



Insecticide resistance in field populations of the tomato fruitworm, *Helicoverpa armigera*, from Senegal

Serigne Omar SENE*, Etienne TENDENG, Mamadou DIATTE, Serigne SYLLA,
Babacar LABOU, Amadou Woory DIALLO and Karamoko DIARRA

Université Cheikh Anta Diop (UCAD), Laboratoire Production et Protection Intégrées en Agroécosystèmes
Horticoles - L2PIA, Faculté des Sciences et Techniques, Dakar, Sénégal.

*Corresponding author; E-mail: pape.o.sene44@gmail.com; Tel : +221 774410498

ABSTRACT

Monitoring of the evolution of insecticide resistance in the field is crucial to prevent pest control issues. The present study was conducted to assess insecticide resistance status of the fruitworm, *Helicoverpa armigera* (Hübner) (Lepidoptera, Noctuidae), the most destructive pest of field-grown tomato in Senegal. A sample of 11-15 field populations were monitored for their susceptibility to abamectin, deltamethrin, and profenofos, using a standard leaf-dip bioassay method. Resistance ratios ranged from 1- to 30-fold to abamectin (4/15 populations with RR>10), 7- to 112-fold to deltamethrin (11/12 populations with RR>10), and 1- to 29-fold to profenofos (3/11 populations with RR>10). This indicates that resistance evolution to deltamethrin was widespread among field populations of *H. armigera*. However, an increasing trend of resistance to deltamethrin was observed from the South to the North of Niayes. Susceptibility to abamectin and profenofos was generally high but showed that resistance might be evolving within some populations. In addition, signs of cross-resistance to abamectin were detected, suggesting possible metabolic resistance mechanisms already selected in pyrethroid-resistant populations. The recorded high levels of pyrethroids resistance are a concern for the control of *H. armigera* in Senegal as the country is being currently embarking into economic expansion of tomato cropping systems.

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Keywords: Insecticide resistance, pyrethroids, avermectins, OPs, *Helicoverpa armigera*, West Africa.

INTRODUCTION

In Senegal, vegetable production experiences an annual variation of 12%. This increase in production is linked to the increase in the area planted. The tomato is the most cultivated crop behind the onion and is one of the most consumed vegetables. Its production experiences an annual variation of around 9% and represents around 11.9% of the total vegetable production estimated at 1,212,911

tons for the 2017/2018 campaign (ANSD, 2019). In the Niayes, tomato crops are grown for fresh market throughout the year with a peak during the dry season, while in the “Walo”, the tomato is grown once a year mostly for industrial processing.

The tomato fruitworm, *Helicoverpa armigera* (Hübner) (Lepidoptera, Noctuidae), is a serious pest causing substantial damages to a wide range of field and vegetable crops

worldwide, including cotton, maize, sorghum, and tomato (Cunningham and Zalucki., 2014; CABI, 2016). It is a polyphagous pest (Tendeng et al., 2017; Diatte et al., 2018) which presents a broad spectrum of distribution. In West Africa, *H. armigera* is the most damaging pest of field-grown tomato crops (Umeh et al., 2002; Huat, 2006; Mailafiya et al., 2014). It has been reported in almost all West African country. Its biological and ecological traits such as high reproduction rate, polyphagy, high mobility, migratory flights, facultative diapause, and propensity to develop resistance to insecticides, make it difficult to control (Torres-Vila et al., 2003; Martin et al., 2005; Achaleke et al., 2009). In Senegal, extensive monitoring of a set of 98 tomato fields in the Niayes area from October 2012 to May 2014 indicated that *H. armigera* was the most destructive pest, with an occurrence of 92% in sampled fields (90/98) (Diatte et al., 2018).

Economic damage caused by *H. armigera* is very significant worldwide (Sharma, 2005). Losses caused in Africa are estimated at more than US \$ 5 billion annually despite application of pesticides (Sharma, 2005). Monetary losses are the result of monitoring and control costs, using insecticides, and the direct reduction of yield. Damage to high-value crops including tomatoes, has a high socio-economic cost. In Senegal, yield losses on tomatoes can be higher than 28% (Diatte et al., 2016). A positive relation between the number of insecticide applications and the incidence of *H. armigera* indicated that insecticide strategies were not effective (Diatte et al., 2018). Such control failure is likely due to the evolution of resistance in *H. armigera* field populations.

Resistance of *H. armigera* to a wide range of insecticides has been reported worldwide (Mironidis et al., 2013). Pyrethroid and organophosphate insecticides have been widely used by both cotton and tomato growers on account of their efficacy in controlling a wide range of pests at low doses and at extremely low cost (Badiane et al., 2015). As a

result, resistance to pyrethroids occurred during the mid-1990s in *H. armigera* populations from West Africa (Martin et al., 2000; Djihinto et al., 2012). Resistance led to the wide adoption of a new spraying program based on the temporary exclusion of pyrethroids (Martin et al., 2005). Field-evolved resistance to almost all the insecticides available for their control has been documented in other regions, including profenofos (Alvi et al., 2012; Qayyum et al., 2015) and abamectin (Alvi et al., 2012) in Asia. To date, no case of resistance to OPs or avermectins has been reported for *H. armigera* in Africa (Achaleke et al., 2009). The possible loss of effectiveness of insecticide molecules due to resistance is a major issue for both farmers and the industry. In addition, subsequent pest outbreaks might lead to generalized use of even more toxic insecticides, with unintended effects on human health, biodiversity within agroecosystems, particularly non-target species such as natural enemies of the pest species, and risk on safety of fresh tomatoes due to insecticide residues. In Senegal, *H. armigera* resistance status has been poorly documented and conventional insecticides continue to be used in vegetable growing areas, with sometimes low efficacy. According to Diarra (personal communication), *H. armigera* is insensitive to pyrethroids and organophosphorus in some areas of the Niayes and the Senegal River Valley.

The objective of the present study was to assess the susceptibility to commonly used insecticides (abamectin/avermectin, deltamethrin/pyrethroid, and profenofos/OP) of *H. armigera* populations in the main vegetable-growing area in Senegal (Niayes). Such monitoring should provide crucial information on the scope, variability and magnitude of insecticide resistance in tomato-growing areas in Senegal, for the sound design of adaptive areawide insecticide resistance management programs and effective pest control strategies.

MATERIALS AND METHODS

Insects sampling

Eleven populations of *H. armigera* larvae were collected from tomato fields over two dry seasons, from February 2014 to March 2015, in the main vegetable-producing area in Senegal, "Niayes" (Figure. 1, Table 1). The environment of Niayes is characterized by dunes and often flooded depressions, and the alternation of a short rainy season (July-September) and a long dry season (October-June). It is subdivided into three different eco-geographic sub-areas because of the existence of a climatic gradient and the variation of the soils encountered between the South and the North. The southern zone, slightly arid, is characterized by a Sudano-Sahelian climate and low hygrometric conditions (temperature, relative humidity), regulated by the sea. The ferruginous tropical soils, little leached are predominant. The central zone, moderately arid, is marked by a Sudano-Sahelian climate. It is dominated by hydromorphic soils of depressions very favorable to the development of market gardening. The highly arid northern zone is characterized by a Sahelian climate and high temperatures. Iso-humic red-brown soils are more important (Ndiaye et al., 2012).

Two additional populations were collected from tomato (Mbour) and cotton (Koussanar) fields out of the two above-mentioned tomato-growing areas. A minimum of 100 larvae (second to fourth instar) were randomly collected by walking through at least two plots of a particular crop in a zig-zag manner to get a mixed population from sampling locations.

Field-collected larvae were individually incubated in the laboratory at 27 ± 2 °C, $60 \pm 10\%$ RH, and a photoperiod of 14:10 (L: D) h, in 6-well culture plates (Fisher Scientific, France) containing cubes of artificial diet (Southland Products, USA). Resulting moths (from 25 to 141 per colony) had free access to 10% honey-water solution. Eggs laid on cheesecloth were collected daily. The laboratory susceptible strain of *H. armigera* was obtained from field collected populations

and reared in the laboratory for two years without any exposure to insecticides.

Insecticides

Insecticide solutions were prepared from technical-grade materials. Cypermethrin (97%) and profenofos (90%) were provided by SPIA (Dakar, Senegal), and abamectin (90%) by Senchim (Dakar, Senegal). Pyrethroids affect the sodium-potassium channel of target insect, whereas OPs act as inhibitors of acetylcholinesterase in the target insect (Ahmad et al., 2007). Abamectin (avermectin B1) is the major fermentation component of avermectins derived from a soil actinomycete microorganism (*Streptomyces avermitilis*) and act agonistically on GABA and glutamate-gated chloride channels. For each insecticide, a stock solution was prepared in a 100-ml glass vial by dissolving the active ingredient in 3 ml of ethanol (90%) and distilled water. Five to seven serially diluted solution of each active ingredient and one control solution with only 3 ml of ethanol (90%) and distilled water were prepared. Two droplets of surfactant (Triton X-100) were added to each solution.

Leaf-dip bioassays

Bioassays were conducted using a standard leaf dipping bioassay method adapted from The Insecticide Resistance Action Committee (IRAC) method No. 7 (IRAC, 2014). Five-centimeter cotton (*Gossypium hirsutum*) leaf discs were dipped into the test solutions for 10 s. Discs were then dried at room temperature for 30-60 min and placed in a Petri dish with adaxial side up. Five newly-moulted second-instar larvae from F1 laboratory cultures were released on to each leaf disc. Petri dishes were then wrapped with Parafilm to prevent leaf desiccation. Two to six replicates were used for each test and control solution. Treated larvae were kept at a constant temperature of 27 ± 2 °C, $60 \pm 10\%$ RH, and a photoperiod of 14:10 (L: D) h. Petri dishes were covered with a black cloth to avoid cannibalism (Ahmad, 2004). Mortality was observed 48 h later. Larvae were considered

dead when no coordinated response was obtained from touch stimulation with a blunt needle. A test was discarded when mortality in the control exceeded 20%.

Data analyses

Bioassay data were analyzed with XLSTAT Version 2018.1 (Addinsoft) using the 'Dose effect' module based on Finney's log-probit method (Finney, 1971). The LC_{50} (the concentration that kills 50% of the test population, expressed in $mg.l^{-1}$ of insecticide solution) and their respective 95% fiducial limits were calculated. Significant differences in the susceptibility of populations were

established by non-overlapping 95% fiducial limits. Resistance ratios (RR) were calculated as LC_{50} of field populations divided by LC_{50} of the susceptible strain (LAB). Resistance ratios >10 were considered as demonstrating field-evolved resistance (Che et al., 2013). Non-parametric Kruskal-Wallis tests were performed to compare insecticide resistance ratios among the three main areas: North, Centre and South of Niayes. Pairwise Pearson-correlation tests of log LC_{50} values of insecticides were done to assess cross-resistance of *H. armigera* populations to tested insecticides.

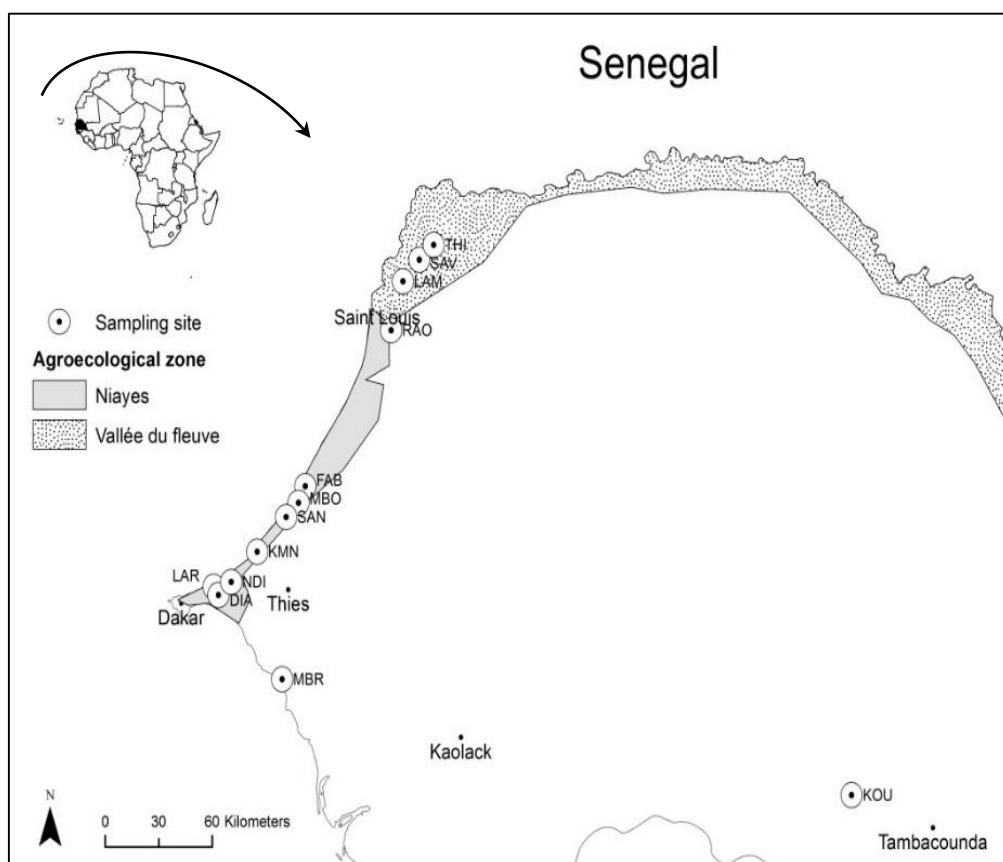


Figure 1: Field sampling of *Helicoverpa armigera* populations from Senegal. LAM (Lampsar), SAV (Savoigne), THI (Thilène), RAO (Rao), FAB (Fass Boye), MBO (Mboro), SAN (Santhié Ndong), DIA (Ndiakhirate), KMN (Keur Mbir Ndao), LAR (Lac rose), NDI (Ndiéguène), KOU (Koussanar), and MBR (Mbour).

Table 1: Location, collection date, and host plant of field-collected populations of *Helicoverpa armigera* tested in bioassays.

| Area | Location | Map code | Collection date | Host plant | Nb of pupae |
|--------|----------------|----------|-----------------|------------|-------------|
| North | Lampsar | LAM | Feb. 2014 | Tomato | 107 |
| Niayes | Savoigne | SAV | Mar. 2015 | Tomato | 55 |
| | Thilène | THI | Mar. 2015 | Tomato | 73 |
| | Rao | RAO | Febr. 2015 | Tomato | 71 |
| Centre | Fass Boye | FAB | Feb. 2014 | Tomato | 124 |
| Niayes | Fass Boye | FAB | Feb. 2015 | Tomato | 141 |
| | Mboro | MBO | Feb. 2014 | Tomato | 118 |
| | Mboro | MBO | Feb. 2015 | Tomato | 113 |
| | Santhié Ndong | SAN | Jan. 2015 | Tomato | 105 |
| South | Ndiakhirate | DIA | Jan. 2015 | Tomato | 121 |
| Niayes | Keur Mbir Ndao | KMN | Dec. 2014 | Tomato | 112 |
| | Lac rose | LAR | Jan. 2015 | Tomato | 117 |
| | Ndiéguène | NDI | Feb. 2014 | Tomato | 89 |
| Others | Koussanar | KOU | Oct. 2016 | Cotton | 121 |
| | Mbour | MBR | Mar. 2015 | Tomato | 78 |

Map code: see Fig. 1. Nb of pupae: number of pupae obtained from field-collected *H. armigera* larvae.

LAM (Lampsar), SAV (Savoigne), THI (Thilène), RAO (Rao), FAB (Fass Boye), MBO (Mboro), SAN (Santhié Ndong), DIA (Ndiakhirate), KMN (Keur Mbir Ndao), LAR (Lac rose), NDI (Ndiéguène), KOU (Koussanar), and MBR (Mbour).

RESULTS

The status of insecticide resistance to commonly used insecticides (abamectin, deltamethrin, and profenofos) was assessed on 11-15 field-collected populations of *H. armigera* (Figure. 2, Table 1). Resistance ratios ranged from 1- to 30-fold to abamectin (4/15 populations with $RR > 10$), 7- to 112-fold to deltamethrin (11/12 populations with $RR > 10$), and 1- to 29-fold to profenofos (3/11 populations with $RR > 10$) (Table 2). This indicates that resistance evolution to deltamethrin was widespread among field populations of *H. armigera*. For abamectin and profenofos, susceptibility was generally high but RR suggested that resistance might be evolving in some populations. No significant difference of RR was observed among

sampling areas for abamectin ($K = 1.70$, $df = 2$, $P = 0.427$) and profenofos ($K = 2.49$, $df = 2$, $P = 0.288$). However, an increasing trend of resistance to deltamethrin was observed from the South to the North of Niayes ($K = 6.16$, $df = 2$, $P = 0.042$). The population of *H. armigera* collected from tomato fields at Mbour (MBR) showed low susceptibility to both abamectin ($RR = 30$) and deltamethrin ($RR = 48$). The population sampled from cotton (KOU) showed high susceptibility to abamectin and profenofos ($RR \approx 1$), and moderate level of resistance to deltamethrin ($RR = 13$). Pairwise correlation of $\log LC_{50}$ values of insecticides showed a significant correlation between abamectin and deltamethrin ($P = 0.025$), indicating cross-resistance.

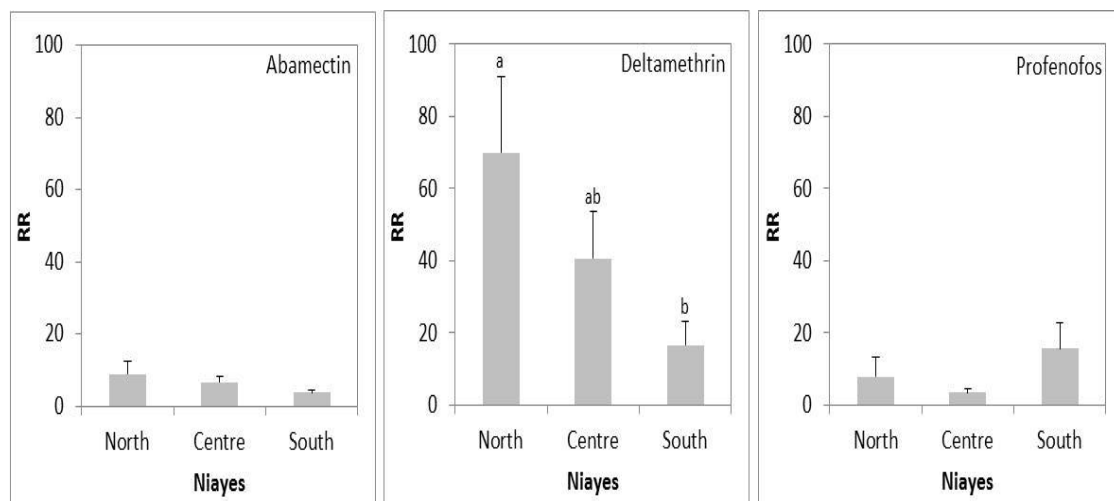


Figure 2: Resistance patterns of *Helicoverpa* populations to abamectin, deltamethrin and profenofos (2014-15) along the Niayes area in Senegal.

Table 2: Susceptibility of field populations of *Helicoverpa armigera* to three commonly used insecticides (abamectin, deltamethrin, and profenofos) in tomato-growing areas in Senegal.

| Insecticide | Population | Slope \pm SE | LC ₅₀ (mg.l ⁻¹) | 95% FL | RR | N |
|-------------|------------|-----------------|---|-----------|------|-----|
| Abamectin | LAB | 3.41 \pm 1.69 | 0.46 | 0.00-0.79 | 1.0 | 210 |
| | LAM | 3.70 \pm 1.73 | 3.48 | 0.07-5.33 | 7.6 | 70 |
| | SAV | 1.19 \pm 0.40 | 8.36 | 4.31-39.2 | 18.2 | 70 |
| | THI | 1.16 \pm 0.40 | 4.02 | 1.56-9.29 | 8.7 | 70 |
| | RAO | 2.49 \pm 0.58 | 0.73 | 0.29-1.09 | 1.6 | 210 |
| | FAB-14 | 2.03 \pm 0.47 | 4.64 | 2.37-7.26 | 10.1 | 105 |
| | FAB-15 | 1.65 \pm 0.38 | 2.39 | 1.33-3.59 | 5.2 | 105 |
| | MBO-14 | 1.32 \pm 0.22 | 2.57 | 1.42-3.95 | 5.6 | 175 |
| | MBO-15 | 3.53 \pm 0.74 | 5.18 | 3.93-6.26 | 11.3 | 210 |
| | SAN | 1.23 \pm 0.34 | 0.35 | 0.03-0.81 | 0.8 | 210 |
| | DIA | 2.27 \pm 0.37 | 1.58 | 1.10-2.04 | 3.4 | 210 |
| | KMN | 3.69 \pm 0.70 | 2.59 | 1.87-3.20 | 5.6 | 190 |
| | LAR | 1.63 \pm 0.32 | 1.75 | 0.90-2.57 | 3.8 | 210 |
| | NDI | 0.61 \pm 0.29 | 0.98 | 0.00-4.49 | 2.1 | 70 |

| | | | | | | |
|--------------|--------|-------------|--------|-----------------------|-------|-----|
| | KOU | 1.40 ± 0.71 | 0.11 | 0.00-0.48 | 0.2 | 210 |
| | MBR | 2.57 ± 0.41 | 13.7 | 10.9-19.1 | 29.8 | 210 |
| Deltamethrin | LAB | 1.13 ± 0.17 | 14.9 | 10-23 | 1.0 | 210 |
| | LAM | 2.23 ± 0.97 | 696.0 | 464-8394 | 46.7 | 75 |
| | SAV | 1.50 ± 0.67 | 766.3 | 425-18318 | 51.4 | 60 |
| | THI | 1.17 ± 0.56 | 1667.2 | 767-5.10 ⁹ | 111.9 | 60 |
| | FAB-15 | 1.22 ± 0.53 | 678.3 | 364-5667 | 45.5 | 90 |
| | MBO-15 | 3.30 ± 0.64 | 897.4 | 738-1081 | 60.2 | 210 |
| | SAN | 1.38 ± 0.43 | 241.8 | 66-372 | 16.2 | 175 |
| | DIA | 1.30 ± 0.34 | 159.4 | 41-266 | 10.7 | 210 |
| | KMN | 3.03 ± 0.73 | 541.26 | 438-746 | 36.3 | 190 |
| | LAR | 0.65 ± 0.32 | 103.1 | 0-297 | 6.9 | 210 |
| | NDI | 1.18 ± 0.51 | 184.1 | 8-359 | 12.4 | 70 |
| | KOU | 1.37 ± 0.33 | 200.0 | 74-310 | 13.4 | 210 |
| | MBR | 1.59 ± 0.34 | 710.1 | 509-996 | 47.7 | 210 |
| Profenofos | LAB | 2.62 ± 0.57 | 1.06 | 0.66-1.38 | 1.0 | 150 |
| | LAM | 2.07 ± 0.69 | 19.4 | 4.55-30.4 | 18.3 | 75 |
| | SAV | 2.99 ± 0.80 | 2.83 | 1.48-4.28 | 2.7 | 50 |
| | THI | 1.03 ± 0.42 | 2.69 | 0.20-7.74 | 2.5 | 70 |
| | FAB-15 | 2.12 ± 0.43 | 1.89 | 1.19-2.66 | 1.8 | 105 |
| | MBO-15 | 2.15 ± 0.29 | 5.70 | 4.52-7.43 | 5.4 | 210 |
| | SAN | 3.70 ± 0.88 | 3.29 | 1.89-4.24 | 3.1 | 150 |
| | KMN | 2.38 ± 0.39 | 30.2 | 22.8-43.5 | 28.5 | 190 |
| | LAR | 1.21 ± 0.45 | 3.51 | 1.69-5.50 | 3.3 | 210 |
| | NDI | 1.49 ± 0.54 | 16.0 | 3.04-27.21 | 15.1 | 70 |
| | KOU | 2.99 ± 0.45 | 1.53 | 1.18-1.87 | 1.4 | 210 |
| | MBR | 2.94 ± 0.39 | 7.17 | 5.91-8.94 | 6.8 | 210 |

Resistance ratio (RR) calculated as $RR = LC_{50}$ of field strain / LC_{50} of the insecticide-susceptible strain (LAB).

DISCUSSION

Tomato production is of strategic importance and has significantly increased to meet urban demand for fresh market or processing tomato (FAO, 2015). It has an annual variation of 10 and -1.1% (respectively for industrial and cherry tomatoes) (ANSD, 2019). The fruitworm, *H. armigera*, is the most destructive pest of field-grown tomato in Senegal (Diatte et al., 2018). In addition, the introduction in 2012 of the tomato leafminer, *Tuta absoluta*, has increased farmer's reliance upon insecticides (Brévault et al., 2014). The monitoring of the susceptibility to commonly used insecticides (abamectin, deltamethrin, and profenofos), using a standard leaf-dip bioassay, showed that *H. armigera* populations collected from tomato fields, have evolved high levels of resistance to pyrethroid insecticides (11/12 populations with $RR > 10$, 7/12 populations with $RR > 30$). Moreira et al. (2002) already suspected resistance to pyrethroids in a *H. armigera* population from tomato in Northern Senegal (Saint-Louis), when they identified two mutations located in the sodium voltage dependent channel gene, the main target of pyrethroids. The level of resistance increased significantly from the South (1/4 populations with $RR > 30$) to the North (3/3 populations with $RR > 30$) of Niayes. In the North of Niayes, field-grown tomato is mostly dedicated to processing industry, occupies large areas (4500 ha), and relies on intensive use of pesticides, compared to small-size tomato plots conducted by smallholders (3-4 ha) in The South and Center of Niayes. In addition, large surfaces of alternative host crops for *H. armigera* such as sweet corn planted by agro-industrial companies, are also heavily sprayed with pyrethroids. The only population collected from cotton in the Eastern part of Senegal showed moderate resistance level ($RR = 13$) compared to populations from tomato, probably because programs of insecticide resistance management temporally excluding pyrethroids have been implemented on the whole cultivated cotton area (Martin et al., 2005; Badiane et al., 2015).

Low to moderate levels of resistance to OPs (8/11 populations with $RR < 10$) or newer chemistries such as abamectin (11/15

populations with $RR < 10$) were observed. Special attention should be paid to some cases of resistance to abamectin as this insecticide has been increasingly used these last years for the control of vegetable pest in Senegal. In addition, cross-resistance with pyrethroid-resistant populations was detected. Elevated metabolic detoxification already selected in pyrethroid-resistant populations might be responsible for this cross-resistance because abamectin does not share target sites with other classes of insecticides. Conventional insecticides such as synthetic pyrethroids generally act on the sodium-potassium channel, while organophosphates act as acetylcholinesterase inhibitors. Abamectin belongs to the avermectins group and acts as chloride channel activator. Different levels of cross-resistance to various insecticides within and outside the pyrethroid group such as abamectin has been reported in the CRR strain of *Musca domestica* (Zhang et al., 2007) and according to Liang et al. (2003) there is little cross-resistance between abamectin and four pyrethroid insecticides (deltamethrin, beta-cypermethrin, fenvalerate and bifenthrin) in a strain of the diamondback moth, *Plutella xylostella* (L). This suggests abamectin is not necessarily an effective tool for the management of pyrethroid *H. armigera* resistance in Senegal. The use of more selective novel-chemistry insecticides with different modes of action such as insect growth regulators (lufenuron, methoxyfenozide, etc.), diamides (chlorantraniliprole, flubendiamide), oxadiazines (indoxacarb), or spinosyns (spinosad, spinetoram), associated to threshold-based sprayings to better target sprays (Silvie et al., 2013), could partly (rotation) or totally (exclusion) replace insecticides at risk (herein pyrethroids).

Better knowledge of the scope and magnitude of resistance of *H. armigera* provides essential information to help farmers to adapt their pest management practices. Priority actions should focus on the rational and concerted use of pesticides at the regional scale, considering major host crops of *H. armigera*, in the framework of an area-wide insect resistance management plan. This entails research efforts for a better understanding of

genetic and demographic flows of *H. armigera* populations among agricultural production areas and crops in Senegal, and more broadly in West Africa. This also entails cooperation between major stakeholders including farmers, agrochemical industry, research, and extension services (Martin et al., 2005). Capacity building of stakeholders to use biorational insecticides, low doses, targeted applications (temporal or spatial), should be also encouraged to enhancing biological control by indigenous natural enemies (Barzman et al., 2015). This approach could effectively support the transition of farming systems to ecologically-based production reducing the reliance on insecticides.

Conclusion

Research in this study has focused on the use of synthetic chemicals in various agroecosystems as strategies to control *H. armigera*. The results revealed high levels of pyrethroid resistance in several populations of *H. armigera* in the Niayes area. A pattern of increasing resistance to deltamethrin was observed from south to north. The study also showed that the susceptibility of larvae to abamectin and profenofos is high in general. However, an evolution of resistance to these two products within some populations has been noted. Low yields and high production costs are among the consequences of this resistance. An agroecological approach, including good cultural practices and biological control methods is therefore necessary. Further investigation will be needed to evaluate effects of biological pesticides for developing more appropriate, cost-effective and sustainable integrated protection strategies.

COMPETING INTERESTS

No potential competing interest was reported by the authors.

AUTHORS' CONTRIBUTIONS

SOS is the principal investigator, ET and MD defined the protocols and participated all the activities. SS, AOD and BL participated in writing the manuscript. KD is the initiator of the project.

ACKNOWLEDGEMENTS

We express our sincere gratitude to L2PIA (Laboratoire Production et Protection Intégrées en Agroécosystèmes Horticoles) for financial support.

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