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Comparing Efficacy of Mixed Larval Diets on the Developmental Attributes of *Anopheles arabiensis* Patton

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Abstract.- A comparison test was conducted on three mixed diets consisting of ingredients from natural grains and commercial brand aquaculture foods. *Anopheles arabiensis* Patton larvae were reared on these diets separately under standard rearing conditions at the Insect Pest Control Laboratories (IPCL), IAEA, Seibersdorf, Austria. Time to pupation, time to emergence, survival to pupal and to adult stages, and adult wing length measurements were recorded and taken into account as parameters for diet comparison. All three diets resulted in development of larvae to pupae and to the adult stage. Significant reductions in time to pupation and to emergence were recorded for larvae fed diet 1 (NIFA1) composed of natural ingredients. Diet 1 resulted in the shortest larval duration, highest survival rate and longest wing length for both male and females. Body length of the 4th instar (L4) was also largest for diet 1, but was not significantly different from other diets. Addition of vitamin mix to diet 1 did not improve its quality as measured by larval duration, survival and emergence success.

Key Words: Mosquito, *Anopheles*, larval diet, malaria, sterile insect techniques.

INTRODUCTION

Malaria is the most damaging insect-transmitted disease of people. Half of the world's human population lives in malaria-affected areas. There are approximately 1 million deaths a year, 250 million cases of clinical malaria each year and about 3.3 billion people at risk of malaria transmission (WHO, 2009a). Being a sub-tropical country, Pakistan has a rich fauna of vectors including mosquitoes, sand flies and other Diptera (Report, 2003, 2006). Presence of vast agricultural lands, open networks of irrigation channels, rivers and several water dams for power generation in the country provide plenty of breeding sources for mosquitoes. *Anopheles culicifacies* and *An. stephensi* are the primary malaria vectors in the country (Reisen and Milby, 1986; Suleman *et al*., 1993). *Anopheles culicifacies* is the most important vector in the rural areas (Covell, 1931; Hick and Majid, 1937; Mahmood *et al*., 1984; Kamimura, 1986; Pervez and Shah, 1989) and *An. stephensi* in the urban areas (Rehman and Muttalib, 1967; Reisen *et al*., 1982). Both species breed in clean water mainly in agricultural drains, irrigation channels, pools, pits puddles paddy fields (Mahmood and MacDonald, 1985) and transmit *Plasmodium falciparum* and *P. vivax*. The major transmission period is post-monsoon from July to October, but a short spring transmission can occur during April and May.

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The World Health Organization (WHO) reports 140 million people in Pakistan are at risk for malaria infection with 18% living in high risk situations (WHO, 2009b). In Pakistan, the public sector is the main source for provision of preventive and curative care to about 25% of the urban and rural population. The remaining 75-80% of therapeutic needs are provided by the private sector. Most often patients are treated at private clinics where records of blood tests are not maintained. The actual cases of malaria load in the country thus may be 5 times higher than what has been reported.

Organized vector control operations are rare in Pakistan. Municipal organizations at the district level, in a few malaria endemic areas' carry out residual spraying operations. The country has dire need for dedicated operations on integrated vector management. Use of pesticides in farmer's fields and in houses may lead to the development of resistance in vector populations and pollution of the environment.

As a component of IPM for vector control, the Sterile Insect Technique (SIT) is an alternative approach which relies on mass rearing and release of non-biting male mosquitoes sterilized by gamma radiation. This technique could be used along with other control strategies for the suppression of the populations of several vector species (Klassen and Curtis, 2005). Under this program, the males used in SIT must be compatible with the wild males in vigor, flight and mates selection (Parker 2005; Benedict *et al*., 2009). Much of this potential is determined by the diet provided in the larval stage (Benedict *et al*., 2009). Keeping in mind the importance of larval diet in a mass rearing system, studies were conducted under a Coordinated Research Program (CRP) to evaluate three diets developed independently by the contract and agreement holders for the development of a standardized mass rearing system for male *An. arabiensis.* A combination mixture diet (NIFA1) was developed from cheap and globally available seeds and grains over the last three years at the Nuclear Institute for Food and Agriculture (NIFA), Peshawar Pakistan (Khan *et al*., 2011). Growth, developmental rate and survival of mosquitoes reared on this diet were compared to mosquitoes reared on two other diets; one modified from diet 1, and other developed by the mosquito group at the Insect Pest Control Laboratories (IPCL) of International Atomic Energy Agency.

MATERIALS AND METHODS

Each of the three diets (Table I) was prepared in 1000 ml deionised water. Sub-samples of the diet were distributed into 50-ml falcon tubes. Aliquots of each diet were placed in Eppendorf tubes and stored in a freezer at -20°C. Thirty-two *An. arabiensis* 1st instars of 2-3 hours age were pipetted from hatching cups and transferred into 9-cm Petri dishes with 32 ml deionised water. For each diet, 5 replicates were done and 640 µl of prepared diet was added daily to each dish. Larval developmental times from L1 (first instar) to pupa and from L1 to adult emergence and their survival were recorded. After appearance of the first pupa, measurements on size from anterior margin of the head capsule to the terminus of the last abdominal segment of ten, 4th instars (2 from each replicate) were performed with a CC-12 camera (Olympus Soft Imaging System GmbH, Germany, 2006) mounted on a stereomicroscope (LeicaMZ16FA) using analySIS_B software (Olympus Soft Imaging System GmbH, Germany, 2006). For wing length measurements on adults, pupae were transferred into replicate plastic tubes each with 7 pupae. On emergence, the wing length measurement (5 males and 5 females) was taken from their left wing (or right if the left was deteriorated or lost) from the distal edge of the alula to the end of the radius vein (excluding fringe scales).

Table I.- Diet composition fed to *Anopheles arabiensis* larval stages.

Diets	Composition ingredients (in g)
Diet 1 (NIFA 1)	Wheat, corn, bean, chick pea, rice, bovine liver (2+3+2+3+3+3.6)
Diet 2 (NIFA 2)	Wheat, corn, bean, chick pea, rice, bovine liver, vitamin mix (2+2+2+2+2+2+2.6)
Diet 3 (IAEA)	Bovine liver, tuna meal, vitamin mix (5+5+4.6)

Statistical analysis

Statistical analyses of differences in developmental time, survival rate, L4 were done in completely randomised design. Larval development time was defined as the number of days between hatching and pupation. Larval survivors were counted as individuals that reached the pupa stage. Wing length data was analysed using Tukey's HSD all pairwise test in factorial design in Statistix 8.1. In all cases, the alpha level was selected at 0.05.

RESULTS AND DISCUSSION

Figure 1 shows larval duration from first instar to pupation and to adult emergence. All three diets resulted in a good development of the immature stages of *An. arabiensis*. Time to pupation was significantly different among the different diets with 7.19, 10.51 and 11.61 days for diet 1, diet 2 and diet 3, respectively. The same trend was observed for the time to adult emergence.

Survival from the first instar (L1) to pupa was highest for diet # 1 (96%) compared to diet # 2 (95%) and diet # 3 (85%), respectively. Figure 2 illustrates survival rate from first instar (L1) to pupa

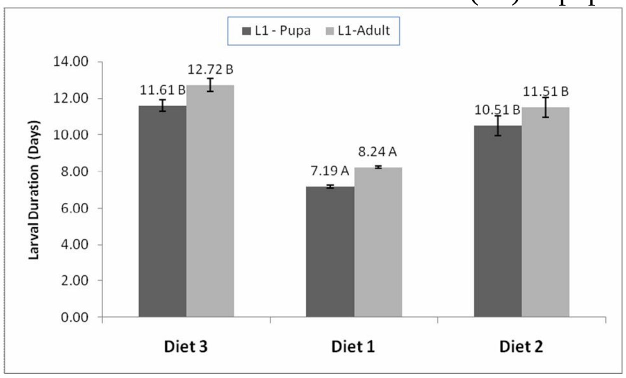

Fig. 1. Comparison of larval duration of *Anopheles arabiensis* from first instar to pupae and to adult stages fed with 3 mixture diets. Vertical bars are standard errors, n=5.

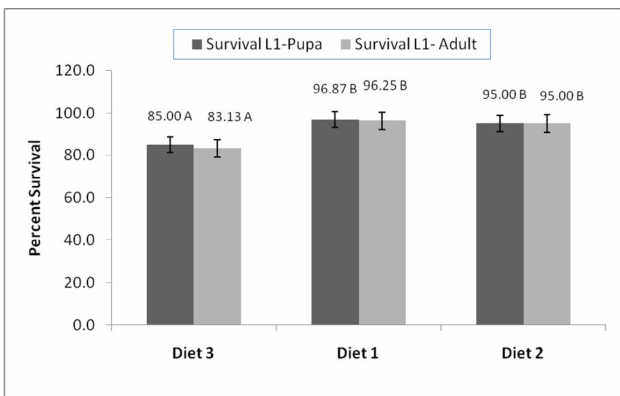

Fig. 2. Survival rates of *Anopheles arabiensis* from 1st instar to pupa and to adult stage fed with 3 mixture diets. Vertical bars are standard errors, n=5.

and adult stage. Survival rate was highest in diet # 1, resulting in 96.9% survival to the pupal stage and 96.3% to adult emergence. There was very little mortality in the pupal stage. When food and other rearing conditions are optimum, 4th instars of mosquitoes consume maximum food and change to pupal stage within 2-3 days. In order to get accurate readings on the fourth instar (L4), we obtained measurements on the L4 size larvae in a very short time window when the first pupa appeared in the rearing medium. The body size of fourth instars at the appearance of the first pupa is shown in Figure 3. Overall, L4 body size was longest for larvae fed diet # 1 and smallest for larvae fed diet # 3. However, the difference in the L4 body size was not significantly different among the three diets.

Wing length of adult mosquitoes emerged from different diets were different between the sexes and among the diets. Females emerged from diet # 1 were significantly large in size (Fig. 4). Our results on wing length measurements are not consistent with previous observations for *An. gambiae* (Reisen, 1975; Mori, 1979; Reisen and Milby, 1986; Briegel, 1990; Lehman *et al.*, 2006). The wing lengths of females emerged from diet # 1 was significantly large from that of males emerged from diet # 2 and diet #3.

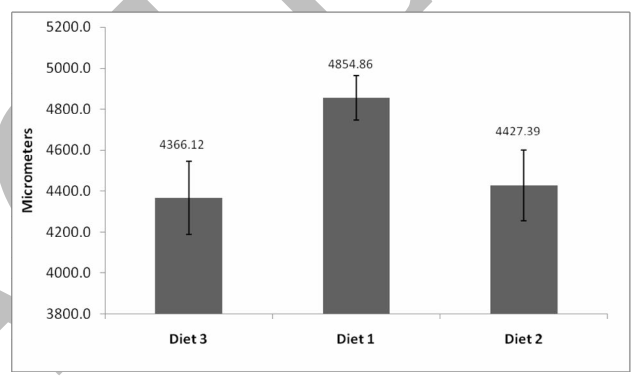

Fig. 3. Length of *Anopheles arabiensis* larva at last instar (L4) measured after appearance of first pupa. Vertical bars are standard errors, n=10.

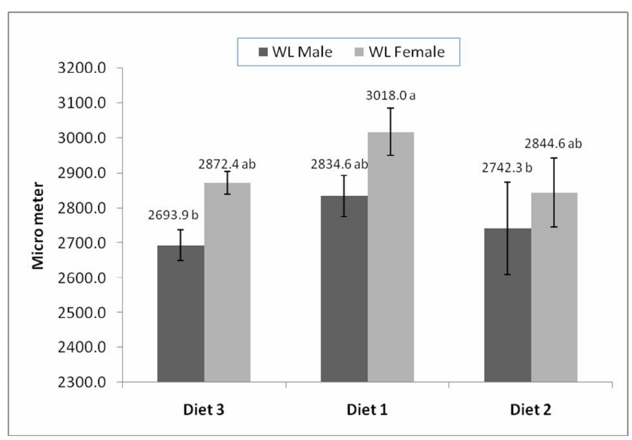

Fig. 4. Wing length (μm) of adult males and females fed different diets at the larval stage. Means followed by the same letter are not significantly different from one another. Vertical bars are standard errors, n=5.

In our diet selection, we tried to compare diets developed from two different sources. NIFA 1 was developed from natural sources of various grains and seeds, while diet # 3 was a mixture diet from aquaculture foods. Diet # 2 was a modified diet from NIFA 1. A vitamin mixture was added to diet # 1with the hope for improvement of NIFA 1. Our findings on comparison of these diets showed the addition of vitamin mix to NIFA 1 did not improve its quality in terms of development and success to emergence as adults. This may be due to the reason that cereals and legumes are rich in vitamins, (Butt *et al.*, 2006) predominantly the B vitamins-thiamin (B1), riboflavin (B2) and niacin (B3). Thus, vitamins supplement in the diet NIFA 1 may not have an additional role in the enhancement of insect development. The second reason may be due to the difference in the nutritive value of various diets. Bovine liver was common in all there diets, and the differences seen in the mosquito's development might be due to combination of cereals and legumes in diet NIFA 1. Cereal grains and legumes contain varying amounts of carbohydrate, fat, protein, water and minerals (Ajah and Madubuike, 1997; Iqbal *et al*., 2006). The carbohydrates, predominantly starch makes up 79-83% of the dry matter of wheat grain (Bibi *et al*., 1988). The fiber especially cellulose and hemicellulose composes approximately 6% of the grain. Wheat, rice and corn contain 1-2% lipid with 72-85% unsaturated fatty acids, primarily, oleic acid and linoleic acid. Although cereals are low in the amino acids tryptophan and methionine, this deficiency might have been compensated by their mixture with chick-pea and beans (Shah *et al*., 1987). Studies on animal diets have shown that cereal legume mixtures give high nutritional value than cereal alone (Del-Angel and Sotelo, 1982).

These experiments demonstrated the impact of the diet on *An. arabiensis* production and emphasized the role of natural ingredients in diet composition for the development of aquatic stages of mosquitoes. Chemico-physical analyses of the different diet are obviously the priority to understand why we observed significant variations in the different diets (survival, duration and wing length). The role of amino acids, fatty acids especially arachidonic acid (AA, C20:4) may not be underestimated in the biology and insect development. Polyunsaturated fatty acids (PUFA) such as arachidonic acid, EPA (C20:5) and docosahexaenoic acids (DHA, C22:6) are also required for normal growth and flight of newly-emerged mosquitoes (Dadd *et al*., 1977; Dadd and Kleinjan, 1978; Dadd and Kleinjan, 1979; Dadd *et al*., 1988). The vitamin level supplements of PUFA also play a significant role in the normal growth and survival of *Culex pipiens* (Dadd *et al*., 1988). Further experiments on mass rearing for *An. arabiensis* should be carried out with these diets prior to launching an SIT of this mosquito. The tests we have performed provide a reasonable foundation and first step towards understanding the role of diet in mass rearing. Further experiments are under way (Khan, 2010) on the role of polyunsaturated fatty acids in the development and flight performance of *An. arabiensis* and studies on the optimal temporal feeding schedule and relationship of diet quantity to larval density for large-scale rearing operations must be performed to enhance the total output and quality of adults in an SIT program.

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