Chapter 13

A Review of Insecticide Resistance Status in Botswana

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Abstract

For many decades, Botswana has been engaged in various malaria control activities that involved programmes that focused on the elimination of the malaria vector *Anopheles arabiensis*, by using DDT and pyrethroids. Despite the numerous and continuous application of these insecticides, studies have shown that there is susceptibility of this vector to DDT and pyrethroids in Botswana. Natural insecticides such as *Bacillus thuringiensis* and Spinosad, as alternatives to the use of chemicals, have shown to be effective against the eggs and larvae of DBM. Insect-resistant crop varieties were also found as alternatives in order to minimise insecticide resistance through the application of insecticides on insect infesting crops. The appearance of esterases B1 and A2–B2 in the Gaborone and Molepolole strains of *Culex*, respectively, indicates dispersion of these esterases through human migration.

Keywords: *Anopheles arabiensis*, insecticide resistance, esterases, pyrethroids

1. Introduction

1.1. What is insecticide resistance?

Resistance has been defined as 'the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species' [1] and also recently as a 'genetic change in response to selection by toxicants that may impair control in the field.' [2]. The resistance status also describes the decreased susceptibility of a pest population to a pesticide that was previously effective at controlling the pest, through natural selection with the genetic traits for resistance being passed on to subsequent offspring.

The development of insecticide resistance is dependent on the genetic composition of a species population. It is preadaptive, in the sense that in most cases the insecticide does not induce

any heritable changes but selects favourable mutations that allow the insect to survive the treatment [3]. The resistant strains thus develop through the survival and reproduction of individuals possessing one or more of many possible mechanisms that allow survival after exposure to an insecticide, each controlled by one or more resistance (R) genes. Strains tend to revert to susceptibility in the absence of insecticide exposure unless they have become homozygous for the R genes [1, 4, 5]. This makes insecticide resistance to be a natural phenomenon controlled by genes that bring about the biochemical, physiological, or behavioural changes on which resistance is based.

Resistance can shorten the long-term effectiveness of a particular insecticide against a species population prompting the use of an alternative insecticide to which there is no resistance; but unfortunately, this often becomes a temporary solution. The development of cross-resistance may occur to compounds within a group with a similar mode of action, especially if their metabolism and their target site attachment are very similar [6].

Cross-resistance can also occur between groups of insecticides with different modes of action and can be mediated by a single gene, i.e., be monogenic due to a single defense mechanism operating against two or more toxicants. It can also be polygenic where multiple mechanisms are available, which may not act equally against different toxicants. Since multiple resistances involve multiple genes, it can be a most serious development, should it occur in the field [6].

2. History of resistance to insecticides

Resistance to insecticides by insect pests has been documented for over 75 years, but its greatest impact has occurred during the last 30 years following the discovery and extensive use of synthetic organic insecticides [7]. Insect resistance was first observed in 1908, reported by Melander [8] in the San Jose scale insects *Aspidiotus perniciosus*, found to have become insensitive to lime-sulphur. Thirty years later, there were further reports of insect resistance towards numerous other pesticides.

When dichlorodiphenyl-trichloro-ethane (DDT) was introduced in 1946, insect resistance to the compound appeared quickly and worldwide. The first sign of resistance towards DDT was shown in the housefly *Musca domestica* [9]. Thereafter, cases from different locations were reported: *Aedes sollicitans* in Florida, *Culex pipiens* in Italy, and *Cimex lectularius* in Hawaii [1]. New insecticides that were later introduced did not last long with regard to their usage as the number of species showing resistance to one or more toxicants doubled every six years between 1948 and 1983 [10].

A number of resistant species are also reported in other agriculturally important orders such as Lepidoptera (67 species, representing 15%), Coleoptera (66 species, representing 15%), Acarina (58 species representing 13%), Homoptera (46 species, representing 4%), and Heteroptera (20 species, representing 4%) [11]. However, studies have shown that resistance develops faster in insects with many generations per year rather than only one, at higher selection pressures than at lower ones. Sawicki [12] noted that resistance is regarded as a problem only when the cost of control becomes unjustified or when excessive use of the control agent presents health and environmental hazards.

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3. Insecticide use in Botswana

The economy of Botswana is mainly dependent on agriculture and mining. The agricultural sector in Botswana covers both crops and livestock production. The industrial growth has brought about awareness in farming systems for both livestock and arable farming. However, this has also brought about an increase in the use of chemicals for pests on animals and crops. Insect pests are very important in crop production because they pose a serious problem to farmers. They reduce the yield and quality of crops resulting in lower prices for the crops and lower returns to the farmer.

Since the introduction and use of DDT in Botswana in the 1950s, other types of insecticides such as organophosphates, pyrethroids, and carbamates have been used in various aspects of agriculture. In crop production, these were used to target pests diamond back moth, aphids, locusts, and armyworms; fruit flies, diamond back moth, aphids, and leaf miners; American bollworm, diamond back moth, aphids cutworms, and bagrada bug, respectively [13].

From the results of experiments carried out during the 1970s in Botswana, carbaryl proved to be the most effective insecticide against *Helicoverpa armigera* on cotton, sorghum, and cowpea when tested against insecticides such as DDT, endosufan, monocrotophos, and tetrachlorvinphos [14]. However, the current pest management option for *H. armigera* in Botswana is the use of pyrethroids from recommendations based on the information from manufacturers and recommendations from other countries [14, 15].

Organophosphates are commonly used for the control of infestations of parasites for livestock and may also be applied as sprays and dips in form of acaricides. The same application of organophosphates has extended to spraying of the quelea birds by the Plant Protection Unit of the Ministry in Botswana [16].

Several control methods have been employed in the management of tsetse fly in Northern Botswana, and all of these methods involved the use of chemicals (Table 1). After the spraying of 2001 and 2002 in the Okavango Delta and 2006 in the Kwando-Linyanti systems, tsetse fly has not been found [17]. There were reports, however, that the deltamethrin spraying negatively affected other nontargeted organisms such as *Cyrtobagous salvinae*, with recovery in abundance after spraying [18].

Year of control	Method of control	Insecticide used
1960–1972	Residual ground spraying	DDT
1970–1990	Nonresidual aerial spraying	Endosulfan and pyrethroids
1990–2000	Traps and targets	Deltamethrin
2000 onwards	Aerial spraying	Deltamethrin

Source: Ingram [17].

Table 1. Insecticides used for the different control methods for tsetse fly.

4. Insecticide resistance studies in Botswana

4.1. Mosquitoes

4.1.1. Esterases in Culex mosquitoes

The global spread of resistant genes acts as an example of evolution in action showing how selective forces, genetic variability, gene flow, migration, and life history can interact to produce changes in gene frequency. Two types of esterases are known and coded for loci est-A and est-B, corresponding to the production of esterases A and B, respectively [19]. These two elevated esterases have been shown to be overproduced as a result of amplification. This being said, several copies of one gene found on the same genome of the structural genes coding for them may happen in isolation or together [20, 21]. However, this is the major mechanism associated with organophosphate resistance in Culicine mosquitoes [22]. The association of these esterases has been found to be globally widespread in *Culex pipiens* complex mosquitoes, with highly active esterases A2 and B2 being shown to be strongly associated with organophosphate resistance in strains of *Culex quinquefasciatus* from California [23], West Africa [24], Kenya [25], Thailand and South Africa [26], and Vietnam [27]. Esterase B1 has been linked to North America and the Far East in *Culex* mosquitoes collected in Foshan [28]. Resistance by this massive overproduction of these esterases is conferred in a way that enzymes are able to detoxify the ester-based organophosphorus insecticides by hydrolysis to produce a nontoxic ionic metabolite. At the same time, the increased production of esterases can effectively sequester the insecticide and prevent it from reaching its target site [29].

A study was conducted in order to establish the concept of migration and the widespread of these esterases and whether they are present in mosquitoes sampled in Botswana. Their presence will be a clear indication of the possibility of resistance demonstrated by the mosquitoes due to the selection pressure from the use of insecticides.

The mosquito larvae were collected from two areas in the southern part of Botswana: Gaborone and Molepolole. The areas are at least 50 km apart and have different economic activities. Gaborone is a city whilst Molepolole is a village. Single larvae at third and fourth instar and the adults were used for the experiment. The adults were identified as that of *Culex* specie from both collection sites. The presence of esterases was determined by subjecting each homogenised larvae and adult to a nondenaturing 7.5% PAGE gel to reveal the esterase bands.

Esterases identified as A2–B2 were revealed in the Molepolole strain, in both the adults and the larvae (Figure 1)

Esterase activity was determined by carrying out esterase and protein assays on the same homogenates of the single larvae and adults. The method used was described by Callaghan [30]. The results showed that esterase activity also varied in the larval and the adult stages for these mosquitoes from the two areas. In the Gaborone strain, the esterase activity for the *Culex* larvae was up to 50 μmol of β-naphthol per microgram of protein, whilst that of the adults ranged from 101 to 150 μmol of β-naphthol per microgram of protein. The results revealed that esterase activity increased in almost 35% of the population tested upon reaching the adult

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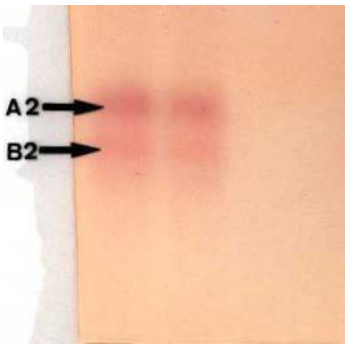
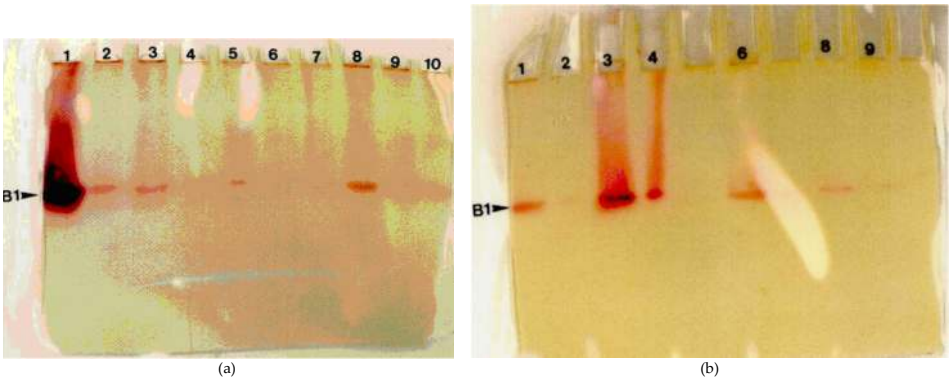


Figure 1. Gel showing the presence of A2B2 esterases from the adult Molepolole strain of *Culex* mosquitoes. The A and B esterases preferentially hydrolyse α- and β-naphthyl acetate, respectively. In the Gaborone strain, both the adults and the larvae revealed the presence of one esterase band B1, which preferentially hydrolysed β-naphthyl acetate (Figure 2a and b).



(a) (b)

Figure 2. (a). Gel showing the presence of B1 esterases from the adult Gaborone strain of *Culex* mosquitoes. (b). Gel showing the presence of B1 esterases from the larvae of the Gaborone strain of *Culex* mosquitoes.

stage. However, contrary to the results of the Gaborone strain, the esterase activity was found to be high at the larval stage.

The origin of the amplified A2–B2 esterases is yet to be identified. The appearance of resistance to organophosphates among populations of *C. pipiens* complex mosquitoes in Africa was reported in 1967 [31] and in Asia around 1968 [32]. On the basis of earlier reports of organophosphate resistance, Africa is thought to be the origin of amplification, but the first use of organophosphates in the continent is not well documented. According to Raymond et al. [33], the occurrence of overproduced esterase B1 is relegated to Asia and North America, but not in Africa. The distribution of B1 unlike the A2 and B2, which have been shown to be closely linked, has not yet been studied to elucidate the cause of its geographical spread and importance in organophosphate insecticide resistance [20, 34]. However, the presence of B1 esterases

as shown in the *Culex* mosquitoes collected from Molepolole indicated that esterase B1 has slowly found its way into Southern Africa.

The distribution of A2–B2 esterases in Africa, Asia, and North America may have been attributed to migration events [33]. This spread was also inevitable in the city of Gaborone. Botswana shares borders with South Africa where the presence of A2–B2 has been reported in organophosphate resistant strain of *Culex pipiens* [26]. This spread could have also been due to frequent migration of people between the two countries through visits, tourism, and trade. It is also not surprising that the spread of esterase B1 found itself into every town and village in the country through the Asian and Chinese traders, where only esterase A2–B2 was known to be dominant, such as in Africa. Thus, the presence of esterase B1 in Gaborone is as a result of migration of the esterase genes from areas of known prevalence such as North America, Asia, and China. Gaborone is an urban area where domestic and industrial pesticide spraying is randomly carried out. The high esterase activity displayed by the adults in the Gaborone strain may have been acquired initially at the larval stage and increased in the adults due to increase in the resistance from the kill of these chemicals.

The study has also shown that the possibility of resistance in mosquitoes is not restricted to one developmental stage. However, the esterase patterns in the developmental stages were the same, indicating that the same esterase genes are responsible for resistance throughout development from the larvae to adult stages, in both strains. Reasons for the increase in esterase activity in the larvae of Molepolole strain could be attributed to the fact that the area is a village within which there are farms lands and rearing of livestock. There is an extensive amount of agricultural practices whereby the application of insecticides on crops or acaricides on livestock is bound from time to time. These may have found their way into the nearby streams and gutters, which make good breeding sites for the mosquitoes.

4.1.2. Susceptibility tests on malaria vector, Anopheles arabiensis

Malaria is distributed in the northern part of the country, and this is a disease that is of public health priority to the government of Botswana, as it accounts for over 95% of malaria cases in Botswana [36]. *A. arabiensis* is the main malaria vector, and studies conducted in 2006 revealed that the species is distributed in all malaria areas of Botswana.

In order to reduce malaria transmission, the government of Botswana has engaged in what is called integrated vector management (IVM), which involves the utilisation of different interventions, including environmental management, safe, careful, and thoughtful use of insecticides. One such intervention is the indoor residual spraying (IRS) of insecticides, which goes back to the 1940s when spraying of human dwellings was initiated [37]. In the 1950s, the use of diethyl-dichloro-trichloroethane (DDT) started in Botswana for the malaria vector control using IRS [35]. In 1997, Botswana then introduced insecticide-treated nets (ITNs) to complement IRS as part of the IVN initiative [38]. Between 1971 and 1973, fenitrothion, which is an organophosphate, briefly replaced DDT. However, due to the poor efficacy of fenitrothion, DDT was reinstated as the main insecticide to serve together with IRS as Botswana's principal vector control intervention against malaria.

The WHO global strategy for the Malaria control is to break the malaria parasite transmission by using indoor residual spraying or pyrethroid impregnated materials such as bed nets. It is during such programmes that the annual vector susceptibility studies are carried out in Botswana.

Similar studies were conducted [39] to confirm the presence of pyrethroid resistance among *Anopheles gambiae* from West Africa (Benin and Burkina Faso), Central Africa (Cameroon), and *A. arabiensis* from Southern Africa (Botswana). WHO test kits for resistance tests were used with the adult mosquitoes being subjected to exposure to permethrin, deltamethrin, and DDT. From the results, permethrin resistance were detected in Benin, Burkina Faso, and deltamethrin resistance was detected also in Cote d' Ivoire. Botswana, on the other hand, showed susceptibility of *A. arabiensis* towards permethrin and DDT. The results obtained by Coetzee [40] on the susceptibility status of *A. Arabiensis* in Botswana using the same three insecticides were found to be in agreement with the susceptibility tests conducted annually for vector susceptibility by the Ministry of Health in Botswana. Table 2 presents the summary of the results.

Insecticide	Source: Chandre et al. [39]	Source: Botswana National Strategic Plan 2006–2011 [36]
DDT	99.6	99.09
Permethrin	86.3	90.71
Deltamethrin	-	92.47

Table 2. Percentage susceptibility levels of *Anopheles arabiensis* towards insecticides.

Both studies have been able to show that the malaria vector *A. arabiensis* does not have an indication of resistance towards the insecticides used in Botswana as it is fully susceptible to DDT and pyrethroids. DDT still remains the most sensitive insecticide, when tested against pyrethroids. Baseline studies were carried out on insecticide resistance in five Southern African countries including Botswana. The results showed that there was also complete susceptibility of *A. arabiensis* to DDT and pyrethroids [40].

5. Studies on the use of alternatives to chemicals in Botswana

Application of insecticides indiscriminately on agricultural crops can reduce or kill the natural enemies of insect pests. Continuous use of insecticides as we have already seen can also induce the resistance development in the targeted pests as well as killing beneficial nontargeted organisms. However, the detriment can also extend to human health through dietary exposure of contaminated crops. This great concern has brought about the need for alternatives to chemical insecticides that can be safe to human and the environment and at the same time affordable to farmers. Most of these natural insecticides are derived from plants and botanical insecticides, and some are of microbial type.

5.1. Microbial and spinosyns

Reports in Botswana have indicated that most insect pests found on agricultural crops have been subjected to chemical control. Diamondback moth *Plutella xylostella*, which is a pest of cabbage, is one such example of insect pest whose control relies heavily on the application of pyrethroids. It has also been demonstrated that DBM quickly develops resistance to many new insecticides [41].

Studies were conducted using *Bacillus thuringiensis* (*Bt*) [41] and Spinosad [42] as alternatives to insecticides to demonstrate their efficacy on the diamondback moth (DBM). *Bt* is a soil dwelling bacterium and is largely used in agriculture worldwide. It is a natural insecticide that produces crystals protein (cry proteins), which are toxic to many species of insects but nontoxic to humans.

Spinosad is derived by fermentation from the soil actinomycete and is effective by both contact and ingestion to numerous insect species [43]. Bioassays using both natural insecticides were carried out on the eggs and 2nd instar larvae of DBM. The results using *Bt* indicated that *Bt* was effective against both the eggs and the larvae, whereas spinosad was shown to be more effective against the eggs than against the larvae. These results were able to demonstrate that both natural insecticides used in the experiments can achieve effective control of the developmental stages: eggs and larvae of DBM. However, more bioassays still remain to be done on other insect pests that cause damage to various agricultural crops commonly grown in Botswana.

5.1.1. Resistant crop varieties

One other option to using insecticides on crops is to plant crop varieties that are found to be insect resistant. However, resistant varieties are usually only resistant to one or a limited number of insect pests. Genetic engineering has been able to allow the transfer of desired genes from one species to another, resulting in a quicker development of pest-resistant varieties or transgenic crops. On the evaluation of nine cabbage varieties for resistance to the cabbage aphid, Munthali [44] concluded that the most resistant cabbage variety would be the one that has a combination of low aphid numbers and low percentage of damaged leaves per plant. Notwithstanding, the use of these partially resistant varieties would also be recommended for use in combination with a low dose of insecticide.

6. Conclusion

The levels of resistance in the two strains of Gaborone and Molepolole for both esterases B1 and A2–B2 are yet to be elucidated by carrying out bioassays against the susceptible strains. This approach will help to determine whether there is any correlation between esterase levels and insecticide resistance in these strains. This will also give an indication to the kind of resistance mechanism that may be conferred in these strains. It is at the DNA level that we can be able to trace the origin and the migration path of these esterases into Botswana.

The continuous use of DDT and pyrethroids on ITNs and IRS has shown that *A. arabiensis* is susceptible to both insecticides. However, there have been reports of DDT resistance in South Africa (ANVR, 2005). Monitoring of the current susceptibility status of the malaria vector *Anopheles arabiensis* using other insecticides should be encouraged as Botswana continues with the campaign to eradicate malaria.

Despite the extensive use of insecticides in the agricultural sector in Botswana, it is encouraging that research is focussing on using alternative insecticides that would not pose any threat to the environment and humans in any way. This way the agriculture and health sectors can be able to manage the evolution of insecticide resistance in insect pests of crops and insect vectors of diseases, respectively.

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