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Insecticide resistance in *Anopheles gambiae sensu lato* (Diptera: Culicidae) across different agroecosystems in Niamey, Niger

Noura Mamane Salé[1,2*]; Rabiou Labbo[2]; Ibrahim Maman Laminou[2]; Ibrahima Issa Arzika[2]; Ali Djibo Souley[2]; Wilfried Zoulkifouly Hounkarin[2]; Halima Zamanka Naroua[2]; Amadou Soumana[2]; Abdoul-Aziz Maiga[3]; Ronan Jambou[2,4] & Ali Doumma[1]

[1]Faculté des Sciences et Techniques, Université Abdou Moumouni de Niamey, Niger
[2]Unité Paludologie et Entomologie Médicale, Centre de Recherche Médicale et Sanitaire, BP10887, Niamey Niger
[3]Université Joseph KI-ZARBO, Ouagadougou, Burkina Faso
[4]Département Santé Globale, Institut Pasteur, Paris, France

**Corresponding author*, E-mail: *mamanesalenoura@gmail.com*

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ABSTRACT

Malaria vector control in Niger is currently based on the distribution of insecticide treated nets. However, vectors resistance to insecticides represents a major threat to the current national strategy against malaria. This study aims to characterize the impact of agroecosystems on insecticide resistance in *Anopheles gambiae s.l.* at Niamey. Larvae collected were reared until emergence. Adults aged 2-5 days were used to assess susceptibility to insecticides (pyrethroids, DDT and bendiocarb) after pre-exposure to piperonyl butoxide (PBO) synergist according to WHO protocols. PCRs were performed to identify the sibling species of *An. gambiae* complex and characterization resistance mutations (*Kdr* and *ace-1*). Overall, An. gambiae s.l. was resistance to pyrethroids and DDT (mortality rates from 1% to 55%) and susceptible to bendiocarb at most sites. Pre-exposure to the PBO synergist resulted in partial restoration of pyrethroid susceptibility. Two species of *An. gambiae* complex were found: *An. arabiensis* and *An. coluzzii.* The presence of *An. coluzzii* was strongly correlated with agricultural practices (99% in rice cultivation sites). *Kdr* mutations were found at all sites with *kdr-w* ranging from 45% to 70% in mosquitoes collected in unirrigated and rice field, respectively, and kdr-e found at 37% to 47% at each type of site, respectively. The *ace-1* mutation was detected at low frequency (1%) and only from two rice cultivation sites. The high levels of pyrethroid and DDT resistance detected in Niamey had a strong link with rice cultivation, shown that agriculture is a driver of resistance that can compromise control malaria efforts.

Keywords: *Anopheles gambiae* sensu lato, insecticide, resistance, agroecosystem, vector control, Niamey, Niger..

RÉSUMÉ

Résistance aux insecticides d'*Anopheles gambiae* sens large (Diptera: Culicidae) dans différents agroécosystèmes de Niamey, Niger

Au Niger, La lutte contre les vecteurs du paludisme repose essentiellement sur l'utilisation de moustiquaires imprégnées. Cependant, la résistance des vecteurs aux insecticides constitue une menace pour cet élan. Cette étude vise à caractériser l'impact des agroécosystèmes sur la résistance d'*Anopheles gambiae* s.l. aux insecticides à Niamey. Des larves collectées ont été élevées jusqu'à l'émergence. Les adultes âgés de 2 à 5 jours ont été utilisés pour évaluer la sensibilité aux insecticides (pyréthrinoïdes, DDT et bendiocarb) et la préexposition au synergiste pipéronyl butoxide (PBO) suivant le protocole OMS. Des PCRs ont été réalisées pour déterminer l'espèce du complexe et les mutations (*Kdr* et *ace-1*). *An. gambiae* s.l. était résistant aux pyréthrinoïdes et DDT (1% à 55% de mortalité) et sensible au bendiocarbe dans la plupart des sites. Une préexposition au PBO a permis de restaurer partiellement la sensibilité aux pyréthrinoïdes. Deux espèces du complexe *An. gambiae* s.l. ont été trouvées: *An. arabiensis* et *An. coluzzii*. La présence d'*An. coluzzii* était fortement corrélée aux pratiques agricoles (99% dans les sites rizicoles). Des mutations *kdrs* ont été trouvées dans tous les sites, allant de 45 % dans les sites non irrigués à 70% dans les moustiques collectés dans les rizières avec *kdr-w* et de 35% à 47% pour *kdr-e* respectivement. La mutation *ace-1* a été détectée à une faible fréquence (1%) uniquement dans les sites rizicoles. Une forte résistance aux pyréthrinoïdes et au DDT a été détectée à Niamey, avec une forte implication de la riziculture dans les résistances observées qui pourraient compromettre les efforts faits pour contrôler le paludisme.

Mots clés : *Anopheles gambiae* sens large, résistance, insecticides, agroécosystèmes, Niamey,

INTRODUCTION

Malaria remains a public health problem in Niger, affecting mostly children under five years of age, and pregnant women Faye (2012). Niger is one of four African countries that account for more than 50% of malaria cases and deaths worldwide WHO (2022). Primary vectors, *Anopheles gambiae sensu lato (*s.l.) and *Anopheles. funestus* s.l., both found in Niger Czeher (2010) are long-lived and highly anthropophilic (Sinka et al., 2010). In addition, *An. gambiae* s.l. vectors have diverse bionomic traits, are highly adaptable, and have a high reproductive capacity (Lacey et Lacey, 1990; Jones et al., 2012) complicating control efforts. The sibling species of *Anopheles gambiae* complex documented in Niger include *An. gambiae s.s*., *An. coluzzii* and *An. arabiensis* - with *An. coluzzii* being the dominant vector in most parts of country (Ibrahim et al., 2014; Labbo et al., 2016; Soumaila et al., 2022). To fight against malaria, the National Malaria Control Program (NMCP) of Niger relies on the following strategies: (i) diagnosis and treatment of malaria with artemisinin-based combination therapies (ACTs), (ii) intermittent preventive treatment for pregnant women (IPTp), (iii) seasonal malaria chemoprevention (SMC) in children, and especially (4) vector control (Soumaila et al., 2022). The latter is a central and essential component of these malaria control strategies Organisation mondiale de la Santé (2012). For the past two decades, malaria vector control relies primarily on two WHO-recommended insecticide-based interventions: indoor residual spraying (IRS) and long-lasting insecticide-treated nets (LLINs) (Organisation mondiale de la Santé, 2012; Van den Berg et al., 2021). One of the major threats to gains achieved against malaria with insecticide-based vector control is insecticide resistance (World Health Organization, 2018; Aikpon et al., 2020). Several studies have reported on the impact resistance mechanisms have on the protective effectiveness of LLINs and IRS (Philbert et al., 2014; Matiya et al., 2019). In Niger, insecticide resistance was documented in 2007 - two years after the first LLIN mass distribution campaign (Czeher et al., 2008). Since then, studies have demonstrated the continued emergence and large-scale dispersion of resistance to insecticides at the national level (Soumaila et al., 2017; Ibrahim et al., 2019b; Soumaila et al., 2022). Though LLINs have been associated with insecticide resistance, IRS has only been implemented in Agadez district between 2013-2015, and its impact has not been evaluated (PLNP 2020 data not published). Six classes of insecticides, namely pyrethroids, organochlorines, organophosphates, carbamates, pyrrole and neonicotinoids are currently used in vector control (Van den Berg et al., 2021). Pyrethroids alone constitute 89.9% of insecticides in vector control in Africa (Van den Berg et al., 2021). They are the primary insecticides used for impregnation of mosquito nets (Organisation mondiale de la Santé, 2012; Van den Berg et al., 2021) due to their relatively low toxicity to mammals and their rapid lethal or knock down effect (Philbert al., 2014). These insecticides are also used in agriculture to control crop pests at a much larger scale (Mouchet 1988; Brévault et al., 2003). Some types of agriculture are associated with high mosquito densities (Gimonneau et al., 2012), e.g. wetland areas used for rice and vegetable cultivation provide suitable larval sites for mosquitoes (Fodjo et al., 2018). In the agroecosystems of Niamey, the impact of the simultaneous and intensive use of insecticides in vector control and agriculture, on the emergence and spread of insecticide resistance in vectors remains to be explored. Similarly the mechanisms involved including mainly acetylcholinesterase inhibitor (*Ace-1*), targeting carbamates and organophosphate insecticides and voltage-dependent sodium channel mutations as *knock down resistance* (*kdr-west* and *kdr-east*) and cytochrome P450 monooxygenases targeting pyrethroids and organochlorines (Sparks et al., 2020). This study aims to characterize and monitor the spatial dynamics of *An. gambiae* s.l. susceptibility to selected insecticides and insecticide resistance mechanisms in different agroecosystems including rainfed, irrigated, rice-growing, and industrial areas (Ludovic and Philippe, 2012) in and around the city of Niamey, Niger.

MATERIALS AND METHODS

Typology and study site description

The study was conducted between October and December 2020 in Niamey, the capital of Niger, located at 13°31'17"S latitude and 2°26'19"E longitude. Niamey is subdivided into five districts, covering 250 km² area and includes 135 villages (Ludovic and Philippe, 2012). With an annual growth rate of 3.83, the population of the city of Niamey was estimated at 1.56 million inhabitants in 2019 with a density of 4876.3 inhabitants/km^2 (Lawali Dambo et Bachir Amadou Gaya, 2021). Climatic conditions are semi-arid with a relatively short rainy season extending over four months (June to September). The average annual rainfall is 550 mm with nearly 80% of the rainfall occurring during the months of July and August. Temperatures range from 41°C in April to 19°C in December Sanda (2010). The hydrological network is dominated by the river Niger, its tributaries, and semi-permanent pools. The study was conducted at six sites (or districts): Tondibiah, Goudel, Lamordé, Gamkallé, Banigoungou and Koira-Tegui (Fig 1). The first five sites are riverside neighborhoods. Tondibiah and Goudel are located upstream of the river and to the north of the city, Lamordé and Gamkallé in the center of the city, and finally Banigoungou is downstream and to the south.

Due to the difference in agricultural practices and their geographical position these sites do not have the same type of ecosystem: Lamordé and Banigoungou are rice-growing sites; Tondibiah, Goudel and Gamkallé are garden irrigated sites, Gamkallé is an industrial site, while Koira-Tegui is a non-irrigated site away from the river.

Collection of larvae

At each study site, several natural or anthropogenic larval sites were investigated for the presence of immature stage of anopheles. Mosquito larvae were collected from positive sites (presence of larvae) by the *dipping* method (Papierok et al., 1975), except in Koira-Tegui where Anopheles larvae where not found. Adult mosquitoes (collected by CDC light traps) was not collected enough to generate a colony to run bioassays. These samples, in addition to the pyrethrum spray collected (PSC) mosquitoes, were used for resistance genotyping. Anopheles mosquito larvae collected were transported to the insectary of Centre de Recherche Médicale et Sanitaire (CERMES) where they were reared to adulthood under controlled conditions of temperature 27±2°C and relative humidity 75±10%. Larvae were fed with Tetramin®. Pupae were sorted daily and placed in cages for emergence and the emerged adults were fed with a 10% sugar solution.

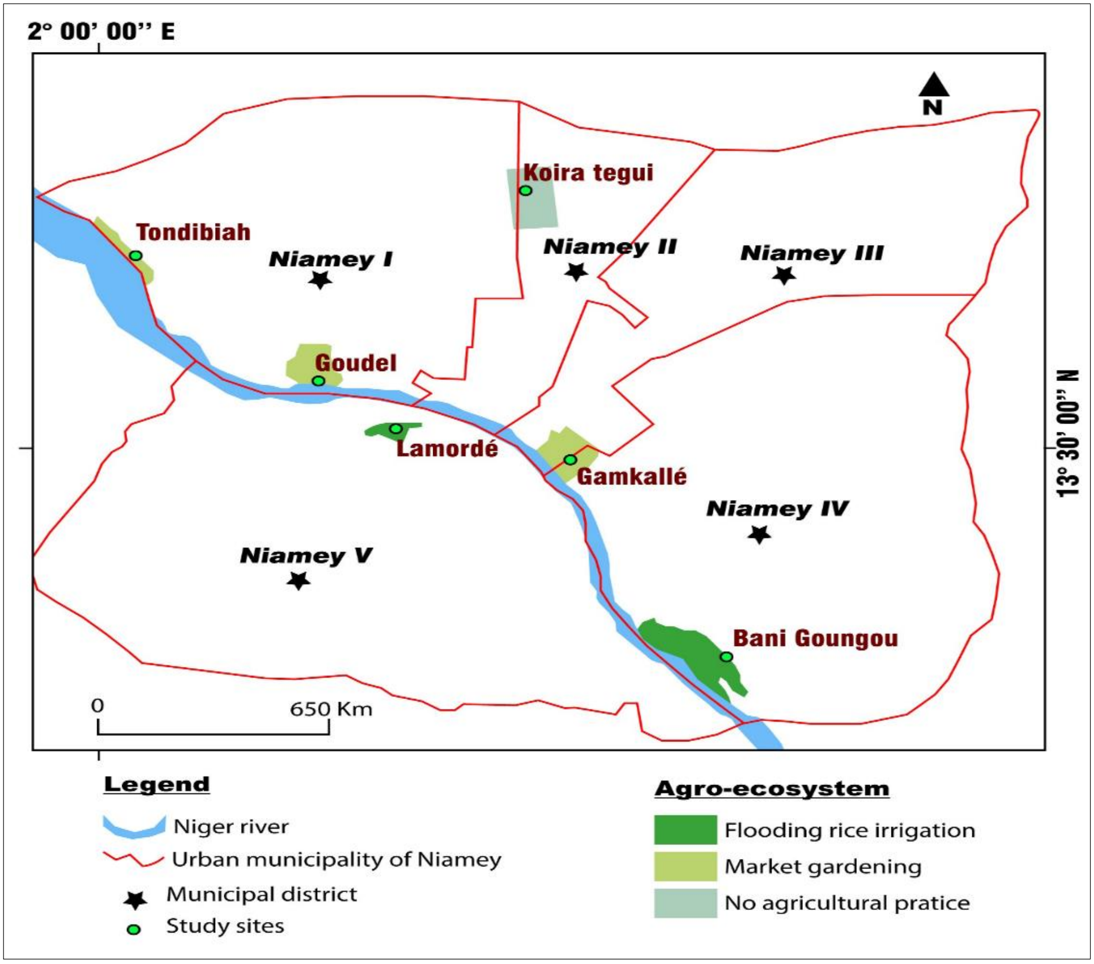

Figure 1. Map showing the collection sites

Insecticide bioassays

WHO Insecticide susceptibility and PBO synergist tests

Insecticide susceptibility tests were performed using 2 to 5 days old female *An. gambiae* s.l. against each selected insecticide according to the WHO standard protocol for resistance evaluation OMS (2017). The susceptible *An. gambiae* Kisumu strain (Yusuf et al., 2021) reared in the insectary of CERMES was used as reference.

A total of five insecticides were tested at the diagnostic dose (1X), i.e., deltamethrin 0.05%, alpha-cypermethrin 0.05% and permethrin 0.75% for pyrethroids; DDT 4% for organochlorines; and bendiocarb 0.1% for carbamates. Approximately, 100 mosquitoes were tested for each insecticide concentration per site. An additional 50 mosquitos were similarly tested using silicone oil treated, risella oil and olive oil treated papers for pyrethroids, DDT and carbamate and organophosphate, respectively, and used as controls. When multiple tests were conducted at the same time, from the same site, the two control tubes were used for all experimental evaluations. Bioassays conducted also included the synergist Piperonyl of butoxide (PBO). This was performed with the diagnostic doses of deltamethrin , permethrin and alpha-cypermethrin following the same WHO tube test protocol OMS (2017) to determine the involvement of monooxygenases (cytochrome P450) in pyrethroid resistance. The same batch of female mosquitoes was first pre-exposed to the synergist PBO (4%) for one hour prior to insecticide exposure as previously described. Mortality readings were done under the same conditions 24 h post-exposure and the results were compared to those obtained with the insecticide without PBO. Temperature and humidity were kept constant at 27±2°C and 75±10% respectively. When the mortality rate was between 5 and 20% in the control tube, the Abbott formula was applied to correct this mortality as follows:

$$\text{Corrected mortality} = \frac{(\%\ \text{observed mortality} - \%\ \text{control mortality})}{(100 - \%\ \text{control mortality})}\ x\ 100$$

Insecticide resistance intensity testing

To evaluate the resistance intensity against pyrethroids insecticides, further WHO tube bioassay tests were performed with 5 and 10 times diagnostic concentrations of deltamethrin (0.25% and 0.5% respectively), Alphacypermethrin (0.25% and 0.5% respectively) and permethrin (3.75% and 7.5% respectively) according to WHO standard protocol (OMS, 2017).

Molecular identification of *An. gambiae* s.l. species and genotyping of *Kdr-w*, *Kdr-e* qnd *Ace-I* mutations:

A total of 100 *An. gambiae* s.l., from dead and live insecticide-exposed mosquitoes, were randomly selected in Tondibiah, Goudel, Lamordé, Gamkallé and Banigoungou for molecular identification of the species of *An. gambiae* complex and insecticide resistance alleles. In Koira-Tégui, as we were unable to collect larvae, 94 dead *An. gambiae* s.l. not exposed to insecticides were used for species and resistance allele identifications.

DNA was extracted from whole mosquito by using the protocol of (Rudbeck et Dissing, 1998). Identification of species from the *An. gambiae* complex was performed using SINE-PCR as described by (Santolamazza et al., 2008). *Kdr-w* (L1014F) and *Kdr-e* (L1014S) mutations genotyping were carried out using the protocol of (Martinez-Torres et al., 1998) and *Ace-I* following the protocol described by (Weill et al., 2004).

Data analysis

Mosquito susceptibility status, or resistance intensity (low, moderate, or high) was assessed 24 hours after exposure and results were interpreted according to WHO criteria (OMS, 2017). For susceptibility tests with diagnostic concentrations, a population was considered susceptible when mortality after 24h was ≥ 98%; resistance is suspected when mortality was between 90 and 97%; and confirmed resistance was indicated by mortality < 90%. For intensity tests at 5X and 10X diagnostic concentrations, outcomes were defined by low resistance (mortality between 98-100% at 5X dose); moderate resistance (mortality < 98% at 5X, and > 98% at 10X dose); and high resistance (mortality < 98% at 10X dose).

Calculations of *Kdr-w*, *Kdr-e* and *Ace-1* allelic frequencies were done as follows:

$$F = \frac{(2RR + RS)}{2(RR + RS + SS)}$$

where RR indicates homozygous resistant, RS heterozygous resistant, SS homozygous susceptible and F the allelic frequency.

Chi-square test was used to compare the subspecies composition of the *An. gambiae* complex at each site and the t-test was used to compare mortality induced by insecticide alone and that induced by the synergist PBO-insecticide combination.

RESULTS

WHO insecticide susceptibility tests: diagnostic dose tests

For all three pyrethroids tested (deltamethrin, alpha-cypermethrin and permethrin), resistance was observed at each site with mean mortality below 60%, 45% and 15% respectively **(Fig 2-4, Table 1).** Furthermore, for all concentrations, the lowest mortality rates were recorded for deltamethrin, alpha-cypermethrin and permethrin at the Banigoungou rice site, located downstream of the city (5%, 7% and 2% respectively). The highest mortality rates were recorded at the irrigated sites of Tondibiah and Gamkallé, located upstream and at the center of the city (55%, 43% and 14% respectively) **(Table 1).**

Table 1. Mortality rates and susceptibility status per surveyed site

Site	Organochlorine		Pyrethroids						Carbamate	
	DDT 4%		Deltamethrin 0.05%		Alpha-cypermethrin 0.05%		Permethrin 0.75%		Bendiocarb 0.1%	
	% Dead (N)	Status	% Dead (N)	Status	% Dead (N)	Status	% Dead (N)	Status	% Dead (N)	Status
Tondibiah	9 (99)	R	55 (100)	R	43 (100)	R	10 (100)	R	100 (100)	S
Goudel	2 (98)	R	53 (88)	R	5 (100)	R	11 (100)	R	99 (98)	S
Lamordé	8 (98)	R	28 (96)	R	41 (93)	R	8 (91)	R	96 (100)	SR
Gamkallé	8 (100)	R	40 (94)	R	8 (99)	R	14 (100)	R	100 (98)	S
Banigoungou	1 (99)	R	5 (100)	R	7 (96)	R	2 (100)	R	97 (100)	SR
Kisumu	98 (100)	S	100 (100)	S	100 (98)	S	100 (98)	S	100 (100)	S

%Dead: Mortality rate; (N) : number of mosquitoes tested ; *R :* resistant ; *S* : susceptible ; SR: suspected resistance

Susceptibility to Bendiocarb was recorded against *An. gambiae* s.l. mosquitoes from Tondibiah, Goudel and Gamkallé, whereas populations from Lamordé and Banigoungou showed suspected resistance with mortality of 96% and 97%, respectively. For DDT, mortality rate below 10% was recorded at all sites (Table 1)

PBO-synergist tests

Pre-exposure to PBO significantly increased mortality rates of *An. gambiae* s.l. without full restoration of susceptibility across sites and according insecticides **(**Fig 2-4). For permethrin, mosquitoes from Tondibiah, Goudel, Lamordé, Gamkallé and Banigoungou demonstrated increased mortality after exposure to PBO from 10%, 11%, 8%, 14% and 2% respectively to 55% (*t*=4,01, *p*= 0.002), 41% (*t*=1.96, *p*=0.07), 38% (*t*=3.07, *p*=0.009), 57% (*t*=3.84, *p*=0.002), and 38% (*t*=4,01, *p*=0,002), respectively (Fig 2). With alpha-cypermethrin, PBO increased mortality rates in *An. gambiae* s.l. from Tondibiah, Goudel, Lamordé, Gamkallé and Banigoungou was recorded from 43%, 5%, 41%, 8% and 7% respectively to 67% (t=1. 72, p= 0. 1), 55% (t=4.44, p= 0.001), 88% (t=4.04, p=0.001), 53% (t=1.94, p=0.07), and 44% (t=3.37, p=0.005), respectively (Fig 3). For deltamethrin, mortality rates in *An. gambiae* s.l. from Tondibiah, Goudel, Lamordé, Gamkallé and Banigoungou also increased from 55%, 53%, 28%, 40%, and 5%, respectively to 85% (*t*=2.65, *p*= 0.02), 86% (*t*=2.51 *p*=0.02), 60% (*t*=3.67 *p*=0.003), 79% (*t*=3.86, *p*=0.002), and 48% (*t*=4.15, *p*=0.001) with PBO (Fig 4).

Intensity of insecticide resistance

Across all sites, results showed a high intensity of resistance to deltamethrin, moderate to alpha-cypermethrin at Tondibiah, Lamordé, and Banigoungou, and high in Goudel and Gamkallé**.** In Tondibiah and Lamordé, moderate resistance to permethrin was recorded, while at Goudel, Gamkallé and Banigoungou, resistance intensity was high (Fig 2-4).

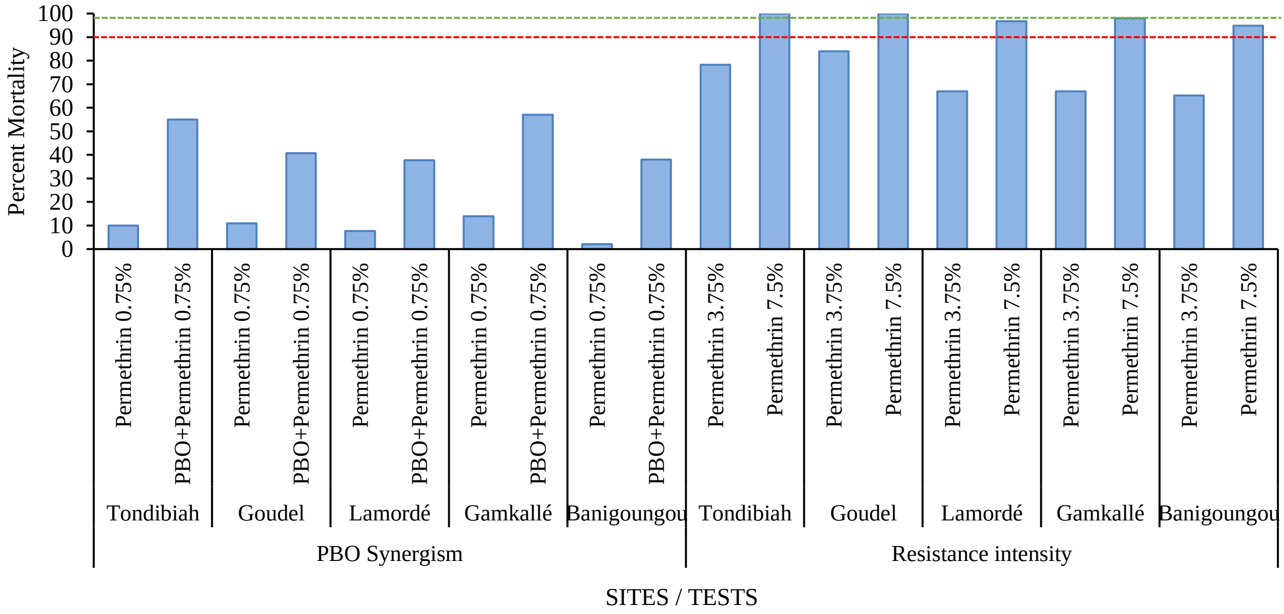

Figure 2. Susceptibility and synergism bioassay results and intensity tests of permethrin (3.75% and 7.5%) against *An. gambiae* s.l. across all sites surveyed. The green and red dotted lines represent the WHO susceptibility and resistance threshold respectively.

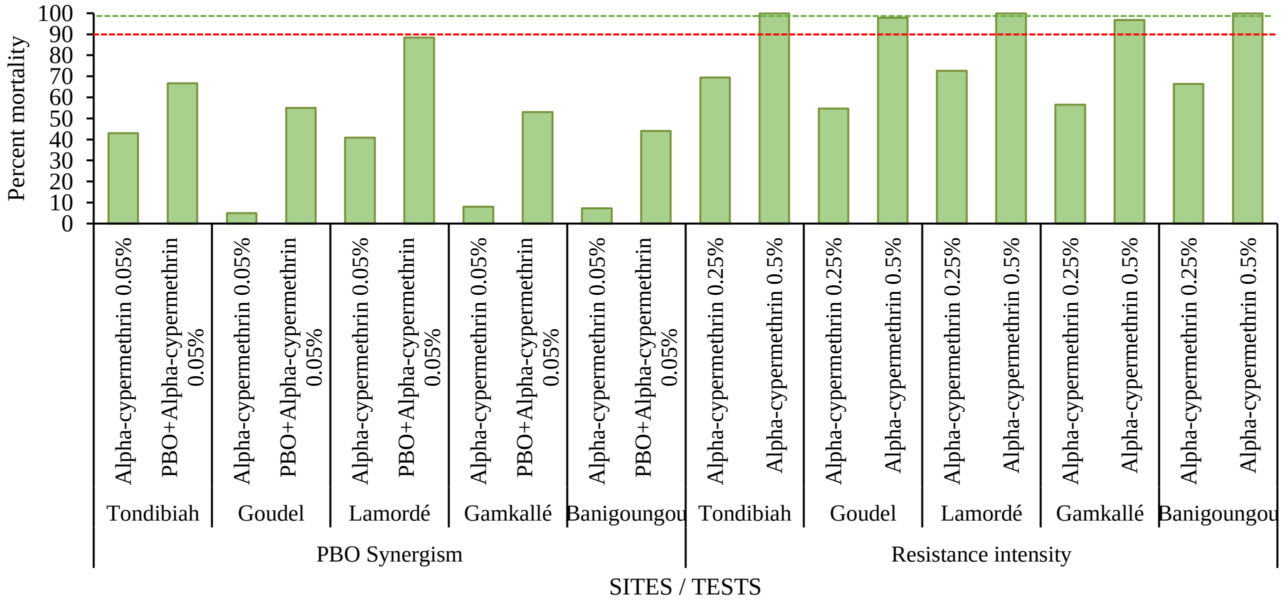

Figure 3. Susceptibility and synergism bioassay results and intensity tests of alpha-cypermethrin (0.25% and 0.5%) against *An. gambiae* s.l. across all sites surveyed. The green and red dotted lines represent the WHO susceptibility and resistance threshold respectively.

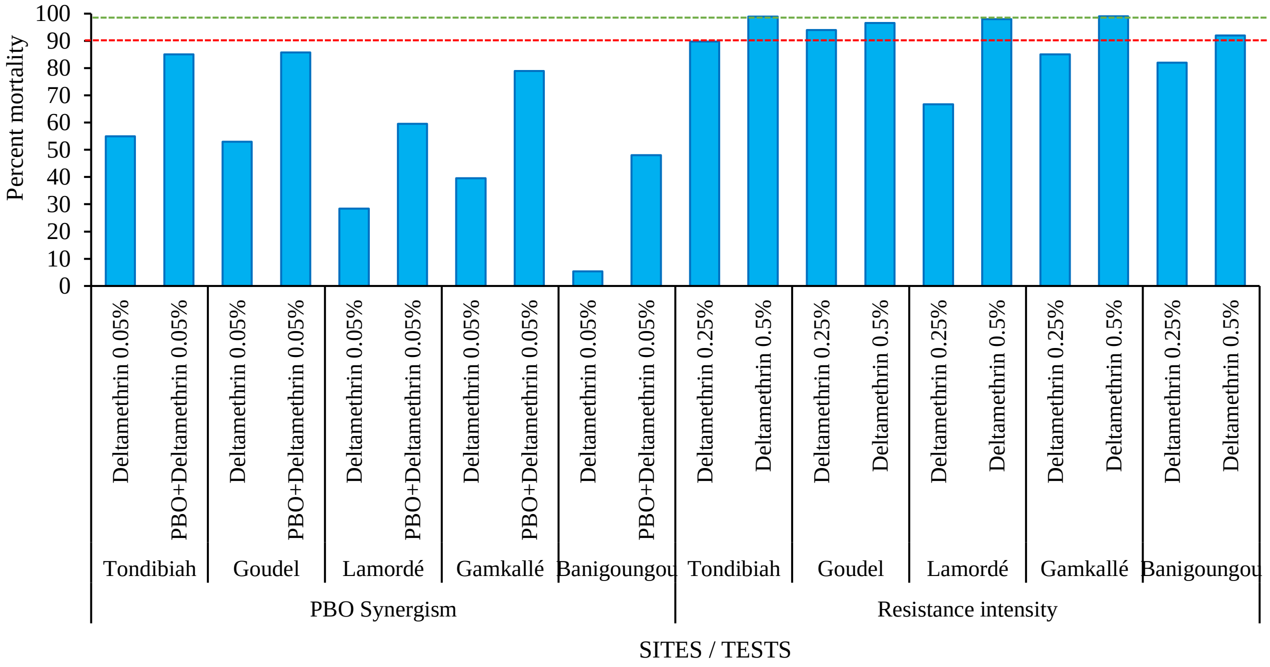

Figure 4. Susceptibility and synergism bioassay results and intensity tests of deltamethrin (0.25% and 0.5%) against *An. gambiae* s.l. across all sites surveyed. The green and red dotted lines represent the WHO susceptibility and resistance threshold respectively.

Molecular identification and spatial distribution of the sibling species of *An. gambiae* s.l.

After PCR analysis, all of 594 mosquitoes analyzed were successfully identified. Two species of the *An. gambiae* complex were identified: *An. coluzzii* and *An. arabiensis*. *Anopheles coluzzii* was the most represented with a proportion of 85% (n = 503/594) whereas *An. arabiensis* represented 15% (n = 91/594) $x^2=264,\ df=1\ p<0.0001$. *Anopheles coluzzii* was the primary species in Lamordé and Banigoungou rice cultivation sites, and represented 99% of collected mosquito. On the other hand, in the market gardening sites of Goudel and Gamkallé, the frequency of *An. arabiensis* reached 37% and 31%, respectively, compared to 63% and 69% for *An. coluzzii*, respectively. At Tondibiah and Koira-Tégui, the frequency of *An. arabiensis* was 17% and 4% respectively (Table 2).

Table 2: Species composition and spatial distribution of *An. gambiae* complex

Species		***An. coluzzii***	***An. arabiensis***
Site	Number Tested	N (%)	N (%)
Tondibiah	100	83 (83)	17(17)
Goudel	100	63(63)	37(37)
Lamordé	100	99(99)	1(1)
Gamkallé	100	69(69)	31(31)
Banigoungou	100	99 (99)	1(1)
Koira-Tegui	94	90 (96)	4(4)
Total	**594**	**503 (85)**	**91 (15)**

Spatial distribution of *Kdr-w* (L1014F), *Kdr-e* (L1014S) and *Ace-1* (G119S) mutations

The L1014F mutation was confirmed in *An. gambiae* s.l. at all sites but with variable frequencies. No difference was observed between *An. coluzzii* and *An. arabiensis* species. The lowest frequency was observed at the non-irrigated site of Koira-Tegui with an allelic frequency of F (R) 45%. The highest frequency was observed at the Banigoungou rice-growing site, located downstream of the town, with an allelic frequency F (R) 70%. The L1014S mutation was also observed at all sites in equally high proportions ranging from 27% at Tondibiah to 47% at Banigoungou. The G119S mutation was only detected in the two rice sites at Lamordé and Banigoungou at a low frequency of 1% each. In addition, no homozygous resistant individuals were observed at all sites**.** Both L1014F and L1014S have presented all three possible genotypes: SS (homozygote sensible), SR (heterozygote associating both susceptible allele and resistant allele), and RR (Homozygote resistant) (Table 3).

Table 3. Spatial distribution of *Kdr-w* (L1014F), *Kdr-e* (L1014S) and *Ace-1* (G119S) mutations

Site	**N**	***Kdr-w***				***Kdr-e***				***Ace-1***			
		RR	RS	SS	F(R)	RR	RS	SS	F(R)	RR	RS	SS	F(R)
Tondibiah	100	36	32	32	52%	7	40	53	27%	0	0	100	0%
Goudel	100	44	42	14	65%	18	34	48	35%	0	0	100	0%
Lamordé	100	50	24	26	62%	7	42	51	28%	0	2	98	1%
Gamkallé	100	42	35	21	61%	27	27	46	41%	0	0	100	0%
Banigoungou	100	51	37	12	70%	29	36	35	47%	0	2	98	1%
Koira-Tegui	94	25	35	34	45%	16	38	40	37%	0	0	94	0%

N : Number tested ; RR : homozygote resistant ; RS : heterozygote ; SS : homozygote susceptible ; F(R) : frequency of resistant allele

DISCUSSION

This study was undertaken to characterize the spatial distribution of phenotypic and genotypic insecticide resistance in *An. gambiae* s.l. populations in the city of Niamey. Susceptibility to bendiocarb was observed at all market gardening sites of Tondibiah, Goudel and Gamkallé, while suspected resistance was seen at the rice-growing sites of Lamordé and Banigoungou, downstream of the town. This resistance observed at the two sites may be attributed to urban pollutants and also the use of carbamate in the formulation of pesticides used to control crop pests in rice-growing areas. A similar study conducted in Chad also showed the susceptibility of *An. gambiae* s.l. to bendiocarb (Demba-Kodindo et al., 2022). In contrast, our results reported a high level of resistance of *An. gambiae* s.l. to pyrethroids (deltamethrin, permethrin, and alpha-cypermethrin), and organochlorine (DDT) at all sites. Resistance intensity bioassays confirmed the extent of the resistance in the different sites. This high intensity of resistance of *An. gambiae* s.l. to pyrethroids corroborates recent results in other parts of the Niger (Soumaila et al., 2017; Ibrahim et al., 2019b; Soumaila et al., 2022). Similar results were recently reported in Benin (Sagbohan et al., 2022), Côte d'Ivoire (Kouassi et al., 2020; Kouadio et al., 2023), and in Chad (Ibrahim et al., 2019a). Nonetheless, the lowest mortality rates recorded at Banigoungou may be related to the strong selection pressure applied through the wide distribution and use of long-lasting insecticide-treated net (LLIN, 100% coverage in this area, according to data form the Ministry of health), the large use of pesticides in rice cultivation and the urban pollutant discharged into the river. Indeed, LLIN coverage has already been associated with pyrethroid resistance of *An. gambiae* s.l. in Niger (Czeher et al., 2008). Excessive use of insecticides for crop protection has been mentioned as one of the main factors of selection pressure for mosquito resistance - especially in *An. gambiae* s.l. in West Africa (Diabate et al., 2002; Akogbeto et al., 2005; Yadouleton et al., 2009; Tia et al., 2017; Chabi et al., 2018; Salako et al., 2018; Orondo et al., 2021), specifically *An. coluzzii*, which tends to prefer agricultural areas, especially rice-growing areas, as larval sites. Mosquito larvae in rice fields undergo selection pressure from agricultural pesticides leading to the emergence of a resistant adult strains (Akogbeto et al., 2005). In addition, some urban sites contain pollutants of various origins including garbage, that have already been associated with the emergence of insecticide resistance (Antonio-Nkondjio et al., 2011). The mortality of *An. gambiae* s.l. to the diagnostic concentrations of pyrethroids alone was significantly lower ($p<0.05$) after pre-exposure to the synergist PBO. Similar results have already been observed in Niger (Ibrahim, et al., 2019b; Soumaila et al., 2022), and in countries such as Chad (Ibrahim et al., 2019a) and Côte d'Ivoire (Kouassi et al., 2020). This trend suggests the involvement of an increase in the activity of monooxygenase in the cytochrome P450 which inhibit the toxicity of insecticides (Cisse et al., 2015; Ntonga-Akono et al., 2017). The study revealed the presence of two species of the *An. gambiae* complex, *An. coluzzii* and *An. arabiensis,* with a high predominance (99%) of *An. coluzzii,* especially in rice-growing sites. However, this species also lived in sympatry with *An. arabiensis* in the market gardening sites. Similar results have been reported by many authors (Yadouleton et al., 2009; Ibrahim et al., 2014; Cisse et al., 2015; Salako et al., 2018; Ibrahim et al., 2019b; Kouamé et al., 2023). The presence of *An. arabiensis* was also reported by Labbo et al. in Goudel in urban areas in 2016 (Labbo et al., 2016). The absence of *An. gambiae* in the surveyed sites could be attributed to the rainfall regime in Niger where rainfall frequencies are very irregular not allowing small natural sites to support the biological development of the species. Indeed, *An. gambiae* has a preference for natural and temporary sites created by rainfall (Gimonneau et al., 2012). Across sites, molecular analysis revealed highly variable allelic frequencies of the *Kdr-w* allele in *An. gambiae* s.l. It is the main mechanism involved in cross-resistance to pyrethroids and DDT in Niger (Czeher et al., 2008; Soumaila et al., 2017; Ibrahim et al., 2019b; Soumaila et al., 2022; Kouadio et al., 2023). The lowest frequencies were observed in Koira-Tegui and Tondibiah with 45% and 52% respectively while the highest frequency was observed in Banigoungou F=70%. These results support a strong genetic basis of the phenotypic resistance mentioned above. The lower level of *Kdr-w* allele frequency detected at Koira-Tegui, a non-riverine and non-irrigated site, may be due to a lower presence of pesticides and pollutants. The coverage rate of LLINs is also lower, as nuisance due to mosquitos is low.

Overall, these differences in the level of resistance of *An. gambiae* s.l. to pyrethroids observed between irrigated and non-irrigated sites (Orondo et al., 2021) were recently obtained in Benin (Aikpon et al., 2020; Sagbohan et al., 2022) and Cameroon (Antonio-Nkondjio et al., 2011), pointing out the major role of agriculture and pollution on the emergence and spread of resistance. Initially described in East Africa (Ranson et al., 2000), the *Kdr-e* (L1014S) mutation was present in all sites of Niamey during this study. This resistance mechanism is becoming more and more prevalent in many West African countries (Djègbè et al., 2011; Ibrahim et al., 2019a; Keïta et al., 2020; Gueye et al., 2020), and was reported for the first time in Niger in 2021 (Soumaila et al., 2022). The *Ace-1* (G119S) mutation was found at two of our sites but with a very low frequencies of 1% at each site, and no vector was homozygous for this mutation. This low frequency is in line with the sensitivity of *An. gambiae* s.l. to carbamates observed in Niamey. Several authors have highlighted the low frequency of the *Ace*-1 allele in the sub-region (Kwiatkowska et al., 2013; Cisse et al., 2015; Namountougou et al., 2019; Soma et al., 2021), however, the two sites where the mutation was found, are rice-growing sites, which pointed out again, to the role of agriculture practices in the spread of resistance. This involvement of rice cultivation in the resistance of *Anopheles gambiae* s.l. to carbamate was recently highlighted in Côte d'Ivoire (Kouadio et al., 2023). Indeed, as Niger is not officially an IRS country, there is no use or application of carbamate for vector control.

No significant difference in distribution of allelic frequencies of *Kdr-w* and *Kdr-e* mutations was found between *An. coluzzii* and *An. arabiensis* living in sympatry at our sites. This confirms that the variation observed between sites can be attributed to environmental selection. The occurrence and spread of these mutations in Niger, in addition to the existing ones, could further compromise the government's vector control efforts.

Conclusion

This study focused on members of the *An. gambiae* s.l. complex, the main malaria vectors in Niger. In Niamey, this complex was dominated by *An. coluzzii*, especially in suburbs of the town along the Niger river, devoted to urban farming. In this area of excessive agricultural insecticide implementation by farmers, the resultat showed susceptibility of vector populations to carbamates with a high resistance to DDT and pyrethroids and partial restoration of susceptibility due the synergist PBO. This high level of resistance varied from a site to another site. These results are important for NMCP which mainly used pyrethroids. The susceptibility of *An. gambiae* s.l. to carbamates is an alternative to be exploited to set up an IRS project for effective vector control. Sensitization of the farmers must be done about this problem and other methods must be implemented to control vectors in areas with high levels of resistance such as rice-growing sites in order to reinforce the existing vector control strategies and to preserve the effectiveness of the insecticides used.

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