 ^d	2.7 ^d	2.6 ^{cd}
24 °C	2.2 ^c	2.1 ^c	2.0 ^c	2.4 ^c
28 °C	1.2 ^b	1.4 ^b	1.4 ^b	1.8 ^b
32 °C	0.6 ^a	0.7 ^a	0.6 ^a	0.8 ^a
36 °C	2.3 ^c	2.1 ^c	2.2 ^c	2.0 ^{bc}

Means±SD followed by same letter within columns indicate no significant difference in Duncan’s multiple range test (P<0.05 value).

Larval toxicity effect of spinosad (microbial pesticide) on filarial vector, *Cx. quinquefasciatus* is given in the Table 2. The percentage of mortality of *Cx. quinquefasciatus* after the treatment of spinosad on the I to IV instar larvae and pupae from 0.01, 0.02, 0.04, 0.06 and 0.08 mg/L were carried out. Higher mortality rate was 80% at 0.08 mg/L concentration in the I instar larval stage. The LC₅₀ value and LC₉₀ values represented as follows: LC₅₀ value of I instar was 0.2496, II instar was 0.2964, III instar was 0.3471, IV instar was 0.3484 and pupa was 0.2746 respectively. LC₉₀ value of I instar was 0.1018, II instar was 0.1018, III instar was 0.1070, IV instar was 0.1141 and pupae was 0.1042 respectively. Among the different larval stages, the I instar larvae was more susceptible than the other instar larvae.

4. Discussion

The distribution and abundance of an insect species depends on its own biological characteristics and the influence of other organisms, on its physical environment. Temperature plays a major role[14,15], as insects are poikilothermic or cold–blooded. Metabolic heat, that is generated by most insects themselves, is limited and has little effect on their body temperature[16]. Therefore, their metabolic rate and thus the growth and development rate of insects depend on the temperature of their direct environment.

Table 2

Larvicidal and pupicidal effect of spinosad on filarial vector, *Cx. quinquefasciatus*.

Larval and pupal stage	Larval and pupal mortality (%)					Value of LC ₅₀ and LC ₉₀ (%)	Regression Co–efficient	95% Confidence Limit		Chi–square Value (χ ²)
	Concentration (mg/L)							LCL	UCL	
	0.01	0.02	0.04	0.06	0.08			LC ₅₀ (LC ₉₀)	LC ₅₀ (LC ₉₀)	
I	37 ^a	49 ^b	61 ^c	74 ^d	80 ^e	0.2496 (0.1018)	16.46855	0.1530 (0.0870)	0.3216 (0.1271)	1.153
II	34 ^a	44 ^b	59 ^c	73 ^d	79 ^e	0.2964 (0.1018)	17.75401	0.2160 (0.8779)	0.3618 (0.1248)	1.078
III	28 ^a	43 ^b	57 ^c	69 ^d	76 ^e	0.3471 (0.1070)	17.71390	0.2739 (0.9225)	0.4115 (0.1313)	2.674
IV	31 ^a	42 ^b	55 ^c	70 ^d	73 ^{de}	0.3484 (0.1141)	16.16937	0.2677 (0.9693)	0.0418 (0.4371)	2.284
Pupa	35 ^a	59 ^b	59 ^b	75 ^c	79 ^{cd}	0.2746 (0.1042)	17.32660	0.1877 (0.1871)	0.0325 (0.0125)	1.012

Means±SD followed by same letter within rows indicate no significant difference in Duncan’s multiple range test (P<0.05 value).

Temperature is an important determinant in the growth, development and survival of mosquito larvae. The relationship between mosquito development and temperature is one of the keys to understand the current and future dynamics and distribution of vector-borne diseases. Many process-based models use mean air temperature to estimate larval development times, and hence adult vector densities and malaria risk[17].

The results showed that the rise in temperature acts as a growth inhibiting factor for mosquitoes. And no development was found in the temperature below 16 °C and above 36 °C. 32 °C was obtained as the maximum sustainable temperature and after which the developmental rate was gradually reduced. The optimal temperature for development was lower than the temperatures at which development was quickest. Earlier reports states that the abiotic factors such as temperature also affect the larval mortality[15,18,19].

Adverse effects of temperature on the developmental stages of *Anopheles stephensi*[7], *Anopheles quadrimaculatus* Say[20], *Aedes aegypti* Linnaeus[21,22], *Culex* and *Anopheles* species[23], *Toxorhynchites brevipapilis* Theobald[24] and *Wyeomyia smithii* Coquillett[25] have been reported, which lie in concordance with the present report.

Larvae developed into adults at temperatures ranging from 16 to 34 °C. Larval survival was shortest (<7 d) at 10–12 °C and 38–40 °C, and longest (>30 d) at 14–20 °C. Earlier report states that within the temperature range at which adults were produced was 18–32 °C. Larval mortality was highest at the upper range 30–32 °C, with death (rather than adult emergence) representing over 70% of the terminal events[26]. Development time from egg to adult was measured under laboratory conditions at constant temperatures between 10 and 40 °C. Rate of development from one immature stage to the next increased at higher temperatures to a peak around 28 °C and then declined. Adult development rate was greatest between 28 and 32 °C, although adult emergence was highest between 22 and 26 °C. No adults emerged below 18 °C or above 34 °C[19].

Spinosad have been brought out significant toxicity on different larval instars of *Cx. quinquefasciatus*. Earlier, laboratory larval bioassays of spinosad on *Aedes aegypti*, *Cx. quinquefasciatus*, and *Anopheles gambiae* (specimens that were either susceptible or resistant to pyrethroids, carbamates, and organophosphates) showed that this product had a lethal action (mortality after 24 h of exposure) regardless of the original status, susceptible or resistant, of the mosquito larvae[27]. The study showed an increase in mortality with the increase in concentration and the early instar larvae are much susceptible than the later ones. The bacterial pesticide spinosad showed that it is an effective mosquito control agent and can be used for further integrated pest management programmes. Earlier we reported that spinosad is more toxic in lower concentrations when compared to NSKE to mosquitoes that are more susceptible than chironomids[28]. The lowest LC₅₀ value obtained from spinosad against *Anopheles stephensi* was 0.00205 mg/L.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Global warming is the rise in the average temperature. Speculations on the potential impact of continued warming on human health often focus on mosquito-borne diseases. Elementary models suggest that higher global temperatures will enhance their transmission rates and extend their geographic ranges. Developing an understanding of the likely effects of climate change on different mosquito species is not only valuable from an insect ecology perspective, but has implications for the transmission of mosquito borne infections.

Research frontiers

The present study states that the global warming may not just cause mosquitoes to proliferate; it may also allow malaria to spread and lead to deaths worldwide. The mosquito vectors cannot develop below the 16 °C. But as the study says due to global warming the winter temperature rises above 16 °C, which could bring a dramatic expansion in mosquito population.

Related reports

Researchers agree that global warming will increase the number of mosquitoes, which can bring outbreak of mosquito-borne diseases throughout the world (Miller, 2012; Reiter, 2008).

The effectiveness of spinosad for larval mosquito control has been demonstrated by a number of researchers (Hertlein et al., 2010; Jiang and Mulla, 2009; Romi et al., 2006; Darriet et al., 2005).

Innovations & breakthroughs

Using wide range of temperature including 16 and below to show the effect of climate change on insects especially mosquitoes is a novel approach. And using spinosad a neurotoxin to control mosquito also valuable.

Applications

The study is applicable for product development (bio-

pesticide) using spinosad. The report is also useful for research work on global warming and their impacts on animals. It will also be helpful for the government and non-governmental organizations who work on vector borne diseases.

Peer review

The present study is valuable indicating the impact of increase in temperature *i.e.*, the raise in global warming on mosquitoes which leads to epidemics of vector borne diseases. The study also pinpoints the importance of bio-insecticides (spinosad) which are environmentally safer, target specific and bio-degradable

References

- [1] Laneri K, Bhadra A, Ionides EL, Bouma M, Dhiman RC, Yadav RS, et al. Forcing versus feedback: epidemic malaria and monsoon rains in Northwest India. *PLoS Comput Biol* 2010; doi: 10.1371/journal.pcbi.1000898.
- [2] Arjunan N, Murugan K, Madhiyazhagan P, Kovendan K, Prasannakumar K, Thangamani S, et al. Mosquitocidal and water purification properties of *Cynodon dactylon*, *Aloe vera*, *Hemidesmus indicus* and *Coleus amboinicus* leaf extracts against the mosquito vectors. *Parasitol Res* 2011; **110**(4): 1435–1443.
- [3] Naresh Kumar A, Murugan K, Madhiyazhagan P. Integration of botanicals and microbials for management of crop and human pests. *Parasitol Res* 2012; **112**(1): 313–325.
- [4] Wilder-Smith A, Ooi EE, Vasudevan SG, Gubler DJ. Update on dengue: epidemiology, virus evolution, antiviral drugs, and vaccine development. *Curr Infect Dis Rep* 2010; **12**: 157–164.
- [5] World Health Organization. Lymphatic filariasis. Geneva: WHO; 2012. [Online] Available from: <http://www.who.int/mediacentre/factsheets/fs102/en/>. [Accessed on April 4th 2013]
- [6] Fairros WYW, Azaki WHW, Alias LM, Wah YB. Modelling Dengue Fever (DF) and Dengue Haemorrhagic Fever (DHF) Outbreak Using Poisson and Negative Binomial Model. *Int J Math Comput Sci Eng* 2010; **4**: 809–814.
- [7] Naresh Kumar A, Murugan K, Shobana K. *Impact of climate change on the malarial vector, Anopheles stephensi and control using Cassia alata implications of climatic change on mosquito-borne diseases and its impact of public health*. New Delhi, India: Excel India Publishers; 2010, p. 106–111.
- [8] Kumar AN, Jeyalalitha T, Murugan K, Madhiyazhagan P. Bioefficacy of plant-mediated gold nanoparticles and *Anthocephalus cadamba* on filarial vector, *Culex quinquefasciatus* (Insecta: Diptera: Culicidae). *Parasitol Res* 2013; **112**(3): 1053–1063.
- [9] Cropping LG, Menn JJ. Biopesticides: a review of their action, applications and efficacy. *Pest Manag Sci* 2000; **56**: 651–676.
- [10] Thompson GD, Dutton R, Sparks TC. Spinosad—a case study: an example from a natural products discovery programme. *Pest Manag Sci* 2000; **56**: 696–702.
- [11] Lyimo EO, Takken W, Koella JC. Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of *Anopheles gambiae*. *Entomol Exp Appl* 1992; **63**: 265–271.
- [12] Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; **18**: 265–267.
- [13] Finney DJ. *Probit analysis*. London, UK: Cambridge University Press; 1971, p. 68–78.
- [14] Randall DA, Wood RA, Bony S, Colman R, Fichetef T, Fyfe J, et al. Climate models and their evaluation. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, et al., editors. *Climate change 2007: The physical science basis contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change*. Cambridge, UK: Cambridge University Press; 2007, p. 589–662.
- [15] Palumbo JC. Weather can have major impact on insects. Yuma Agricultural Center: Yuma AZ; 2010. [Online] Available from: <http://cals.arizona.edu/crop/vegetables/advisories/docs/WeatherandInsects.pdf>. [Accessed on April 15th 2013]
- [16] Wild M, Gilgen H, Roesch A, Ohmura A, Long CN, Dutton EG, et al. From dimming to brightening: decadal changes in solar radiation at earth's surface. *Sci* 2005; **308**: 847–850.
- [17] Paaijmans KP, Imbahale SS, Thomas MB, Takken W. Relevant microclimate for determining the development rate of malaria mosquitoes and possible implications of climate change. *Malar J* 2010; **9**: 196.
- [18] Kheder RB, Moal J, Robert R. Impact of temperature on larval development and evolution of physiological indices in *Crassostrea gigas*. *Aquaculture* 2010; **309**: 286–289.
- [19] Bayoh MN, Lindsay SW. Effect of temperature on the development of the aquatic stages of *Anopheles gambiae* sensu stricto (Diptera: Culicidae). *Bull Entomol Res* 2003; **93**: 375–381.
- [20] Huffaker CB. The temperature relations of the immature stages of the malarial mosquito *Anopheles quadrimaculatus* Say, with a comparison of the developmental power of constant and variable temperatures in insect metabolism. *Ann Entomol Soc Am* 1944; **37**: 1–27.
- [21] Bar-Zeev M. The effect of temperature on the growth rate and survival of the immature stages of *Aedes aegypti* (L.). *Bull Entomol Res* 1958; **49**: 157–163.
- [22] Tun-Lin W, Burkot TR, Kay BH. Effects of temperature and larval diet on development rates and survival of the dengue vector *Aedes aegypti* in north Queensland, Australia. *Med Vet Entomol* 2000; **14**: 31–37.
- [23] Shelton RM. The effect of temperatures on the development of eight mosquito species. *Mosq News* 1973; **33**: 1–12.
- [24] Trips M. Development and predatory behaviour of *Toxorhynchites brevipalpis* (Diptera: Culicidae) in relation to temperature. *Environ Entomol* 1972; **1**: 537–546.
- [25] Bradshaw WE. Thermoperiodism and the thermal environment of the pitcher plant mosquito, *Wyeomyia smithii*. *Oecologia* 1980; **46**: 13–17.
- [26] Bayoh MN, Lindsay SW. Temperature-related duration of aquatic stages of the Afrotropical malaria vector mosquito *Anopheles gambiae* in the laboratory. *Med Vet Entomol* 2004; **18**: 174–179.
- [27] Darriet F, Duchon S, Hougard JM. Spinosad: a new larvicide against insecticide-resistant mosquito larvae. *J Am Mosq Control Assoc* 2005; **21**(4): 495–496.
- [28] Kumar AN, Murugan K, Madhiyazhagan P, Prabhu K. Spinosad and neem seed kernel extract as bio-controlling agents for malarial vector, *Anopheles stephensi* and non-biting midge, *Chironomus circumdatus*. *Asian Pac J Trop Med* 2011; **4**(8): 614–618.