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Fitness cost in field *Anopheles labranchiae* populations associated with resistance to the insecticide deltamethrin

Ahmed Tabbabi*, Jabeur Daaboub

Department of Hygiene and Environmental Protection, Ministry of Public Health, Tunis, Tunisia

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ABSTRACT

We evaluated in the present study the effect of deltamethrin resistance on the fitness cost of the filed populations of *Anopheles labranchiae*. A susceptible population was used as reference to do different comparisons. We selected the most resistant larvae population collected from northern Tunisia. Eggs were used for study of life history traits including developmental time, larvae mortality, fertility, hatchability and adult sex-ratio. Our results showed that deltamethrin resistance affected negatively ($p < 0.05$) the developmental time with the median range of 70 h, mortality with the rate of 7 folds in resistant population and hatchability which are lower than in susceptible population. Whereas, no significant differences were detected in adult sex-ratio and fertility of the two studied populations. Our results could help to determine the evolution of population dynamics of the resistant studied population in the areas where insecticide resistance is reported and resistance management is needed.

Introduction

The *Plasmodium*, which is transmitted by mosquito, is the biggest killer mosquitoes born diseases (WHO, 2014). It remains a serious health problem for many countries classified as malaria free through cases imported from endemic regions. In the case of Tunisia, several studies showed the increase of the annual incidence of imported cases of malaria related to the existence of *Anopheles* mosquitoes in numbers high enough to ensure the risk of a resumption of the disease transmission in Tunisia (Chadli et al., 1985; Ben Rachid et al., 1984; Gmara, 2006; Bouratbine et al., 1998).

Anopheles (An.) labranchiae (Falleroni, 1926) is known as an important vector of malaria throughout its global distribution (Becker et al., 2010). Its role as vector has been suggested in northern Tunisia where malaria was transmitted until its elimination in 1980 (Tabbabi et al., 2015). Historically, their abilities to transmit strains of *Plasmodium falciparum in natura* and under laboratory conditions have been suggested (Toty et al., 2010). There is evidence also of their capacities to transmit *Plasmodium malariae* (Toty et al., 2010). Their responsibilities in the transmission of *Plasmodium vivax* have been recently reported (Baldari et al., 1998).

The control of *An. labranchiae* can be difficult. This species remains established in Tunisia despite the eradication of malaria in 1980 (Tabbabi et al., 2015). Chemical and biological pesticides including DDT, and later deltamethrin, fentrothion, pyrethroids, and larvivorous fish such as *Gambusia* have been used against mosquitoes' larvae but *An. labranchiae* is still persisting in high densities in northern parts of the country (Tabbabi et al., 2015).

It is known that the massive application of insecticide during malaria eradication program actually tends to favor insecticide resistance which remains a serious threat in mosquitoes control programs (Ben Cheikh et al., 1998; Nauen, 2007; Daaboub et al., 2008). The toxic effect of insecticides could be expressed in insects by different ways such as behavioral, physiological and genetic expressions (Kliot and Ghanim, 2012). Fitness costs resulting from resistance to insecticides has been reported in many insects from different orders including mosquitoes (Tabbabi and Ben Cheikh, 2017; Sanil and Shetty, 2012; Brown et al., 2013; Jaramillo et al., 2014). However, other studies did not observe any biotic disadvantage in resistant studied populations (Okoye et al., 2007; Bielza et al., 2008; Lyons et al., 2016).

According to our knowledge, the effect of insecticide resistance on the *An. labranchiae* life history traits has not been explored. Different parameters including developmental time, larvae mortality, fertility, hatchability and adult sex-ratio were studied using two field collected populations that previously showed high and low level of resistance to deltamethrin, respectively (Tabbabi and Daaboub, 2017).

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* Corresponding author.
E-mail: tabbabiahmed@gmail.com (A. Tabbabi).

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Fig. 1. Geographic origin of Tunisian populations of *Anopheles labranchiae*.

Materials and methods

Mosquitoes

Two populations of *An. labranchiae* were collected in 2016 in northwestern (Le Kef) and northeastern (Ben Arous) Tunisia (Fig. 1) and identified as susceptible and resistant populations, respectively. We choose the most resistant (frequent control mosquitoes using chemical insecticides) and the most susceptible population (absence of mosquitoes control) to do comparisons of studied biological parameters.

Table 1
Resistance to deltamethrin in *An. labranchiae* from Tunisia.

Population	LC_{50} (μg/L)		RR_{50}
	95% *CI*	Slope ± SE	
Susceptible population	0.12 (0.05–0.17)	2.10 ± 0.32	–
Resistant population	1.50 (0.50–2.20)	1.22 ± 0.17	12.50 (10.20–14.10)

RR_{50}, resistance ratio at LC_{50} ($RR_{50} = LC_{50}$ of the population considered/LC_{50} of sensitive strain).

Mosquito rearing

Eggs were taken in breeding sites of resistant and susceptible populations and transferred to plastic basins containing water and rabbit crop which served as food for hatched larvae. Rearing proceeded until the adult stage.

Larval bioassays

Resistance to deltamethrin insecticide (99.5% [AI]) was evaluate in late third and early fourth instar larvae from both populations according to standard methods of Raymond et al. (1986). Lethal concentrations (LCs) and resistance ratios (RR50 and RR95) (Table 1) were calculated via probit analysis of Raymond et al. (1993) based on Finney (1971). Bioassays included 5 concentrations (100, 10, 1, 0.1, and 0.01 ppm) providing between 0 and 100% mortality and 3 replicates per concentration on sets of 20 larvae in a total volume of 100 mL of water containing 1 mL of ethanol solution of each tested insecticide. We repeated the assay if the rate of mortality in the control group exceeded 10%. It should note that the study was carried out under laboratory conditions and not under field conditions.

Fertility

Fertility was measured as number of larvae hatched from each egg. Eggs were qualified as big when the number of larvae exceeded 150, average when the number of larvae was located between 150 and 100 and small when it not exceeded 100 larvae.

Hatchability

This life history trait was measured in percentage as the number of hatched eggs by the total number of collected eggs.

Larval developmental time and mortality rate

Developmental time and larvae mortality were assessed by following larval development of resistant and susceptible populations from first instars larvae to emergence of adults. We used three ranges to neutralize the effect of density: low density (50 larvae/500 mL), average density (100 larvae/500 mL) and high density (200 larvae/500 mL). Mortality was recorded daily.

Adult sex-ratio

The pupae were transferred daily to a small cup containing 200 mL to be able to identify them in male and females.

Statistical analysis

Data obtained for each parameter evaluated were compared using *t* tests for quantitative parameters and χ^2 analysis for qualitative variables, as indicated in the results.

Table 2
Hatchability and egg fertility of sensitive and resistant strains.

	Number of fertile eggs	Egg size[a]	Number of fertile eggs/number total (%)	Big eggs (%)	Average eggs (%)	Small eggs (%)
Susceptible population	78	122.17 ± (38.15)	98.45	8.55	28.45	64.00
Resistant population	24	89.42 ± (52.22)	35.27	12.18	33.79	54.03

[a] Standard errors in parentheses.

Table 3
Average number of larvae, percentage of emerged adults, mortality rate and development time of resistant strain.

Density	Larvae	Adults	Males	Females	Mortality rate	Development time (h)		
						Male		Females
ALDR	50 ± (0.00)	45 ± (7.89)	21.55 ± (1.27)	23.45 ± (5.25)	5.00 ± (7.88)	298.15 ± (14.24)	301.87 ± (18.26)	304.00 ± (13.07)
AMDR	100 ± (0.00)	50.47 ± (11.32)	20 ± (3.79)	30.47 ± (9.04)	50.53 ± (14.97)	390.55 ± (17.45)	412.75 ± (6.27)	422.99 ± (16.50)
AHDR	200 ± (0.00)	35.33 ± (11.07)	15.75 ± (6.37)	19.58 ± (8.74)	164.66 ± (12.45)	390.00 ± (14.78)	450.33 ± (17.08)	420.33 ± (20.87)
AA	116.66 ± (76.37)	43.6 ± (14.35)	19.1 ± (2.59)	24.5 ± (12.07)	73.33 ± (13.28)	359.56 ± (44.58)	388.31 ± (54.77)	382.44 ± (55.22)

h, hours; standard errors in parentheses; ALDR, Average of the three Low-Density Repetitions; AMDR, Average of the three Medium Density Repetitions; AHDR, Average of the three High-Density Repetitions; AA, Average of Averages.

Table 4
Average number of larvae, percentage of emerged adults, mortality rate and development time of sensitive strain.

Density	Larvae	Adults	Males	Females	Mortality rate	Development time (h)		
						Male		Female
ALDR	50 ± (0.00)	49.33 ± (4.53)	20 ± (5.69)	29.33 ± (5.48)	1.66 ± (4.72)	120.00 ± (9.65)	150.66 ± (24.45)	180.87 ± (22.19)
AMDR	100 ± (0.00)	96.66 ± (3.48)	29.66 ± (5.87)	57.33 ± (10.42)	3.66 ± (8.40)	145.50 ± (28.77)	185.50 ± (17.43)	220.08 ± (17.38)
AHDR	200 ± (0.00)	175 ± (4.79)	75 ± (3.78)	100 ± (6.45)	25 ± (8.08)	290 ± (20.41)	320.05 ± (22.37)	350 ± (12.27)
AA	116.66 ± (76.37)	106.99 ± (32.27)	41.55 ± (21.07)	62.22 ± (8.77)	10.10 ± (5.27)	185.16 ± (42.78)	218.73 ± (75.88)	250.31 ± (75.85)

h, hours; standard errors in parentheses; ALDR, Average of the three Low-Density Repetitions; AMDR, Average of the three Medium Density Repetitions; AHDR, Average of the three High-Density Repetitions; AA, Average of Averages.

Results

Insecticide resistance status of studied populations

As shown in Table 1, the lethal concentration (LC_{50}) of the resistant population is very high compared with the susceptible population calculated by log probit analysis (1.50 (0.50–2.20) and 0.12 (0.05–0.17) ug/L, respectively).

Hatchability

The mean hatching rate of susceptible population was 98.45%, whereas, resistant population scored a mean range of 35.27% (Table 2). Statistical analysis showed a significant difference between the two populations ($p < 0.05$).

Fertility

No significant difference in fertility was observed between the two studied samples ($p > 0.05$, Table 2).

Developmental time

It should be note that the study of the development time of the two studied populations was investigated until the adult emergence. The comparison of this trait history parameter showed a significant difference between the two populations ($\chi^2 = 298.7$, df = 1, $p < 0.05$, Tables 3 and 4). The larval developmental time for resistant population was longest (median = 388 h) compared to susceptible population (median = 218 h). On the other hand, the density seemed to affect the development time. Indeed, this life history trait was shorter in low densities than in high ones. It should be note that any significant difference ($p > 0.05$) was detected between development time of males and females.

Mortality rate

Mean larval mortalities were 73.33 and 10.10 for resistant and sensitive populations, respectively (Tables 3 and 4). Statistical analysis revealed significant difference in mortality rate between strains ($p < 0.05$). On the other hand, mortality rate was higher in high densities than in low and medium ones of the two studied populations. The significant difference in larval development time of the two studied populations could be explained by the important mortality rate of resistant population.

Adult sex-ratio

Based on our results, unbalanced sex-ratio was observed in all studied populations (Tables 3 and 4). The eggs tend to give more females than males. This difference was significantly observed in resistant strain ($p < 0.05$).

Discussion

In the present study, we tried to estimate the effects of deltamethrin resistance on several life histories of two *An. labranchiae* populations (resistant and susceptible) collected from

Northern Tunisia. It should be note that the fitness cost of several mosquito vectors due to insecticide resistance has been reported. However, studies on fitness cost of *An. labranchiae* have not, to our knowledge, been explored despite their public health importance (Tabbabi et al., 2015). Our finding showed the negative effect of deltamethrin resistance on some life history parameters of resistant population. Similar results were found on other mosquito species using different insecticides (Martins et al., 2012; Jaramillo et al., 2014).

Among the affected life history parameters, an increase of the development time was recorded in the resistant population. These finding are in agreement with previous studies which reported an increase of the larval development time in resistant *Aedes aegypti* (Diniz et al., 2015). In contrast, Plernsub et al. (2013) showed that resistance did not affect larval development time of *Aedes* mosquitoes in Thailand. It is important to mention that this parameter affect the dissemination of mosquito population in the field (Martins et al., 2012). In natural environment, it may affect the adaptive advantages of an individual by cause of several extrinsic factors, such as physical destruction of breeding sites (Berticat et al., 2004), and the presence of predators or parasites can reduce the larval survival rate (Agnew and Koella, 1999) which help in the reduction of generations. We should note that developmental time varied according to the sex. Indeed, slowing of the development of female could be explained probably by their need to accumulate resources for reproduction (Clements, 1992).

Our results showed that resistance tends to increase the mortality rate of *An. labranchiae*. Similar results have been observed in many previous studies on mosquito's species (Guillemaud et al., 1998; Lenormand et al., 1998). The fact that mortality was higher in the high-density let us suggest the role played by density in higher mortality. However, previous studies showed that larval density affects larval growth dramatically, but larval mortality was independent of larval density (Duffy and Epifanio, 1994). It should note that food played also some role in the larval death by polluted water quickly enough to kill. On the other hand, a female-biased sex ratio has also been reported in *Anopheles gambiae* from Kenyia (Mutuku et al., 2006). Skewed sex ratios of females at high larval densities have been reported in many insects. Cipollini (1991) stated that male-biased mortality at higher larval densities produced a female-biased sex-ratio in *Acanthoscelides obtectus*. In the present study, the magnitude of density-dependent development and mortality thus caused the distorted sex ratio.

In our study, changes in behavioral aspects were also observed. Indeed, a reduction in hatchability and fertility of resistant population was recorded. Similar results were observed in *Culex pipiens* and *Aedes aegypti* (Li et al., 2002; Martins et al., 2012). In contrast, some reproductive advantages have been reported in pyrethroid resistant malaria vector, *An. funestus* (Okoye et al., 2007).

The detection of high deltamethrin resistance in *An. labranchiae* mosquitoes from Tunisia can be explained by deltamethrin/DDT cross-resistance. In fact, the DDT was used as the main the main insecticide in the framework of the National Program for the Eradication of Malaria during the 60s and 70s against malaria vectors. Previous study (Tabbabi and Daaboub, 2017) showed the involvement of target site alteration (Kdr mutation) in the recorded resistance. However, detoxification enzymes were not involved. In support of these results, the resistance mechanism involved exhibit a reduction in fitness. Authors noted that the increase of mortality rate and the decrease of fecundity females were probably due to the modified acetylcholinesterase that appears associated with a higher cost than that associated with overproduced esterase (Lenormand et al., 1998; Lenormand and Raymond, 2000). In this context, we should note that it is not clear if the insecticide resistance can affect the vectorial capacity of malaria vectors. However, Alout et al. (2013) have reported the negative impact on competency of *An. gambiae* to transmit *P. falciparum*.

The evaluation of fitness cost parameters was carried out in the laboratory conditions which differ with field conditions. The artificial and optimum conditions can sur- and/or under-estimate the results. Indeed, several environmental factors including the quality of food, larval density, temperature and humidity could be deleterious for resistant populations in the nature (Belinato and Martins, 2016).

Conclusion

The negative impact of insecticide resistance on different fitness cost parameters is very important against the maintenance and dispersion of the resistant individuals in the field. Further investigations are needed to evaluate this impact on vectorial capacity of *An. labranchiae* which is an important malaria vector. It should be noted that our results could help to determine the evolution of population dynamics of the resistant studied population in the areas where insecticide resistance is reported and resistance management is needed. However, further investigations are needed to elucidate the mechanism of resistance using molecular and biochemical methods and therefore knowing whether insecticide resistance mutations involve fitness costs.

Conflicts of interest

The authors declare no conflicts of interest.

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