

Efficacy of two pesticides against *Helicoverpa Armigera* (Lepidoptera: Noctuidae) found on tomato plants in the bafing region, Touba, Côte d'Ivoire

Diabate Dohouonan^{1*}, Fondio Drissa², Diby Yao Kan Séraphin³, Akpessa Akpa Alexandre Moïse³, Tano Yao⁴

¹Department of Agronomy and Forestry, UFR Agronomy, Forestry and Environmental Engineering, University of Man, BP Man, Côte d'Ivoire

²Department of Entomology and Plants Protection, UFR Agroforestry, University Jean Lorougnon Guédé, BP Daloa, Côte d'Ivoire

³Department of Biosciences, UFR Biosciences, University Félix Houphouët Boigny, BP Abidjan, Côte d'Ivoire

⁴Department of Biosciences, UFR Biosciences, University Nangui Abrogoua, BP Abidjan, Côte d'Ivoire

Abstract

Helicoverpa armigera is a major pest of tomato crop. It reduces tomato fruit quality despite pesticides spraying. This study carried out to evaluate the efficacy of two pesticides commonly used by the farmers against the tomato fruit borer *Helicoverpa armigera* in Touba department, Côte d'Ivoire. The number and damage of *Helicoverpa armigera* larvae on tomato plants were recorded four times, from 41st to 62nd day after the transplanting of tomato plants on a quarter-hectare plot established on March 1, 2022. This plot was subdivided into three equal subplots. Two subplots of tomato plants were treated with the pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC®, respectively and the third subplot was untreated. Each subplot was subdivided into four elementary plots. The results showed that the pesticides K-OPTIMAL 35EC® (1.5 larvae) and SAUVEUR 62EC (1.25 larvae) significantly reduced *Helicoverpa armigera* larvae on tomato crop compared with the control plots (5.5 larvae) per 12 plants ($p < 0.0001$). Tomato fruits attack rates were 25.26%, 5.01% and 2.08% in untreated tomato plots and in plots treated with K-OPTIMAL 35EC® and SAUVEUR 62EC, respectively ($p < 0.0001$). The percentage reduction of tomato fruit damaged by *Helicoverpa armigera* compared to untreated tomato plots was 80.19% and 91.76% for K-OPTIMAL 35EC® and SAUVEUR 62EC, respectively. Yield loss percentages were 30.69%, 4.58% and 2.43% for the control, K-OPTIMAL 35EC® and SAUVEUR 62EC®, respectively ($p < 0.0001$). The pesticide SAUVEUR 62EC® can be used to protect tomato crops against *Helicoverpa armigera*.

Keywords: Attack rate, *Helicoverpa armigera*; *Lycopersicon esculentum*, pesticides, yield loss

Introduction

The tomato fruit borer *Helicoverpa armigera* Hübner (Lepidoptera : Noctuidae) is a major pest of tomato crops (Wakil *et al.*, 2009 ; Diabaté, 2016; Kumar *et al.*, 2020; Rathour and Ravi, 2023) [1-4]. This insect pest is a prominent phytophagous that attacks tomato fruits and has been responsible for tomato yield losses (Ghosh *et al.*, 2010 ; Diabaté *et al.*, 2014a ; Deb & Bharpoda, 2017) [5-7]. Tomato yield losses caused by *Helicoverpa armigera* was estimated at 42% worldwide as well as in Côte d'Ivoire (Sapkal *et al.*, 2018 ; Diabaté *et al.*, 2014a ; Diabaté 2016) [2, 6, 8]. The average daily temperature of 23.5°C was reduced *H. armigera* population's per year and was also reduced tomato fruit damaged by this insect pest (Walker and Cameron, 1990) [9]. *H. armigera* damage was regularly distributed during the dry season and tomato fruits damaged were lower during rainy season (Bouchard *et al.*, 1992 ; Talekar *et al.*, 2006) [10; 11]. *H. armigera* populations showed resistance to several insecticides, such as organophosphates, carbamates, indoxacarb, pyrethroids, benzoates and spinosad (Diabaté *et al.*, 2014a ; Qayyum *et al.*, 2015; Diabaté, 2016 ; Bade and Bhamare, 2023) [2, 6, 12, 13] and to pesticides based on *Bacillus thuringiensis* (Zhang *et al.*, 2011) [14]. *H. armigera* larvae can optionally enter on diapause (Kranti *et al.*, 2002 ; Torres-Villa *et al.*, 2002 ; Bade and Bhamare, 2023) [13, 15, 16]. At that time, more than 30% of world's pesticides were used to control *H. armigera* worldwide (Ahmad, 2007) [17]. *H. armigera* resistance to the pesticides used by the farmers was thought to be related to the inability of the insecticides

to bin with acetylcholinesterase (Srinivas *et al.*, 2004) [18]. Then, to protect the tomato cultivations against *H. armigera* and increase tomato yields, farmers used large quantities of insecticides that are harmful to humans, animals and environment. In Touba, farmers have notice that a decline in the efficacy of the pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® against *H. armigera*. This has led to overuse of pesticides. Furthermore, the ineffectiveness of the pesticides against *H. armigera* may be related to their active ingredients, or to the methods of application of the pesticides. Therefore, the study were carried out in Touba department to evaluate the efficacy of these two pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® against *H. armigera* in tomato farms, while scrupulously respecting good pesticide application practices. In fact, insecticide applications take into account climatic factors and, in particular, the spraying period. In this study, the impact of foliar applications of these two pesticides on *H. armigera* number on tomato plants, on the attack rates and the yield losses caused by *H. armigera* larvae were recorded.

Materials and methods

Study area

The field experiment was conducted from March to May, 2022 in department of Touba (8°49'9''N and 7°52'47''W). The climate of this area is characterized by two seasons, a rainy season from April to October and a dry season from November to March. The annual rainfall and temperature

avarages were 1370 mm and 26°C, respectively (Bamba *et al.*, 2020) [19].

Tomato variety UC 82, GOLDEN QUALITY, GALASSI SEMENTI, Italy was grown in a quarter-hectare plot established on March 1, 2022 at a spacing of 40 cm x 40 cm. After transplanting, dead plants were replaced. This plot was divided into three equal subplots. Two subplots of tomato plants were treated with the pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC®, respectively and the third subplot was untreated. Each subplot was divided into four elementary plots.

Evaluation of the efficacy of the two pesticides on *H. armigera* larvae

The two pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® were sprayed every week, from 6 am to 8 am from the 38th to the 59th day after transplanting. The control plots were not sprayed with the pesticides. ALMANEB 80 (Maneb 80%) was sprayed two time on tomatoes plot. Sampling of *H. armigera* larvae and their damage on tomato fruits was carried out on 12 tomato plants per elementary plot, four times, from 41st to 62th day after transplanting, from 6 am to 8 am, three days after treatment. Our study focuses on *H. armigera* larvae. In fact, this study focuses on *H. armigera* larvae because it is the larval stage that causes damage to tomato leaves and fruits. *H. armigera* larvae were collected and then identified in the laboratory and from the literature. Percentage reduction of tomato fruits attacked by *H. armigera* larvae, the attack rate and yield losses were recorded.

Attack rate on tomato fruits by *H. armigera*

From the 41st day after transplanting to 62th day after transplanting, tomato fruits were harvested at 4 day intervals on 1 m² of each elementary. Tomato fruits damaged by *H. armigera* and the healthy fruits were recorded and the attack rate on tomato fruits was calculated by using the following formula

$$Ta = \frac{Nfa * 100}{Nt} \quad (1)$$

Ta = attack rate (%) ;

Nfa = number of tomato fruits attacked by *H. armigera* per treatment ;

Nt = total number of tomato fruits harvested per treatment.

Percentage reduction of tomato fruits damaged by *H. armigera*

The percentage reduction of tomato fruits attacked by *H. armigera* was calculated by using the following formula

$$PRFat (\%) = \frac{(C - T) * 100}{T} \quad (2)$$

with:

PRFat= percentage reduction of tomato fruit attacked ;

C= number of tomato fruits damaged on untreated plots ;

T= number of tomato fruits damaged on treated plots.

Yield losses due to *H. armigera* on tomato plots

In each elementary plot of the treatments (control, K-OPTIMAL 35EC® and SAUVEUR 62EC®), tomato fruits attacked by *H. armigera* and healthy tomato fruits were weighed and the yield losses (YL) were calculated using the following formula

$$Y L (\%) = \frac{(Wt-Wh) * 100}{Wt} \quad (3)$$

With:

YL: Yield losses ;

Wt: Total weight of tomato fruits collected ;

Wh: total weight of healthy tomato fruits.

Data analysis

Data of *H. armigera* larvae recorded, attack rates, percentage reduction of tomato fruits damaged and yield losses were subjected to an analysis of variance (ANOVA main effect) using SPSS software version 22.0. The means were discriminated by using the Fisher test (LSD, p < 0,05) of XLSTAT 2016.

Results

Number of *H. armigera* larvae found on tomato plants in the different treatments

The pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® significantly reduced *H. armigera* larvae number on tomato crop compared to the untreated plots (5.5 larvae per 12 plants). These two pesticides had a similar efficacy on *H. armigera* larvae (F = 74.455; p < 0.0001). However, the number of *H. armigera* larvae on tomato plants sprayed with the pesticide K-OPTIMAL 35EC® was higher than that of tomato plants treated with the pesticide SAUVEUR 62EC®, being 1.5 larvae and 1.25 larvae per 12 tomato plants, respectively (Figure 1).

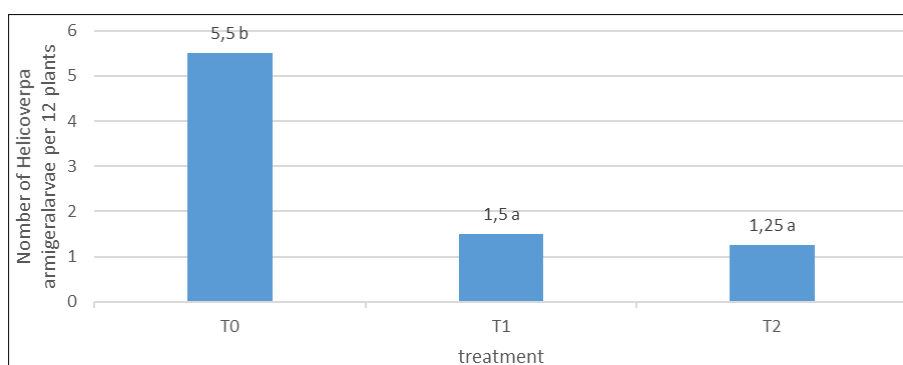


Fig 1: Efficacy of the pesticides K-OPTIMAL 35EC® (T1) and SAUVEUR 62EC® (T2) on the level of infestation of tomato plants by *Helicoverpa armigera* larvae

SE= standard error ; T0= control plots; T1= K-OPTIMAL 35EC® ; T2= SAUVEUR 62EC®.

Means with the same letter are not statistically different (Fisher test, LSD, P < 5%).

Effect of the treatments on tomato fruit damaged by *H. armigera* larvae

Helicoverpa armigera larvae damaged tomato fruits and reduced the market value of tomato fruits (Figure 2). The total number of fruits harvested per 1 m² was higher in the plots treated with the pesticides K-OPTIMAL 35EC® (201.00 ± 54.95 fruits) and SAUVEUR 62EC® (179.25 ± 5.38 fruits) than in the control plot (97.25 ± 17.54 fruits). The two pesticides had similar efficacy on the tomato fruits harvested (F= 10.704 ; p= 0.004). Moreover, the number of healthy tomato fruits was statistically the same in the plots sprayed with the pesticides K-OPTIMAL 35EC® (190.50 ±

49.88 fruits) and SAUVEUR 62EC® (175.50 ± 4.51 fruits). The lowest number of tomato fruits harvested was recorded on the control plots with a value of 72.25 ± 13.30 tomato fruits (F = 18.520 ; p = 0.001). While, the lowest number of tomato fruits attacked by *H. armigera* collected in the plots sprayed with the pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® and the highest number of tomato fruits damaged were recorded on the control plot (F = 8.493 ; p = 0.008). The tomato fruit attack rates were 25.27%, 5.01% and 2.08 in the control plots, in the plots treated with the pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC®, respectively (F = 20.596; p < 0.0001). The reduction rate of *H. armigera* damage on tomato fruits compared to the controle were 80.19% and 91.76% for the pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC®, respectively (Table 1).



Fig 2: Damage caused by *Helicoverpa armigera* (a) and it damage on tomato fruit

Table 1: Total number of tomato fruits recorded, tomato fruits attacked rate by *H. armigera* and the reduction rate of tomato fruits attacked by the pesticides

Treatment	Total fruits harvested ± SE	Healthy fruits ± SE	Fruits attacked ± SE	Fruit attacked rate (%)	Reduction rate of attacked fruits (%)
T0	97.25 ± 17.54 ^b	72.25 ± 13.30 ^b	25.00 ± 11.80 ^b	25.27 ^b	-
T1	201.00 ± 54.95 ^a	190.50 ± 49.88 ^a	10.50 ± 5.07 ^a	5.01 ^a	80.19
T2	179.25 ± 5.38 ^a	175.50 ± 4.51 ^a	3.75 ± 1.26 ^a	2.08 ^a	91.76
F	10.704	18.520	8.493	20.596	-
p	0.004	0.001	0.008	< 0.0001	-

SE= Standard error; T0= Control plots; T1= K-OPTIMAL 35EC® ; T2= SAUVEUR 62EC®.

Means with the same letter within the same column were not statistically different (Fisher test, LSD, p<5%).

Yield losses due to *H. armigera* larvae on tomato plot

The pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® have similar efficacy on the total weight of tomato

fruits recorded, on the weight of healthy tomato fruit and on the weight of tomato fruits attacked. The total weight of tomato fruit harvested from the plots treated with the pesticides K-OPTIMAL 35EC® (16.325 ± 3.827 kg) and SAUVEUR 62EC® (14.350 ± 0.451 kg) was statistically higher than that harvested from the control plots with 5.750 ± 1.392 kg per 1 m² (F = 22.597 ; p < 0.0001). The weight of healthy tomato fruit harvested from plots treated with K-OPTIMAL 35EC®, SAUVEUR 62EC® and control plots were 15.550 ± 3.487 kg, 14.00 ± 0.408 kg and 3.925 ± 0.854 kg, respectively (F=36.610 ; p < 0.0001). However, the highest weight of tomato fruits damaged by *H. armigera* was recorded in the control plots (1.825 ± 0.960 kg) and the lowest weight were on the plots treated with the pesticides K-OPTIMAL 35EC® (0.775 ± 0.340 kg) and SAUVEUR 62EC® (0.350 ± 0.058 kg) (F = 6.641 ; p = 0.017). These two pesticides significantly reduced yield losses caused by *H. armigera* larvae on tomato fruits (F = 22.501 ; p < 0.0001). The percentages of the yield losses caused by *H. armigera* larvae were 30.69%, 4.58% and 2.43% for the control, K-OPTIMAL 35EC® and SAUVEUR 62EC®, respectively.

Table 2: Yield losses of tomato fruits caused by *Helicoverpa armigera* larvae

Treatment	Total fruit weight (kg)	Healthy fruit weight (kg)	Weight of attacked fruit (kg)	Percentage of yield loss (%)
T0	5.750 ± 1.392 ^b	3.925 ^b ± 0.854 ^b	1.825 ^b ± 0.960 ^b	30.689 ^b
T1	16.325 ± 3.827 ^a	15.550 ^a ± 3.487 ^a	0.775 ^a ± 0.340 ^a	4.580 ^a
T2	14.350 ± 0.451 ^a	14.000 ^a ± 0.408 ^a	0.350 ^a ± 0.058 ^a	2.434 ^a

F	22.597	36.610	6.641	22.501
p	< 0.0001	< 0.0001	0.017	< 0.0001

SE= Standard error, T0= control plots ; T1= K-OPTIMAL 35EC® ; T2= SAUVEUR 62EC®.

Means with the same letter within the same column were not statistically different (Fisher test, LSD, $p < 5\%$).

Discussion

The pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® significantly reduced *H. armigera* larvae number on tomato plants, tomato fruits attacked and yield losses, compared to the control plots. These two pesticides are neurotoxic and act on *H. armigera* larvae by contact and by ingestion. The pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® caused hyperactivity of the insects, tremors and convulsions, leading to the paralysis and the death of the insects (Ghosh *et al.*, 2010) [5]. In addition, these pesticides exert antiappetant and repellent effects on the insects through contact and ingestion (Diabaté *et al.*, 2014b; Diabaté *et al.*, 2022) [20-21]. They act on respiratory mechanisms (Park *et al.*, 2003 ; Diabaté *et al.*, 2014b; Diabaté *et al.*, 2022) [20-22]. They disrupt the normal function of synapses. Pesticides bind to acetylcholinesterase (AChE) to form the AChE-insecticide complex, which is highly stable and resistant to hydrolysis. With the enzyme immobilized by the insecticide, acetylcholine can no longer play its normal role as a neuromediator (Shi *et al.*, 2004) [23]. The insect's metabolism is thus disrupted. The ineffectiveness of these two pesticides against *H. armigera* larvae on tomato farms would be related to the poor application of K-OPTIMAL 35EC® and SAUVEUR 62EC®. Furthermore, the pesticide K-OPTIMAL 35EC® is less effective than the newly introduced pesticide SAUVEUR 62EC®. This would result in the emergence of the lowest resistance of *H. armigera* to the pesticides K-OPTIMAL 35EC®. This lower resistance of *H. armigera* to the pesticide K-OPTIMAL 35EC® would be related to the inability of the pesticides to bind to acetylcholinesterase (Srinivas *et al.*, 2004) [18]. The ineffectiveness of these pesticides against *H. armigera* is sometimes related to the poor conditions of the application of the pesticides on tomato plants. Farmers should respect the spray times and recommended doses of pesticides. The pesticides are sometime sprayed on the tomato plants when weather conditions are not optimal and this would induce slower resistance of *H. armigera* larvae populations. Farmers then have to spray tomato farms early in the morning or at the end of the day, when relative humidity is higher, wind speed is lower and the temperatures are lower. This allows the pesticides to their targets, increases their effectiveness, and prevents pesticide drift and pollution. Furthermore, respecting the conditions of pesticide application will increase pesticide efficacy, limit the emergence of pesticide resistance of insects and protect animal and plant biodiversity. We recommend that the doses of pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® recommended by the manufacturer be respected, thus preserving biodiversity and avoiding contamination of food and the environment.

Conclusion

The study showed that the pesticides K-OPTIMAL 35EC® and SAUVEUR 62EC® significantly reduced *H. armigera* larvae number on tomato crop, the tomato fruits damaged rates and the yield losses caused by *H. armigera*. The pesticides K-OPTIMAL 35EC and SAUVEUR 62EC® can

still be recommended to farmer against *H. armigera* in tomato cultivation. However, judicious use of these pesticides can help the farmer to increase the efficacy of these pesticides on *H. armigera*. Moreover, biopesticides can be combined with chemical pesticides to the integrated pest management in tomato farms.

Competing interests

The authors declare that they have no conflict of interest regarding this article.

References

1. Wakil W, Ashfaq M, Kwon YJ, Ghazanfar MU. Trends in integrated pest management strategies for the control of *Helicoverpa armigera* (Hübner) caterpillars on chickpea (*Cicer arietinum* L.). *Entomol Res*,2009;39:84-8.
2. Diabaté D. Impact et mode d'action de quelques biopesticides et insecticides classiques en culture maraîchère dans la région du Moronou (Bongouanou, Côte d'Ivoire). Thèse unique de Doctorat, Université Félix Houphouët-Boigny, Côte d'Ivoire, 2016, 148.
3. Kumar S, Umarao SR, Kumar A, Patel KV, Debnath R, Kumar A. Evaluation of the efficacy of insecticides and biopesticides against *Helicoverpa armigera* (Hübner) on tomato. *J Entomol Zool Stud*,2020;8(3):555-8.
4. Rathour B, Ravi B, Ravi S. *In vivo* appraisal of phytoextracts, essential oils and insecticides against tomato fruit borer (*Helicoverpa armigera* Hübner). *Indian J Hill Farming*,2023;36(1):167-71.
5. Ghosh A, Chatterjee M, Roy A. Bio-efficacy of spinosad against tomato fruit borer (*Helicoverpa armigera* Hub.) (Lepidoptera: Noctuidae) and its natural enemies. *J Hortic For*,2010;2(5):108-11.
6. Diabaté D, Gnago AJ, Koffi K, Tano Y. The effect of pesticides and aqueous extracts of *Azadirachta indica* (A. JUSS) and *Jatropha curcas* L. on *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) found in tomato plants in Côte d'Ivoire. *J Appl Biosci*,2014;80:7132-43. <http://dx.doi.org/10.4314/jab.v80i1.14>
7. Deb S, Bharpoda TM. Field evaluation of biopesticides against *Helicoverpa armigera* (H.) in tomato, *Lycopersicon esculentum* Mill. under middle Gujarat condition. *Green Farming*,2017;8(1):188-93.
8. Sakpal SD, Sonkamble MM, Gaikwad BB. Seasonal incidence of tomato fruit borer, *Helicoverpa armigera* (Hübner) on tomato, *Lycopersicon esculentum* (Mill) under protected cultivation. *J Entomol Zool Stud*,2018;6(4):1911-4.
9. Walker GP, Cameron PJ. Pheromone trapping and field scouting for tomato fruitworm in tomatoes and sweet corn. *Proc N Z Weed Pest Control Conf*,1990;43:17-20.
10. Bouchard D, Oueraogo A, Boivin G. Vertical distribution, spatial dispersion and sequential sampling plan for fruit damage by *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on tomato crop in Burkina Faso. *Trop Pest Manag*,1992;38(3):250-3.

11. Talekar NS, Opena RT, Hanson P. *Helicoverpa armigera* management: a review of AVRDC's research on host plant resistance in tomato. *Crop Prot*,2006;25:461-7.
12. Quayyum AM, Waqas W, Muhammad JA, Shahbaz T, Sahi NAS, Derek AR. Multiple resistances against formulated organophosphates, pyrethroids and newer chemistry insecticides in populations of *Helicoverpa armigera* (Lepidoptera: Noctuidae) from Pakistan. *J Econ Entomol*,2015;108(1):286-93.
13. Bade AS, Bhamare VK. Insecticidal resistance in *Helicoverpa armigera* (Hübner) infesting chickpea. *Indian J Entomol*, 2023, 1-4. DOI: 10.55446/IJE.2023.1348.
14. Zhang H, Yin W, Zhao J, Jin L, Yang Y, Wu S, *et al.* Early warning of cotton bollworm resistance associated with intensive planting of Bt cotton in China. *PLoS One*,2011;6(8):1-8.
15. Kranthi KR, Jadhav DR, Kranti S, Wanjari RR, Ali SS, Russel DA. Insecticide resistance in five major insect pests of cotton in India. *Crop Prot*,2002;21:449-60.
16. Torres-Vila LM, Rodriguez-Molina MC, Lacasa-Plasencia A, Bielza-Lino P. Insecticide resistance of *Helicoverpa armigera* to endosulfan, carbamates and organophosphates: the Spanish case. *Crop Prot*,2002;21:1003-13.
17. Ahmad M. Insecticide resistance mechanisms and their management in *Helicoverpa armigera* (Hübner): a review. *J Agric Res*,2004;45(4):319-35.
18. Srinivas R, Udikeri SS, Jayalakshmi SK, Sreeramulu K. Identification of factors responsible for insecticide resistance in *Helicoverpa armigera*. *Comp Biochem Physiol C Toxicol Pharmacol*,2004;137:261-9.
19. Bamba LK, Kouamé AC, Kouadio KE, Kouadja GS, Assemien-Diarrassouba OS, Kouassi CN. L'élevage ovin (*Ovis aries*) en Côte d'Ivoire: caractéristique démographique du cheptel des localités de Touba (Ouest), Korhogo (Nord) et Bouaké (centre). *Afrique SCIENCE*,2020;16(5):8-16.
20. Diabaté D, Gnago AJ, Tano Y. Toxicity, antifeedant and repellent effect of *Azadirachta indica* (A. JUSS) and *Jatropha curcas* L. extracts against *Plutella xylostella* (Lepidoptera: Plutellidae). *J Basic Appl Sci Res*,2014;4(11):51-60.
21. Diabaté D, Golly KJ, Ahon GM, Tano Y. Bioefficacy of *Azadirachta indica* A. Juss and *Jatropha curcas* L. seeds aqueous extracts on *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Int J Agron Agric Res*,2022;21(6):68-76.
22. Park IK, Lee SG, Choi DH, Ahn YJ. Insecticidal properties of constituents identified in the essential oil from leaves of *Chamaecyparis obtusa* against *Callosobruchus chinensis* (L.) and *Sitophilus oryzae* (L.). *J Stored Prod Res*,2003;39(4):375-84.
23. Shi AM, Lougarre A, Alies C, Fremaux I, Tang ZH, Stojan J. Acetylcholinesterase alterations reveal the fitness cost of mutations conferring insecticide resistance. *BMC Evol Biol*,2004;4(5):1-8.