The Incidence and the Implications of Natural Infection with *Nomuraea rileyi* (Farlow) Samson on the Life-Table Parameters of Laboratory-Reared *Culex pipiens* L. Mosquitoes

Author(s): E.M. Abouzied
Source: African Entomology, 25(1):137-147.
Published By: Entomological Society of Southern Africa
DOI: http://dx.doi.org/10.4001/003.025.0137
URL: http://www.bioone.org/doi/full/10.4001/003.025.0137

The incidence and the implications of natural infection with *Nomuraea rileyi* (Farlow) Samson on the life-table parameters of laboratory-reared *Culex pipiens* L. mosquitoes

E.M. Abouzied

Department of Zoology, Faculty of Science, Fayoum University, Tawfik El-Hakeem, P.O. Box 63514, Al Fayoum, Egypt. E-mail: ema04@fayoum.edu.eg

During routine mass rearing of *Culex pipiens* L., 46 % mortality rate was recorded among 3-day-old larvae in the late summer, 2014, at Cairo University, Giza, Egypt. An analysis of the water in the rearing plastic cups and the dead larvae revealed the presence of the fungus *Nomuraea rileyi* (Farlow) Samson. A comparative study was carried out using life-table parameters of the fungal-infected population of *Cx. pipiens* and fungus-free population (control) under laboratory conditions (30 ± 0.10 °C, RH 52.5 ± 7.8 % and photoperiod 16L:8D). Fungal-infected stages of *Cx.* pipiens exhibited a decrease in longevity (larvae, 5.89 ± 0.063 days; adult males, 7.8 ± 0.75 days; adult females, 10.83 ± 1.6 days). The mean generation time of the healthy population (22.09 ± 0.82 days) was not significantly different from that of the infected population (22.25 ± 0.08 days), nearly the same mean generation time. The fungal-infected *Cx. pipiens* population replicated itself 4.62 ± 1.45 times with a very low exponential rate of daily increase of 0.0688 ± 0.0154/day. However, the fungus-free population showed a higher replication rate of 38.10 ± 11.80 times, with a higher daily rate of increase (0.1648 ± 0.0167/day). Females of the fungal-infected *Cx. pipiens*, exhibited a decrease in the fertility, 25.67 ± 7.70 eggs/female, as compared to the females from the control population, 163.29 ± 40.64 eggs/female. Data of the present study suggested the possibility of using *N. rileyi* as a safe bio-control agent against the development of the larvae of *Cx. pipiens*.

Key words: *Culex pipiens*, *Nomuraea rileyi*, life , natural infection, survival rate.

INTRODUCTION

Members of the mosquito genus *Culex* (Diptera: Culicidae) are the chief vectors of human lymphatic filariasis (Taylor *et al.* 2010; WHO 2011). Michael & Bundy (1997) reported that more than 115 million persons worldwide were affected by lymphatic filariasis (LF) due to infection with *Wuchereria bancrofti***.** In 1996, of 120 million people estimated worldwide infected with filariasis, 40 million were incapacitated, particularly in urban areas (WHO 2000, 2004, 2011). Also, *Culex* is the predominant vector of West Nile virus (WNV) including *Cx. pipiens*, *Cx. tarsalis* Coquillett and *Cx. quinquefasciatus* Say in the U.S.A. (Komar 2003; Bernard *et al.* 2001; Ciota *et al.* 2011). Kilpatrick *et al*. (2005) suggested that *Cx. pipiens* L. and *Cx. restuans* Theobald may be responsible for up to 80 % of human WNV infections in northeastern U.S.A.

In the last decade, the demand for non-hazardous compounds for pest control has been pronounced in order to decrease the non-target effects of insecticides (Alphey 2014). The use of entomopathogenic fungi is one the most promising tools for controlling mosquitoes (Scholte *et al.* 2004, 2005, 2008). The following authors (Geetha & Balaraman 1999; Federici 1981; Lacey *et al.* 1988; Vyas *et al.* 2007; Mohanty & Prakash 2007) investigated the use of different fungi (*Beauveria tenella* (Sacc.), *Culicinomyces* sp., *Coelomomyces* sp., *Metarhizium anisopliae* (Metschn.), *Lagenidium giganteum* Couch, and *Chrysosporium lobatum* Scharapov) as bio-insecticides for mosquito larvae control. Mohanty *et al.* (2008a) studied the control of adult *Cx. quinquefasciatus* using *Fusarium pallidoroseum* (Cooke). Da Costa *et al.* (1998) studied the pathogenicity of several *Penicillium* species against *Aedes aegypti* L., *Ae. fluviatilis* (Lutz), *Anopheles quasalis* Curry, and *Cx. quinquefasciatus*. The authors recorded different mortality rates ranging from 0 to 100 % depending on the concentration of the fungus.

Fungal reproduction includes both asexual stage (zoospores) and sexual stage (oospores) (Federici 1981). There are two ways for the fungus to infect mosquito larvae. The first is when motile zoospores attach to and penetrate the cuticle of the mosquito larva (in the case of *Lagenidium giganteum* Couch producing hyphae). Hyphae develop, and start

ISSN 1021-3589 [Print]; 2224-8854 [Online]
DOI: http://dx.doi.org/10.4001/003.025.0137

forming sporangia. Exit vesicles are formed with either zoospores or oospores. The second is where the biflagellate zygote infects the haemocoel of the mosquito larvae producing hyphagens, which later form hyphae (for species of *Coelomomyces*). Hyphae develop in the haemocoel forming the resting sporangia. Meiospores are released from the sporangia, which infect a copepod and produce gametophytes. Each one will form gametangia that release gametes of a single-mating type. Gametes of opposite mating type fuse inside or outside the copepod, forming a biflagellate zygote that completes the cycle by infecting another mosquito larva (Scholte *et al.* 2004). Larval mortality may be due to the release of toxins produced by the fungus. Toxins, especially destruxins, produced by *M. anisopliae* conidia were considered as the cause of the death of the mosquito larvae, after digestion in the gut (Crisan 1971; Al-Aidroos & Roberts 1978; Tantral *et. al.* 2004). On the other hand, proteases released by the conidia of *M. anisopliae* activated the apoptotic pathways of *Ae. aegypti* larvae. Larval mortality seemed to be due to autolysis (Butt *et al.* 2013).

Larval mortality as high as 46 % was recently reported in a population of *Cx. pipiens*, originally established in 2012 and cultured for *c.* 11 generations at the Entomology Department Insectary, Faculty of Science, Cairo University, Giza, Egypt. Larvae died after three days. It was suspected that a microbial infection was the cause of the mortality. The fungus, *Nomuraea rileyi* (Farlow) Samson was isolated from water in the rearing media as well as from the dead larvae.

The age-stage, two-sex life table is useful for studying insect ecology of laboratory populations (Chi 1988). Use of this approach will help to elucidate the effects of *N. rileyi* infection on the survival of *Cx. pipiens* post infection. The objective of this study was to evaluate the effects of the Hypocreal fungi *N. rileyi* on the life-table parameters of this laboratory *Cx. pipiens* culture.

MATERIAL AND METHODS

Inside the insectary, two maximum/minimum thermo-hygrometers were hung opposite to each other at the corners of the laboratory. The whole study was carried out during late summer to early autumn in 2014. The air temperatures ranged from 27–32 °C, with an average of 30 ± 0.10 °C. The relative humidity reached 35–60 % with an average of 52.5 ± 7.8 % throughout the study. A photoperiod of 16L:8D was used by means of white fluorescent lamps.

Rearing of mosquito larvae to adults followed the protocol of Gerberg (1970). Two to four hours after hatching from a single isolated egg raft, *Cx. pipiens* larvae were removed using glass Pasteur pipettes under dissecting stereo microscopes. Ten larvae were placed inside a white plastic cup (250 ml) with dechlorinated water and a few pellets of commercial fish diet. Each cup was examined daily to detect the daily mortality and to record the date of larval development to pupae. Rearing cups with pupae were transferred into rearing cages (30 × 30 × 30 cm) supplied with sugar crystals and a glass bottle containing a wet cotton plug as a water source. Each cage was labelled with the number of pupae and date of pupation. Cages were checked daily for pupal mortality, adult emergence, and male and female mortality. Female mosquitoes were supplied with fresh blood diet twice a week. The golden speckled quail (*Coturnix japonica* Temminck & Schlegel) was used to feed female mosquitoes. Blood feeding of female mosquitoes lasted for 4–6 h per meal. Following each blood meal, a 250 ml cup filled with dechlorinated water was placed inside each cage for female oviposition. Cups were checked daily for egg rafts. Each egg raft was soaked in 10 % sodium hypochlorite, for 4–6 h, to separate the eggs from each other. Eggs were counted under stereo dissecting microscopes in order to calculate the daily average number of eggs/female.

The ingredients of pellets supplied to feed the larvae included whole fish meal (whole salmon, herring and other mixed fish), whole wheat flour, soybean meal, whole dried krill, dried yeast, squid meal, kelp meal, wheat germ, fish oil, corn gluten meal, spirulina, garlic, natural astaxanthin, marigold powder, chili powder, spinach, choline chloride, calcium propionate (a preservative), L-ascorbyl-2-polyphosphate (source of vitamin C), vitamin A acetate, cholecalciferol (source of vitamin D3), riboflavin supplement, vitamin B12 supplement, niacin, menadione sodium bisulphite complex (source of vitamin K activity), folic acid, thiamine, pyridoxine hydrochloride (B6), calcium pantothenate, biotin, DL-alphatocopherol (E), manganese sulphate, cobalt sulphate, ferrous sulphate, copper sulphate. This diet included crude protein (min.) 41 %, crude fat (min.) 7 %, crude fibres (max.) 3 %, moisture (max.) 8 %, and 1 % phosphorus (min.).

Detection of the infection, isolation and identification of the fungus

Ten cups, each containing 10 larvae (n = 100), were used for each experiment. Once the fungal infection was detected, mosquito larvae were counted and removed by sterilised glass Pasteur pipettes, preserved in 75 % ethanol, labelled, and forwarded for microbial analysis. The remaining surviving larvae were moved to new plastic containers for rearing to adults (infected population).

For the purpose of fungus identification, the cadavers were swabbed with 75 % ethanol solution for 1 min, then washed with sterile ddH_2O and homogenised with Teflon homogeniser. The homogenate was inoculated by streaking into plates containing Sabouraud dextrose agar medium (SDA; Oxoid) with chloramphenicol (50 μg/ml). SDA plates were incubated at 26 ± 1 °C for 4 days. To enhance the growth of fungi, various subcultures were prepared and the developed colonies were removed and placed on potato dextrose agar (PDA) plates and purified by using the single spore technique (Choi *et al.* 1999). The fungus isolation and identification were carried out at the laboratory of the Microbiology Division, Microanalytical Centre, Cairo University, Egypt.

Control experiment

Nine 250 ml cups containing 10 larvae (total 90 larvae) supplied with dechlorinated fresh water and a few pellets of the commercial fish diet. Cups were examined daily to record the mortality, and the larval duration until the transformation into the pupal stage. Once pupation occurred, each cup was placed into wooden rearing cages (30 × 30 × 30 cm) labelled with the number of living pupae and the date of pupation. Each wooden box was supplied with a small bottle containing a wet cotton plug as a source of water for the emerged adults. In addition, a glass dish containing powdered sugar crystals was placed inside each wooden cage. Cages were checked daily for pupal mortality, adult emergence or adult mortality. Female adults of *Cx. pipiens* were supplied with fresh blood meals twice/week. Feeding periods lasted for 4–6 h. The golden speckled quail was again used as the source of the fresh blood meal. After feeding, a cup containing dechlorinated water was placed inside the cage and checked daily for egg rafts. Egg rafts were removed and eggs were counted under a stereomicroscope, in order to record the average number of eggs/day/female, until the death of all adult individuals.

Life-table analysis

The raw data of both of the infected *Cx. pipiens* as well as the control populations were both analysed according to Chi & Liu (1985) and Chi (1988) using the program TWOSEX, available at http://140.120.197.173/Ecology/ and http://nhsbig.inhs.uiuc.edu/wes/chi.html (Illinois Natural History Survey, U.S.A.). The age-stage survival rate, the distribution of mortality rate, the age-stage life expectancy and the stable age-stage distribution were calculated. Furthermore, the means and standard errors of the intrinsic rate of increase (r), the net reproductive rate (R_0), the mean generation time (T) and the finite rate of population increase (λ), were calculated using the bootstrap test (Efron & Tibshirani 1993; Huang & Chi 2013). A paired bootstrap test was used to test the difference between the corresponding parameters of each experiment at 5 % significance level. Standard errors were estimated by using 200 000 bootstrap re-sampling. Analyses were checked and revised by H. Chi (Insect Ecology and Life Table Theory, Faculty of Agricultural Sciences and Technologies, Niğde University, Turkey) (pers. comm.).

Statistical analysis

Student's t-test analyses were carried out in order to evaluate the effect of fungus infection on each stage of *Cx. pipiens* population's life cycle. Analysis was carried out online, using 'The Statistics Calculator', available at https://www.statpac.com/statistics-calculator/statistics-calculator.html

Specific calculations and abbreviations used

1. Age-specific survivorship: $l_x = yx/y_0$, where yx = the number of males or females alive on each day, x.
2. The age-specific fecundity (m_x) = ½ number of offspring born to parent of age x, m_x is usually measured as female offspring per female of age x.
3. Net reproductive rate, $R_0 = \Sigma l_x m_x$. This is average lifetime reproduction, summed across all ages.
4. Mean generation time: $T = \Sigma x l_x m_x / R_0$. T = egg to egg time, or newborn to newborn time.
5. Intrinsic rate of increase: r = antilog e R_0/T, where log e is the natural logarithm; it is the difference between the rate of births/unit time,

and the rate of deaths/unit time (individuals produced per unit time).

6. Finite rate of increase: λ = antilog *e rm*, the change in population size from one generation to the next, or it is the number of individuals 'replacing' each adult in the previous generation.
7. The reproductive value (V_{xj}): is the expected current and future reproductive output for an individual of age *x* and stage *j*.

RESULTS

Age-specific survival rate (l_x) of both controlled and infected *Culex pipiens* populations

The age-specific survival rate curve (l_x) of the infected population indicated a three stage decrease (Fig. 2). Four days after egg raft deposition, the survival rate of the infected individuals (during the larval stage) had decreased to 54 % (Figs 1, 2). This was followed by a gradual decrease from day 6 (40 %) to 27 % on day 18 (Fig. 2). On day 19, the survival rate decreased from 16 % to 9 % on day 20. The survival rate diminished gradually up to day 39 (Fig. 2).

In contrast, individuals in the control population began to die earlier (day 2) recording a higher survival rate of 87 %. The survival curve decreased smoothly towards the end of the surviving females by day 79 (Figs 1, 2).

The effect of *Nomuraea rileyi* fungus infection on the longevity of individuals of *Culex pipiens*

Larvae infected with *N. rileyi* showed a highly significant decrease in longevity (t = 2.65, d.f. = 125, P = 0.0009) at 5.89 ± 0.063 days, as compared to 8.59 ± 0.23 days for the control larvae (Table 1). The maximum longevity of male and female infected *Cx. pipiens* was 23 and 39 days, respectively, as compared to 55 days (males) and 79 days (females) for the control (Fig. 1). A significantly shorter lifespan was apparent (t = 3.77, d.f. = 81, P = 0.0005) for the infected males (7.8 ± 0.75 days) and females (10.83 ± 1.6 days), compared to the control males and females (20.83 ± 3.09 and 24.38 ± 5.49 days, respectively). Only during the pupal stage, the durations were similar (2.05 ± 0.075 *vs* 2.04 ± 0.03 days), since no significant difference was found between infected and uninfected pupae (t = 0.13, d.f. = 81, P = 0.89).

Mortality rates of the developmental stages of the infected mosquito population

Larvae of the infected *Cx. pipiens* population showed a substantially higher mortality (46 %) compared to mortality of the control larvae (1 %). Mortality due to infection began on day 4 after egg raft deposition (Fig. 1). However, pupae of the control suffered 49 % mortality compared to the pupae of the infected population. The mortalities among adults of the infected population were lower (males 2 % and females 18 %) as compared to 27 % in the males and 23 % in the females of the control population.

The effect of *Nomuraea rileyi* on the life table analysis of fungus infected and uninfected *Culex pipiens*

Bootstrapping revealed that the control population of *Cx. pipiens* indicated a significantly higher intrinsic rate of increase (0.16 ± 0.017; P = 0.0003) compared to 0.069 ± 0.015) in the infected mosquito population (Table 2). In addition, the finite rate of increase of the controlled population significantly exceeded (1.18 ± 0.02) that of the infected *Cx. pipiens* population (1.07 ± 0.016; P = 0.0003).

Table 1. Comparison between longevity (in days) of the developmental stages of *Culex pipiens* infected with *Nomuraea rileyi* fungus and a healthy population (control).

Stage	Control		Infected		Statistics		
	Mean ± S.E.	*n*	Mean ± S.E.	*n*	*t*	d.f.	*P*
Larva	8.59 ± 0.23	89	5.89 ± 0.063	38	2.65	125	0.0009*
Pupa	2.04 ± 0.03	45	2.05 ± 0.07	38	0.13	81	0.896
Male	20.83 ± 3.09	24	7.8 ± 0.75	20	3.77	42	0.0005*
Female	24.38 ± 5.49	21	10.83 ± 1.6	18	2.21	37	0.0331*
Adult	22.49 ± 3.02	45	9.24 ± 0.88	38	3.91	81	0.0002*

*Refers to statistical significant difference, *n*: number of individuals.

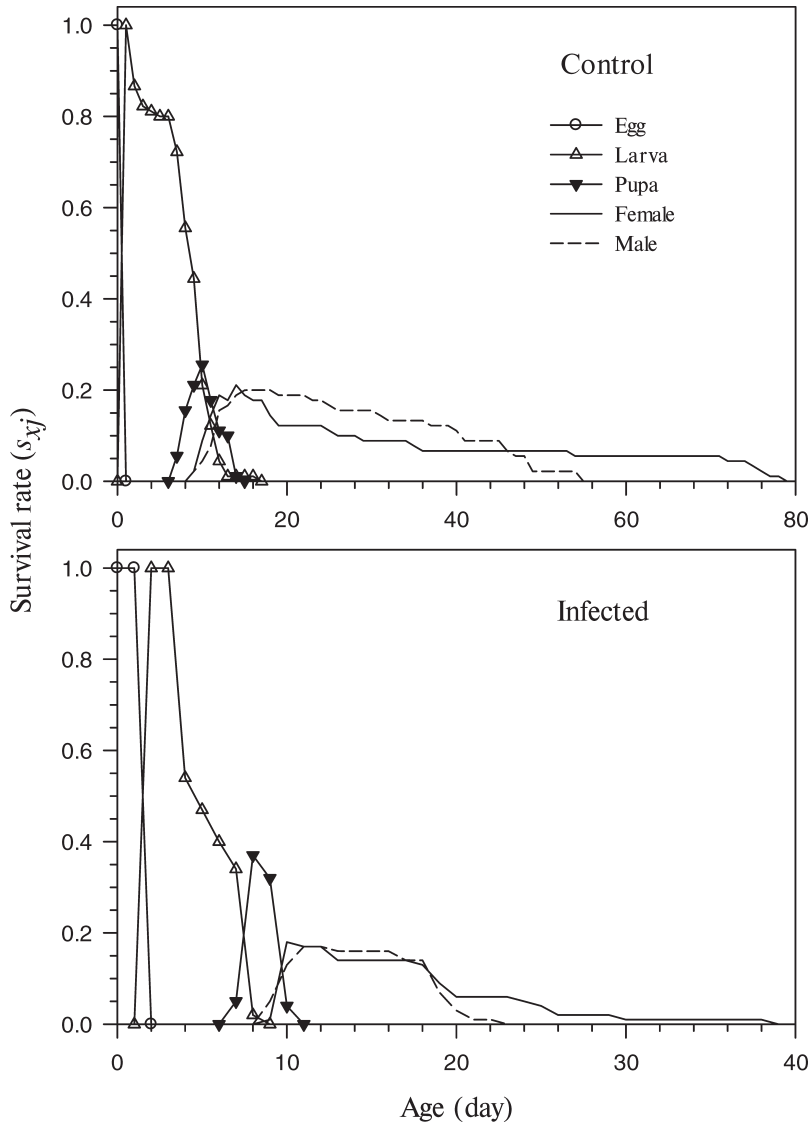

Fig. 1. The age-stage survival rate (S_{xj}) of *Culex pipiens* with and without the infection of *Nomuraea rileyi*.

The age-specific fecundity (m_x) as well as the age-specific maternity ($l_x m_x$) of the infected female *Cx. pipiens* showed a single peak on days 21 and 22, with an average daily fecundity of 48.64 and 17.14 eggs, respectively. Females of the control exhibited 13 peaks of age-specific fecundity (m_x) and the age-specific maternity ($l_x m_x$) was recorded at discrete time intervals (Fig. 2). The major peak was recorded on day 21 with an average daily fecundity of 18.57 eggs.

Infected *Cx. pipiens* females exhibited a significant decrease (P <0.05) in the fecundity and net reproductive rate of 25.67 ± 7.70 and 4.62 ± 1.45, respectively, as compared to females of the control population (163.29 ± 40.64 and 38.10 ± 11.80, respectively) (Table 2). It is noteworthy that only two females of 18 females laid egg rafts from the infected population. The reproductive value (V_{xj}) of the infected female *Cx. pipiens* showed a single peak on day 20 (70.46 eggs/day) and on day 21 (75.67 eggs/day), terminating abruptly on day 24 (Fig. 3). The control females of *Cx. pipiens* had a major peak of reproductive value (V_{xj}) on day 19 (125.97 eggs/day) and day 20 (123.67 eggs/day), end-

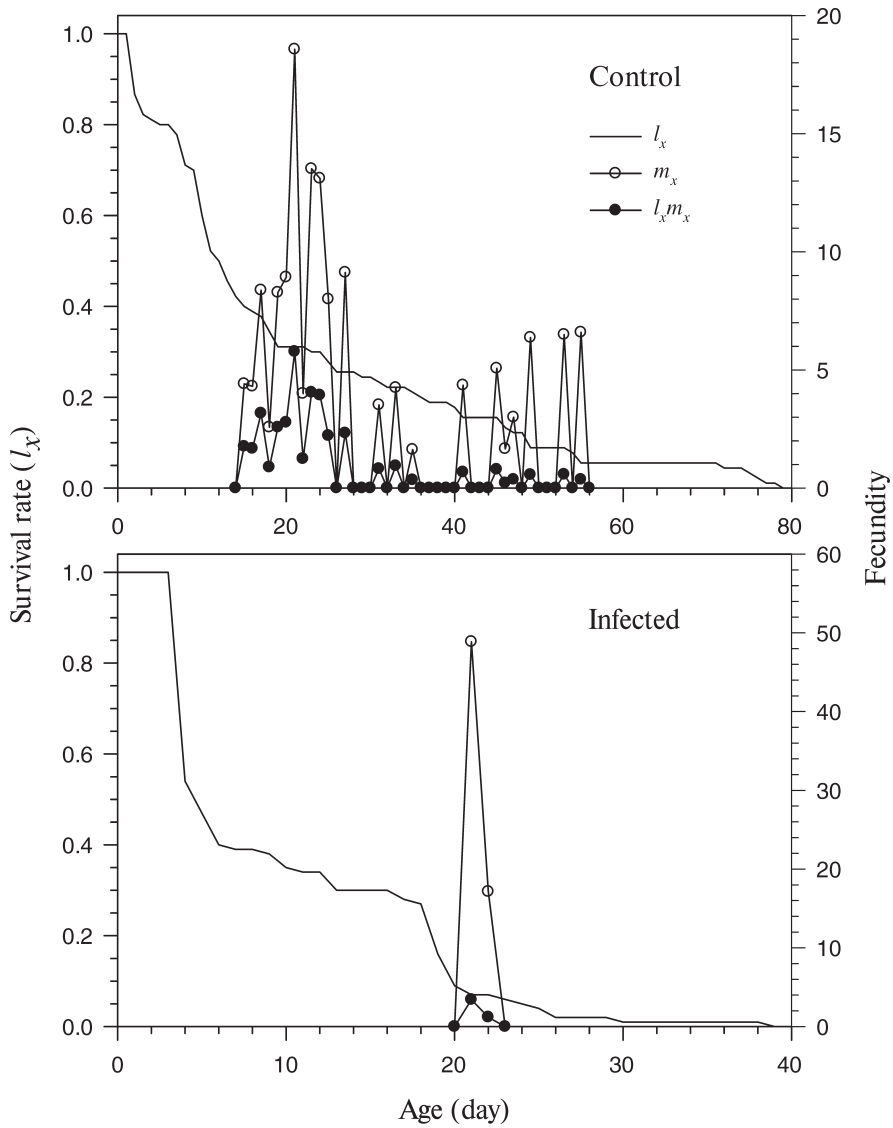

Fig. 2. The age-specific survival rate (l_x), fecundity (m_x), and net maternity (l_xm_x) of *Culex pipiens* infected with *Nomuraea rileyi* and the control.

ing egg laying on day 55 (Fig. 3). The calculated mean generation time of the control and infected mosquito populations was nearly equal (22.09 ± 0.82 and 22.25 ± 0.08 days; $P > 0.05$, respectively).

DISCUSSION

The natural occurrence of the fungus *N. rileyi* has been reported previously by Namasslvayam *et al*. (2013). These authors isolated *N. rileyi* from four sites out of 10 sampling agricultural areas around Tamil, India. Isolates of *N. rileyi* were associated with *Metarhizium* sp. (a soilborne fungus) and *Beauveria* sp, which is known to develop in water but not in the soil.

In this paper, the commercial diet contained whole wheat flour, soybean meal, wheat gluten meal and dried yeast, which may contain inactive conidia of the fungus (agricultural products). Also the protein-enriched diet (41 %), vital essential minerals (manganese sulphate, cobalt sulphate, ferrous sulphate and copper sulphate), a good

Table 2. The effect of *Nomuraea rileyi* fungus infection on the life table characteristics of a *Culex pipiens* laboratory population.

Parameters	Control		Infected		*P*
	n	Mean ± S.E.	*n*	Mean ± S.E.	
r (per day)	90	0.1648 ± 0.0166	100	0.0688 ± 0.0245	0.0003*
λ (per day)	90	1.1792 ± 0.0195	100	1.0712 ± 0.0262	0.0003*
R_0 (offspring)	90	38.10 ± 11.79	100	4.62 ± 2.89	0.0084*
T (days)	90	22.09 ± 0.82	100	22.25 ± 0.18	0.9010
F (eggs/female)	21	163.29 ± 40.55	18	25.67 ± 15.60	0.0032*

*Significant difference between treatments was observed by using paired bootstrap test ($P < 0.05$). *n* = sample size; *r* = intrinsic rate of increase; λ = the finite rate; R_0 = the net reproductive rate; *T* = mean generation time.

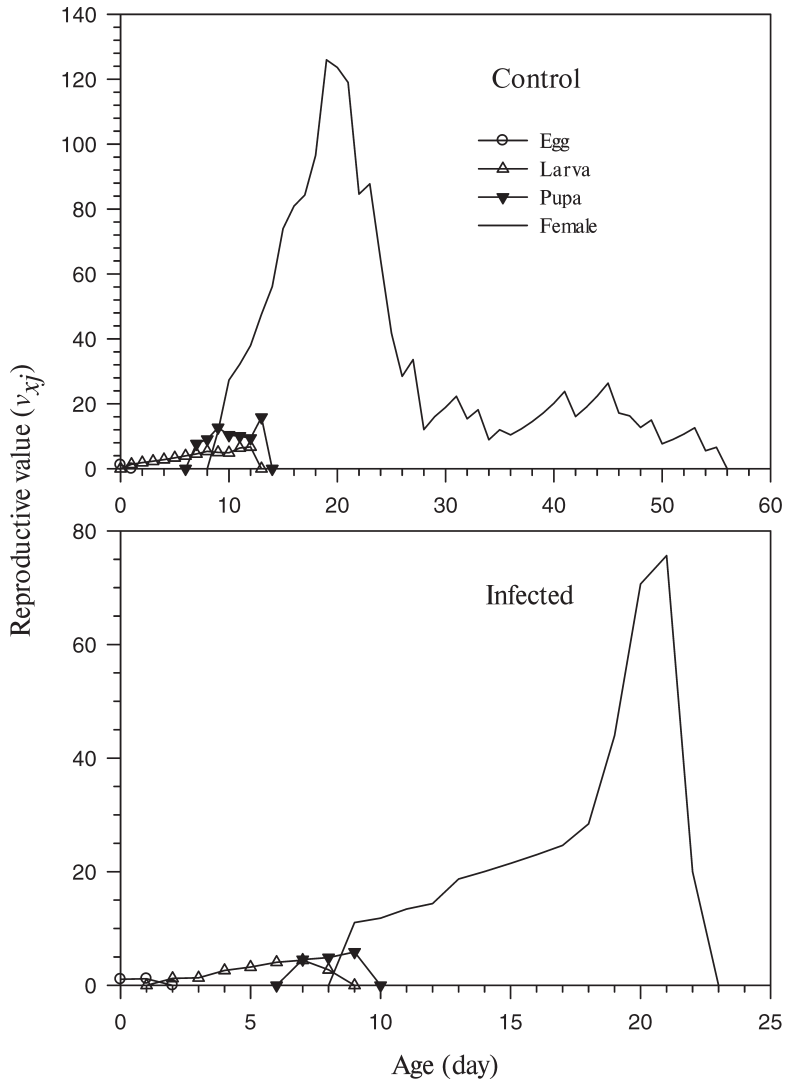

Fig. 3. The age-stage reproductive value (V_{xj}) of *Culex pipiens* L. infected with *Nomuraea rileyi* (Farlow) and the control.

mixture of vitamins A, K, B12, B6, D3 and C and other components of crude fats (7 %), phosphorus (1 %), in addition to the aqueous media for mosquito larvae, the high temperature (30 ± 0.10 °C) and relative humidity scales (52.5 ± 7.8 %) inside the insectary, all facilitated the growth of the fungus. This hypothesis was supported by the results obtained by Namasslvayam *et al.* (2013). These authors isolated *N. rileyi* from soil samples characterised by high organic matter, available nitrogen and phosphorus.

Physical conditions inside the insectary

The high temperature range (27–32 °C, with an average of 30 ± 0.10 °C) as well as the high relative humidity range (35–60 %) with an average of (52.5 ± 7.8 %) facilitated the development of *N. rileyi* inside the plastic containers of the larvae rearing. Similarly, Hart & Mcleod (1955) found that the optimal relative humidity for the germination of *Beauveria* conidia was 94 %. Meanwhile, the optimal conditions for the germination of *Metarhizium* conidia to germinate were recorded as 27–28 °C and 92 % RH (Ferron 1981). Schaerffenberg (1964) and Ferron (1981) stated that the infection with *Beauveria* was not dependent on temperature. In contrast, Suh & Axtell (1999) found that the temperature affected greatly the toxic activity of *Langenidium giganteum* fungus. The mortality rate of the mosquito larvae was greatest at 25 °C, decreased to less than 20 % at 19 °C, while there was no infection at 17 °C, when all mosquito larvae were infected at a concentration of more than 150 zoospores/ml of water.

Pathogenicity of the fungus *Nomuraea rileyi* to larvae of *Culex pipiens* population

On the third day of the larval stage, the survival rate of *Cx. pipiens* was dramatically decreased to 0.54. Similar results were reported by Prayitno (2014) who found that at the level of LC_{50}, *N. rileyi* caused a mortality rate of 0.4 to the closely related species *Cx. quinquefasciatus* and 20 % mortalities to *Ae. aegypti* during the third larval instar. In addition, Maketon *et al.* (2014) reported that *N. rileyi CKN-045* at the concentration 1×10^8 conidia/ml caused high mortality (4.44 ± 1.11) *to Cx. quinquefasciatus* larvae. Additionally, Orduz & Axtell (1991) demonstrated a high mortality rate among 1–2-day-old larvae, moderate mortality during 3-day-old larvae and a very low mortality rate at 4–5-day-old larvae, when infected with *L. giganteum* zoospores. Maketon *et al.* (2014) recorded 100 % mortality after 2 h treatment of *Cx. quinquefasciatus* larvae (third instar) treated with a suspension of *Penicillium* (CM-010; 10^6 conidia/ml). Nnakumusana (1985) showed that *Pythium* caused a mortality rate of 50–100 % for early instars of five culicine and one anopheline species under laboratory conditions. Su *et al.* (2001) isolated *Pythium carolinianum* Matthews infecting *Cx. quinquefasciatus* larvae collected from Guizhou Province, China. The infection rate ranged from 13.3–100 %. *Langenidium giganteum* Couch caused high mortalities in *Culex* larvae collected from California and North Carolina (Merriam & Axtell 1982; Jaronski & Axtell 1983). Golkar *et al.* (1993) observed 99 % mortality of *Cx. pipiens*, while 100 % mortality was recorded among several *Culex* and *Aedes* larvae, infected with *L. giganteum* (McCray *et al.* 1973). In the laboratory, *Crypticola clavulifera* Humber infected four species of Culicinae (Frances 1991). Clark *et al.* (1968) demonstrated that the closely related fungus, *Beauveria bassiana* (Balsamo) infected larvae of *Cx. tarsalis* and *Cx. pipiens*, when applied as conidial dust to the water surface. *Beauveria bassiana* was virulent against three species of mosquito larvae including *Cx. pipiens* (Geetha & Balaraman 1999; Pinnock *et al.* 1973) and also in the case of *Cx. tritaeniorhynchus* (Sandhu *et al.* 1993). On the other hand, *Metarhizium anisopliae* (soilborne fungus) caused 50 % mortality in *Cx. pipiens* larvae when used as dry conidia (1 mg/16 cm^2) (Daoust *et al.* 1982). However, unformulated conidia of *M. anisopliae* affected nine species of mosquito larvae including *Cx. pipiens* (Roberts 1970). A dose of 300 mg conidia/m^2 reduced 91 % of the larvae of *Cx. pipiens*, while a dose of 600 mg conidia/m^2 reduced *Cx. pipiens* larvae by 94 % within 3 days (Roberts 1974).

The recorded survival rate of the infected adult male and female *Cx. pipiens* (0.20 and 0.18) were lower than that recorded for the control (0.27 and 0.23). In agreement with our results, Scholte *et al.* (2004) reported that the fungus *Beauveria bassiana* reduced the survival rate of *An. stephensi* Liston 1901 mosquitoes by 14 days. Maketon *et al.* (2014) reported that adult *Cx. quinquefasciatus* suffered a higher mortality rate when treated with *Aspergillus flavus* (CM-011) compared to *Metarhizium anisopliae* (CKM-048).

Obtained results illustrated that infected females of *Cx. pipiens* had a decrease in the fecundity, and net reproductive rate (25.67 ± 7.70 and 4.62 ± 1.45,

respectively) compared to females of the control population (163.29 ± 40.64 and 38.10 ± 11.80, respectively). Similar findings were reported by Scholte *et al.* (2008). Female *An. gambiae*, infected with a low and high dose of *M. anisopliae*, produced fewer eggs (54.81 ± 2.47 and 45.48 ± 4.7 eggs/gonotrophic cycle, respectively) compared to (65.55 ± 2.96 eggs) in case of uninfected females.

Infected males of *Cx. pipiens* in this study showed a shorter lifespan (7.8 days) as did the females (10.83 days) compared to control males and females (20.83 and 24.38 days, respectively). In agreement with these findings, Scholte *et al.* (2005) recorded a significantly shorter lifespan of infected males (3.7 days) and females (3.43 days) of *An. gambiae* infected with the fungus *M. anisopliae* as compared to uninfected males and females (5.88 days and 9.33 days, respectively). Mosquito longevity is considered as the most important factor in the capacity of the mosquito as a vector of various diseases (MacDonald 1957). The reduction of the female longevity of *Cx. pipiens* greatly reduces the possibility of different disease transmission. The effect of *N. rileyi* on the longevity of male and female *Cx. pipiens* is a promising control strategy.

In this study *Cx. pipiens* was capable of replicating 38.10 times with a high exponential rate of daily increase ($\lambda = 0.16$/day) within a short period of time ($T = 22.09$ days). However, under the stress of the fungus infection the laboratory population could only replicate 4.62 times during nearly the same period of time ($T = 22.25$ days), with a low exponential rate of increase of $\lambda = 0.069$/day.

The demographic effects of the fungus *N. rileyi* on the population of *Cx. pipiens* suggested that it may be developed as an environmentally harmless biological control agent (larvicide) in Egypt. Studies on the production of toxins and enzymes by the fungus *N. rileyi* may provide important information for the future use in mosquito control. Additionally, further detailed investigations to determine the lethal and sub lethal doses are essentially required before field application.

REFERENCES

ALPHEY, L. 2014. Genetic control of mosquitoes. *Annual Review of Entomology* **59**(1): 205–224.

AL-AIDROOS, K. & ROBERTS, D.W. 1978. Mutants of *Metarhizium anisopliae* with increase virulence toward mosquito larvae. *Canadian Journal of Genetics and Cytology* **20**: 211–219.

BERNARD, K.A. , MAFFEI, J.G., JONES, S.A., KAUFFMAN, E.B., EBEL, G.D., DUPUIS, A.P. II, NGO, K.A., NICHOLAS, D.C., YOUNG, D.M., SHI, P.Y., KULASEKERA, V.L., EIDSON, M., WHITE, D.J., STONE, W.B. & KRAMER, L.D. 2001.Comparison of WNV infection in birds and mosquitoes in New York State in 2000. *Emerging Infectious Disease* **7**: 679–685.

BUTT, T.M., GREENFIELD, B.P.J., GREIG, C., MAFFEIS, T.G.G., TAYLOR, J.W.D. *et al.* 2013. *Metarhizium anisopliae* pathogenesis of mosquito larvae: A verdict of accidental death. *PLOS ONE* **8**(12): e81686. DOI: 10.1371/journal.pone.0081686.

CHI, H. 1988. Life-table analysis incorporating both sexes and variable development rates among individuals. *Environmental Entomology* **17**(1): 26–34.

CHI, H. & LIU, H. 1985. Two new methods for the study of insect population ecology. *Bulletin of the Institute of Zoology, Academia Sinica* **24**: 225–240.

CLARK, T.B., KELLEN, W., FUKUDA, T. & LINDEGREN, J.E. 1968. Field and laboratory studies on the pathogenicity of the fungus *Beauveria bassiana* to three genera of mosquitoes. *Journal of Invertebrate Pathology* **11**: 1–7.

CHOI, Y.W., HYDE, K.D., HO, W.H. 1999. Single spore isolation of fungi. *Fungal Diversity* **3**: 29–38.

CIOTA, A.T., MEOLA, M.A. & KARMER, L.D. 2011. The costs of infection and resistance as determinants of west Nile virus susceptibility in *Culex* mosquitoes. *BMC Ecology* DOI: 10. 1186/1472-6785-11-23.

CRISA, E. 1971. Mechanism responsible for release of toxin by *Metarhizium* spores in mosquito larvae. *Journal of Invertebrate Pathology* **17**: 260–264.

DAOUST, R.A., WARD, M.G. & ROBERTS, D.W. 1982. Effect of formulation on the virulence of *Metarhizium anisopliae* conidia against mosquito larvae. *Journal of Invertebrate Pathology* **40**: 228–236.

DA COSTA, G.L., DE MORAES, A.M.L. & DE OLIVEIRA, P.C. 1998. Pathogenic action of *Penicillium* species on mosquito vectors of human tropical diseases. *Journal of Basic Microbiology* **38**: 337–341.

EFRON, B. & TIBSHIRANI, R.J. 1993. *An Introduction to the Bootstrap.* Chapman & Hall, New York, U.S.A.

FEDERICI, B.A. 1981. Mosquito control by fungi *Culicinomyces*, *Coelomomyces* and *Coelomomyces*. In: Burges, H.D. (Ed.) *Microbial Control of Pests and Plant Diseases.* 555–572. Academic Press, London, U.K.

FERRON, P. 1981. Pest control by the fungi *Beauveria* and *Metarhizium*. In: Burgess, H.D. (Ed.) *Microbial Control of Pests and Plant Diseases*. 465–482. Academic Press, London, U.K.

FRANCES, S.P. 1991. Pathogenicity, host range and temperature tolerance of *Crypticola clavifera* (Oomycetes: Lagenidiales) in the laboratory. *Journal of the American Mosquito Control Association* **7**: 504–506.

GEETHA, I. & BALARAMAN, K. 1999. Effect of entomopathogenic fungus, *Beauveria bassiana* on larvae of three species of mosquitoes. *Indian Journal of Experimental Biology* **37**: 1148–1150.

GERBER, E.J. 1970. *Manual for Mosquito Rearing and*

Experimental Techniques. American Mosquito Control Association Inc., CA, U.S.A.

GOLKAR, L., LE BRUN, R.A., OHAYON, H., GOUNON, P., PAPIEROK, B. & BREY, P.T. 1993. Variation of larval susceptibility to *Coelomomyces giaganteum* in three mosquito species. *Journal of Invertebrate Pathology* **62**: 1–8.

HART, M.P. & MACLEOD, D.M. 1955. An apparatus for determining the effects of temperature and humidity on germination of fungus spores. *Canadian Journal of Botany* **33**: 289–292.

HUANG, Y-B. & CHI, H. 2013. Life tables of *Bactrocera cucurbitae* (Diptera: Tephritidae): with an invalidation of the jackknife technique. *Journal of Applied Entomology* **137**: 327–339.

JARONSKI, S. & AXTELL, R.C. 1983. Persistence of the mosquito fungal pathogen *Coelomomyces giganteum* (Oomycetes; Lagenidiales) after introduction into natural habitats. *Mosquito News* **43**: 332–337.

KILPATRICK, A.M., KRAMER, L.D., CAMPBELL, S.R., ALLEYNE, E.O., DOBSON, A.P. & DASZAK, P. 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerging Infectious Disease* **11**: 425–429.

KOMAR, N. 2003. West Nile virus: epidemiology and ecology in North America. *Advances in Virus Research* **61**: 185–234.

LACEY, C.M., LACEY, L.A. & ROBERTS, D.R. 1988. Route of invasion and histopathology of *Metarhizium anisopliae* in *Culex quinquefasciatus*. *Journal of Invertebrate Pathology* **52**: 108–118.

MACDONALD, G. 1957. *The Epidemiology and Control of Malaria*. Oxford University Press, London, U.K.

MAKENTON, M., AMNUAYKANJANASIA, A. & KAYSORNGUP, A. 2014. A rapid knockdown effect of *Penicillium citrinum* for control of the mosquito *Culex quinquefasciatus* in Thailand. *World Journal of Microbiology and Biotechnology* **30**: 727–736.

McCARY, E.M., UMPHLETT, C.J. & FAY, R.W. 1973. Laboratory studies on a new fungal pathogen of mosquitoes. *Mosquito News* **33**: 54–60.

MERRIAM, T.L. & AXTELL, R.C. 1982. Evaluation of the entomogenous fungi *Culicinomyces clavosporus* and *Coelomomyces giganteum* for control of the salt marsh mosquito, *Aedes taeniorhynchus*. *Mosquito News* **42**: 594–602.

MICHEAL, E. & BUNDY, D.A. 1997. Global mapping of lymphatic filariasis. *Parasitology Today* **13**: 472–476.

MOHANTY, S.S. & PRAKASH, S. 2007. Laboratory and field evaluation of the fungus *Chrysosporium lobatum* against the larvae of the mosquito *Culex quinquefasciatus*. *Parasitology Research* **102**: 881–886.

MOHANTY, S.S., RAGHAVENDRA, K., RAI, U. & DASH, A.P. 2008a. Efficacy of female *Culex quinquefasciatus* with entomopathogenic fungus *Fusarium pallidoroseum*. *Parasitology Research* **103**: 171–174.

NAMASLVAYAM, R.K., BHARANI, R.S. & ANSARI, M.R. 2013. Natural occurrence of potential fungal biopesticide *Nomuraea rileyi* (Farlow) Samson associated with agricultural fields of Tamil Nadu, India and its compatabiltity with metallic nanoparticles. *Journal of Biofertilizers and Biopesticides* **4**(1): 1–7.

NNAKUMUSANA, E.S. 1985. Laboratory infection of mosquito larvae by entomopathogenic fungi with particular reference to *Aspergillus parasiticus* and its effects on fecundity and longevity of mosquitoes exposed to sporal infections in larval stages. *Current Science* **54**: 1221–1228.

ORDUZ, S. & AXTELL, R.C. 1991. Compatibility of *Bacillus thuringiensis* var. *israelensis* and *Bacillus sphaericus* with the fungal pathogen *Coelomomyces giganteum* (Oomycetes: Lagenidiales). *Journal of the American Mosquito Control Association* **7**: 188–193.

PINNOCK, D.E. & GARCIA, C.M. 1973. *Beauveria tenella* as a control agent for mosquito larvae. *Journal of Invertebrate Pathology* **22**: 143–147.

PRAYITNO, T.A. 2014. Studies on utilization of the entomopathogenic fungus *Nomuraea rileyi* as a biological control agent against larval *Culex quinquefasciatus* and *Aedes aegypti*. Published M.Sc. thesis, Malang State University, Indonesia. http://mulok.library.um.ac.id/index3.php/65602.html.

ROBERTS, D.W. 1970. *Coelomomyces giaganteum*, *Entomophtora*, *Beauveria*, and *Metarhizium* as parasites of mosquitoes. *Miscellaneous Publications of the Entomological Society of America* **7**: 140–155.

ROBERTS, D.W. 1974. Fungal infections of mosquitoes. In: Aubin, A., Bellonick, S., Bourassa, J.P., La Coursiere, E. & Pellissier, M. (Eds) *Le Controle des Moustique/Mosquito Control.* 143–193. Presses de l'Universite du Quebec, Canada.

SANDHU, S.S., RAJAK, R.C. & SHARMA, M. 1993. Bioactivity of *Beauveria bassiana* and *Metarhizium anisopliae* as pathogens of *Culex tritaeniorhynchus* and *Aedes aegypti*: effect of instar, dosages and time. *Indian Journal of Microbiology* **33**: 191–194.

SCHAERFFENBERG, B. 1964. Biological and environmental conditions for the development of mycoses caused by *Beauveria* and *Metarrhizium*. *Journal of Insect Pathology* **6**: 8–20.

SCHOLTE, E.J., KNOLS, B.G., SAMSON, R.A. & TAKEN, W. 2004. Entomopathogenic fungi for mosquito control: a review. *Journal of Insect Science* **4**: 19–42.

SCHOLTE, E.J., KNOLS, G.J. & TAKKEN, W. 2008. An entomopathogenic fungus (*Metarhizium anisopliae*) for control of adult African malaria vector *Anopheles gambiae*. *Entomologische Berichten* **68**(1): 21–26.

SCHOLTE, E.J., NG'HABI, K., KIHONDA, J., TAKKEN, W., PAAIJMANS, K., ABDULLA, S., KILLEN, G.F. & KNOLS, B.G. 2005. An entomopathogenic fungus for control of adult African malaria mosquitoes. *Science* **308**: 1641– 1642.

SU, X., ZOU, F., GUO, Q., HUANG, J. & CHEN, T.X. 2001. A report on a mosquito-killing fungus, *Pythium carolinianum*. *Fungal Diversity* **7**: 129–133.

SUH, C.P. & AXTELL, R.C. 1999. *Coelomomyces giganteum* zoospores: effects of concentration, movement, light, and temperature on infection of mosquito larvae. *Biological Control* **15**: 33–38.

TANTRAL, L., MALATHI, K., KOHYAMA, S., SILANE, M., BERENSTEIN, A. *et al.* 2004. Intracellular calcium release is required for caspase-3 and -9 activation. *Cell Biochemistry and Function* **22**: 35–40.

TAYLOR, M.J., HOERAUF, A. & BOCKARIE, M. 2010. Lymphatic filariasis and onchocerciasis. *The Lancet* **376**(9747): 1175–1185.

VYAS, N., DUA, K. & PRAKASH, S. 2007. Efficacy of *Lagenidium giganteum* metabolites on mosquito larvae

with reference to non target organisms. *Parasitology Research* **101**: 385–390.

WHO. 2000. The African summit on roll back malaria. WHO, WHO/CDS/RBM/2000.17, Geneva, Switzerland.

WHO. 2004. Community participation and tropical disease control in resource-poor settings. TDR/STR/SEB/ST/04.1. World Health Organisation, Geneva, Switzerland.

WHO. 2011. Lymphatic filariasis. A manual for elimination program. WHO Library Cataloguing-in-Publication Data. World Health Organisation, Geneva, Switzerland. Online at: http://www.who.int/about/licensing/copyright_form/en/index.html).

Accepted 14 November 2016