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Effectiveness of a formulation based on Indoxacarb 120 g/l + flonicamid 200 g/l (SC) in controlling sucking biting insects and carpophagous Lepidoptera larvae of cotton in Côte d'Ivoire

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Abstract
An invasion of jassids has been observed on cotton plants in Côte d'Ivoire during the 2022-2023 season. Insecticides used in plant protection programs were unable to stop damages caused by these pests. The goal of this survey was to assess a formulation based on the flonicamid + indoxacarb combination, in order to include it in protection programs. The test has been realized from June up to December, during the 2023-2024 cotton campaign. The scientific mechanism is in Fisher block design, with 5 objects and 4 replicates. The seeds has been settled in holes separated by 0.80 m among lines and 0.30 m on the same lines. Three doses (187.5 ml/ha, 250 ml/ha and 375 ml/ha) were tested. Six (6) treatments spaced 14 days apart were realized. Surveys were realized by recording the number of individuals on 30 plants per elementary plot taken in groups of 5 plants consecutively on the 8 central lines. Observations were made for the main sucking pests (jassids, aphids and whiteflies) and carpophagous caterpillars (*Earias sp*ecies, *Helicoverpa armigera* and *Diparopsis watersi*). The results show a good control of the formulation tested on all the target pests, with significant variations in the activity of the product depending on the insect species. Concerning sucking pests, the level of jassid and aphid infestation was significantly reduced by the three doses tested, compared with the reference product and the untreated control. The indoxacarb + flonicamid formulation proved effective against *H. armigera*, *Earias spp*. and *D. watersi* larvae at all three doses tested. It is therefore an ideal alternative for controlling cotton pests in Côte d'Ivoire.

Keywords: Côte d'Ivoire, flonicamid, indoxacarb, jassids, cotton pests

Introduction
In Côte d'Ivoire, the economy of savannah areas is still mainly based on cotton growing regarded to important incomes it provides. Cotton ranks 4th among agricultural exports and contributes 1.7% to national GDP. Cotton production stand for around 7% of exports [1]. Cotton is grown every year by around 130, 000 producers and feeds nearly 3.5 million people [2]. The total area sown is around 300, 000 hectares, with production exceeding 400,000 tonnes [3]. However, cotton growing still faces a number of constraints. These include the adverse effects of climate change, soil impoverishment and pest pressure. Cotton is one of the plants most frequently attacked by several species of arthropod pests. If plant protection is lacking, these pests can cause crop losses of up to 80% [4]. To date, the use of synthetic chemical insecticides remains the only means of controlling these pests to ensure optimum yields. Insecticides from the pyrethroid family were highly effective until the emergence of resistance in the noctuid *Helicoverpa armigera* in 1998 [5].
The damages caused by this dreaded bollworm has prompted the introduction of a pyrethroid resistance management and prevention strategy based on 'window programmes' [6]. These programmes recommend rationing pyrethroids and replacing them with alternative molecules such as spynosins, sulphoximins and diamids for the first insecticide treatments of cotton plants during the vegetative phase. The "window programmes" have kept *H. armigera* infestations at relatively low levels since 1999 [7]. However, in recent years, cotton growers have been and still are faced with a succession of new pest problems.

The emergence of other carpophagous caterpillars, such as *Pectinophora gossypiella* and *Earias sp*ecies [6], has been revealed in farming areas, while sucking pests of the Cicadelidae family, including *Jocobiella fascialis* and *Empoasca papayae*, are on the increase [8, 9]. Since the early 2010s, many growers have also been complaining about massive outbreaks and population damage caused by the whitefly *Bemisia tabaci* [10]. During the 2022-2023 cropping season, production losses estimated at over 50% were recorded due to severe attacks by a new species of jassid, *Amrasca biguttula* [11–14]. Particularly against this species, the insecticides commonly used by growers have proved ineffective. Given the level of risk posed by these jassids, it is essential to find new formulations to recommend.

This would enable protection programs to be readapted in the face of these new components of the cotton pest complex, which pose a considerable threat to farmers' incomes. The aim of this analysis is to help guarantee cotton productivity in Côte d'Ivoire by proposing new insecticide formulations. The candidate formulation for this study is a combination based on flonicamid and indoxacarb. The choice of this formulation is justified by efficiency of indoxacarb against various carpophagous and phyllophagous caterpillars [15] and the excellent efficacy of flonicamid against sucking biters [16]. This will make it possible to highlight the spectrum of activity of the indoxacarb + flonicamid combination, as well as the optimal application rate.

Materials and Methods

Study area

The test leaded at 2 sites in the cotton production area. This is subdivided into two major districts in relation to the 9th parallel: the northern zone for all cotton-growing localities above and the southern region for all cotton-growing localities below the 9th parallel. The first site in the southern zone is the cotton research station based in Bouaké (7°42'2.801 'North, 5°0'14.716 'East). The second site is located at Nambingué (10°3'20.308 'N, 5°21'17.028 'W) in the north (Figure 1). According to Halle and Bruzon 2006 [17], the climate of the southern zone is of the Baouléen kind, characterized by 2 rainy seasons and two distinct dry seasons, with irregularities between the different seasons.

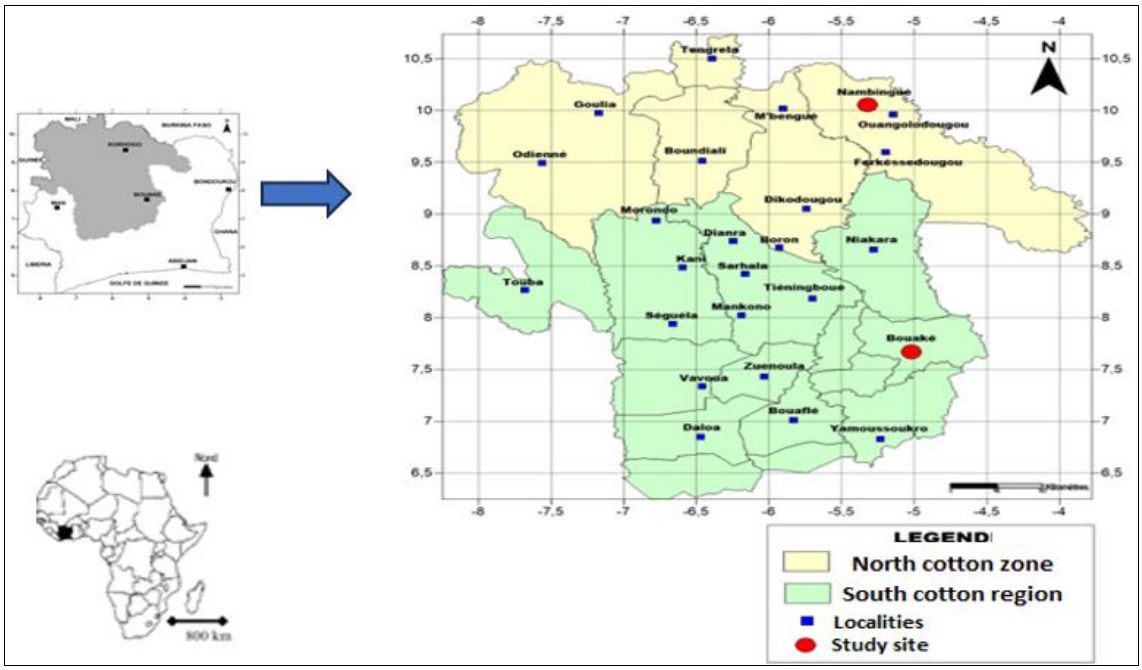

Fig 1: Study areas

Annual rainfall is in the region of 1,300 to 1,750 mm, with temperatures ranging from 24 to 33 °C and relative humidity of 60 to 70%. In the north, the climate is Sudanian. It is characterized by a dry season lasting from 6 months (November to April) to 8 months (October to May) and a rainy season with maximum intensity in August. Annual rainfall varies from 1,150 to 1,400 mm/year, with average temperatures of 26 to 27.5 °C and 40 to 50% relative humidity.

Experimental design

The insecticides used for this test were ANGEL 320 SC (Indoxacarb 120 g/l + Flonicamid 200 g/l) tested product, supplied by the firm SAVANA. CROTALE 46 EC (Indoxacarb 30 g/l + Acetamiprid 16 g/l) formulation, already on the market, was used as a control. The cotton variety involved in this test is C1123 developed by the National Center for Agronomic Research of Côte d'Ivoire.

Essays were guided from June up to December during the 2023-2024 cotton season. The scientific mechanism is a completely randomized block (Fisher), with 5 objects and 4 replications. The dimensions of the elementary plot are 10 m long and 8 m wide, giving a surface area of 80 m². Of the 10 rows, 8 were treated (the border rows were left untreated). The seeds were sown in blocks 0.80 m apart between the rows and 0.30 m apart on the same row. The distance between blocks was 2 m.

Comparative treatments

The treatments are constituted of 3 doses of the new formulation (Indoxacarb 120 g/l + Flonicamid 200 g), a reference product (CROTALE 46 EC at 1 l/ha) and an untreated control (Table 1).

Table 1: Comparative treatments

Treatment	Description	Dose (g. m.a/ha)	Formulation dose (ml/ha)	Source
A	Untreated	-	-	-
B	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	9 + 37,5	187,5	ANGEL 320 SC
C	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	30 + 50	250	ANGEL 320 SC
D	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	18 + 75	375	ANGEL 320 SC
E	Indoxacarb 30 g/l Acétamiprid 16 g/l + (EC)	30 + 16	1000	CROTALE 46 EC

- None

Six (6) treatments spaced 14 days apart were carried out for objects B, C, D and E. Insecticide applications were made using a backpack, pressure-maintained, horizontal boom sprayer delivering 60 l of spray solution/ha.

Effect of treatments on pests

Subjects has been realized were carried out at T-1, T+3, T+7 and T+13 (T being the date of insecticide application). They were carried out on 30 plants per elementary plot taken in groups of 5 plants consecutively on the 8 central rows, using the diagonal method [4]. The following observations were made according to following pest groups.

- **Jassids:** Adults are counted by examining 5 well-developed leaves at the top of the plant. Attacked plants-5 well-developed leaves are examined at the top of the plant. The plant is. Considered attacked if any of these 5 leaves show symptoms of attack.
- **Carpophagous caterpillars:** The entire plant is observed and to record the number of caterpillars of each of these species (*H. armigera*, *Earias* spp. and *Diparopsis watersi*).
- **Whiteflies (*Bemisia tabaci*):** Fixed forms are looked for on the underside of the 5 terminal leaves. The plant is considered to be attacked if one of these 5 leaves bears at least one fixed form.
- **Aphids:** The 5 terminal leaves are examined. The plant is considered to be attacked if one of the 5 terminal leaves bears both symptoms of attack and live aphids.

Data analysis

The data collected were submitted to analyses of variance in order to determine the effect of treatments (Objects) on various entomological variables. In event of significant differences, homogeneous groups were determined using Tukey's HSD test with a threshold of 5%.

Results

Efficacy of Indoxacarb 120 g/l + Flonicamid 200 g/l (SC) formulation against biting and sucking insects

Aphids

Infestations were only observed at the Bouaké site (Table 2). At this site, doses of 250 ml/ha and 375 ml/ha of the combination Indoxacarb 120 g/l + Flonicamid 200 g/l (SC) significantly reduced aphid attacks compared with the untreated control ($p<0.05$), being equivalent to the reference product (Indoxacarb 30 g/l + Acetamiprid 16 g/l EC).

Jassids

Tables 3 and 4 show the population levels and damage levels of jassids on the two trial sites respectively. At both sites, the three doses of Indoxacarb 120 g/l + Flonicamid 200 g/l (SC) were more effective than the reference product. These three doses significantly reduced ($p<0.0001$) jassid populations and attacks, compared with the untreated control and the reference control.

Whitefly *Bemisia tabaci*

Table 5 shows the level of whitefly damage on 30 cotton plants as a function of treatments at Bouaké and Nambingué. At these two sites, no treatment was effective against whitefly compared with the untreated plot.

Effectiveness of the combination Indoxacarb 120 g/l + Flonicamid 200 g/l (SC) against carpophagous caterpillars *Helicoverpa armigera*

Larval infestations of *H. armigera* were only observed at the Nambingué site (Table 6). At this site, the combination of Indoxacarb 120 g/l + Flonicamid 200 g/l (SC) significantly ($p<0.0001$) reduced the level of infestation compared with the untreated control and the reference product.

***Earias* spp.**

Average numbers of Earias larvae according to treatments are shown in Table 5 below. At the Bouaké site, the various treatments did not reduce infestations compared with the untreated control. At Nambingué, on the other hand, plots treated with Indoxacarb 120 g/l + Flonicamid 200 g/l (SC) and the reference product significantly ($p = 0.003$) reduced the number of *Earias spp*. larvae. However, it should be noted that the level of infestation was low at this site.

Diparopsis watersi

D. watersi larvae were only observed at Nambingué (Table 6). At this site, the three doses of the combination of Indoxacarb 120 g/l + Flonicamid 200 g/l (SC) resulted in a significant reduction ($p<0.0001$) in the number of larvae compared with the untreated control and the reference product.

Table 2. Levels of aphid attack on cotton plants in the various experimental plots

Average number of plants attacked/30 plants ± Standard deviation				
Treatment	**Description**	**Dose p.c(ml/ha)**	**Bouaké**	**Nambingué**
A	Untreated	-	1, 829±0,48 b	0, 00 a
B	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	187,5	1, 056±0,48 b	0, 00 a
C	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	250	0, 917±0,83 a	0, 00 a
D	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	375	0, 778±0,26 a	0, 00 a
E	Indoxacarb 30 g/l Acetamiprid 16 g/l + (EC)	1000	0, 629±0, 26 a	0, 00 a
Pr > *F*			0, 036	-
Meaning			S	-

- None, S significant. For Bouaké and Nambingué columns, the means bearing the same letters are not significantly different

Table 3: Jassid densities on cotton plants in the various experimental plots

Average number of individuals/30 plants ± SD				
Treatment	**Description**	**Dose p.c ml/ha)**	**Bouaké**	**Nambingué**
A	Untreated	-	134, 57±60, 11 c	44, 75±17, 39 c
B	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	187,5	12, 94±9, 86 a	13, 02±5, 04 b
C	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	250	11, 69±9, 71 a	10, 25±4, 34 ab
D	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	375	10, 67±9, 80 a	5, 65±4, 55 a
E	Indoxacarb 30 g/l Acetamiprid 16 g/l +(EC)	1000	97, 37±39, 80 b	42, 93±17, 33 c
Pr > *F*			<0,0001	<0, 0001
Meaning			VHS	VHS

- None, VHS Very Highly Significant. For Bouaké and Nambingué columns, the means bearing the same letters are not significantly different.

Table 4: Levels of jasside species attacks on cotton plants in the various experimental plots

Average number of plants attacked/30 plants				
Treatment	**Description**	**Dose p.c (ml/ha)**	**Bouaké**	**Nambingué**
A	Untreated	-	40, 34±16,60 a	21, 42±12,46 c
B	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	187,5	1, 22±0,50 c	5, 78±5,01 b
C	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	250	0, 56±0,10 a	4, 50±4,34 ab
D	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	375	0, 50±0,32 a	0, 93±0,51 a
E	Indoxacarb 30 g/l Acetamiprid 16 g/l + (EC)	1000	34, 57±19,71 b	21, 18±12,40 c
Pr > *F*			<0,0001	<0,0001
Meaning			VHS	VHS

- None, VHS Very Highly Significant. For Bouaké and Nambingué columns, the means bearing the same letters are not significantly different.

Table 5: Whitefly *Bemisia tabaci* densities on cotton plants in various experimental plots

Average number of plants attacked/30 plants				
Treatment	**Description**	**Dose p.c (ml/ha)**	**Bouaké**	**Nambingué**
A	Untreated	-	0, 40 a	13, 88 a
B	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	187,5	0, 50 a	13, 57 a
C	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	250	0, 56 a	13, 52 a
D	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	375	0, 53 a	13, 15 a
E	Indoxacarb 30 g/l Acetamiprid 16 g/l + (EC)	1000	0, 66 a	11, 78 a
Pr > *F*			0, 933	0, 804
Meaning			NS	NS

- None, NS Not significant. For the Bouaké and Nambingué columns, the means bearing the same letters are not significantly different

Table 6: *Helicoverpa armigera* larval infestation levels in experimental plots

Average number of plants attacked/30 plants				
Treatment	**Description**	**Dose p.c (ml/ha)**	**Bouaké**	**Nambingué**
A	Untreated	-	0, 00 a	0, 83±0,07 b
B	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	187,5	0, 00 a	0, 23±0,06 a
C	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	250	0, 00 a	0, 22±0,09 a
D	Indoxacarb 120 g/l + Flonicamid 200 g/l (SC)	375	0, 00 a	0, 17±0,08 a
E	Indoxacarb 30 g/l Acetamiprid 16 g/l + (EC)	1000	0, 00 a	0, 43±0,07 b
Pr > *F*			-	<0,0001
Meaning			-	VHS

- None, VHS Very Highly Significant. For Bouaké and Nambingué columns, the means bearing the same letters are not significantly different

Discussion

The objective of this study was to evaluate the indoxacarb + flonicamid combination on the main lepidopteran and cotton-sucking pests in Côte d'Ivoire. The objectve was to identify the product's strengths and weaknesses with a view to integrating it into recommended plant protection programs. The insects targeted by this study were found on the 2 sites with varying levels of infestation. With the exception of *H. armigera* and *D. watersi*, which were only present at the Nambingué site (In the north) with low levels of infestation, while aphids were only present at Bouaké (In the south). Jassid and whitefly populations recorded the highest levels of infestation. However, jassid infestations were higher in the south. This distribution depends on the current distribution of cotton pests as defined by Kouakou *et al*. 2021 [9]. The Nambingué zone in the north is characterized by a strong presence of most pests (*H. armigera*, *D. watersi*, *B. tabaci*, jassid species) and a scarcity of aphids. On the other hand, Bouaké, located in the southern zone, stands out for its low levels of infestation by *D. watersi*, *Earias sp* and *H. armigera*. The low levels of *H. armigera* infestation indicate the effectiveness of protection programs against this dreaded bollworm. The results of this study also show the good control exerted by the formulation tested (indoxacarb + flonicamid) on all the target pests, with significant variations in the activity of the product depending on the insect species.

Among sucking biters, the level of jassid and aphid infestation was significantly reduced by the three doses tested (187.5 ml/ha, 250 ml/ha and 375 ml/ha) in the 2 localities, compared with the reference product and the untreated control.

Data on the efficacy of the flonicamid + indoxacarb combination on lepidoptera and sucking pests is virtually non-existent in the literature. Those available refer either to flonicamid or indoxacarb, or to one or other of these active

ingredients in combination with other molecules. One of the few documents to mention the mixture flonicamid + indoxacarb [2], indicates its high efficacy on sucking biters (Jassid *Amrasca biguttula*, aphids and *B. tabaci*) as well as bollworm. (*H. armigera*, *Earias spp*, *T. leucotreta* and *P. gossypiella*). The same report also mentions that flonicamid alone does not control *B. tabaci*, *H. armigera* and *Earias spp*.

Flonicamid has been shown to be effective against sucking biters [16], while indoxacarb provides excellent control of various carpophagous and phyllophagous caterpillars at all stages of their development [15]. In addition to good control of lepidoptera, indoxacarb is also highly effective against sucking biters such as jassids [18]. Thus, the flonicamid + indoxacarb formulation would a priori control the spectrum of pests that occur throughout the cotton crop cycle.

The efficiency of the flonicamid + indoxacarb mixture observed on aphids and jassids could then be attributed to a synergistic effect of the two active ingredients. In the case of flonicamid, several studies attest to its high toxicity on sucking biters in general and jassids in particular [16,19–21]. This insecticide acts by contact and ingestion, irreversibly inhibiting feeding activity leading to the insect's death [20]. This mode of action gives it good tolerance for beneficial insects and mites and an absence of cross-resistance with other conventional insecticides [19]. This would also make it possible to overcome the resistance of certain pests to other insecticides. The flonicamid + indoxacarb combination is therefore particularly interesting as an adjunct product in the management of jassids, which caused more than 50% of crop losses during the 2022-2023 season. This damage was attributed to the species *A. biguttula* when it appeared on the parasitic facies of cotton in Côte d'Ivoire [9, 12, 14].

While the flonicamid + indoxacarb combination provides good control of jassids, this is not the case for the whitefly species *B. tabaci*, which has a certain level of tolerance to the product. Some authors have shown that indoxacarb can cause low mortality in *B. tabaci* [22]. In addition, several studies refer to the very high toxicity of individual flonicamid on these insects. On the other hand, other authors show that flonicamid is not very effective against whiteflies [28].

However, these studies do not specify the nature of the *B. tabaci* biotypes involved in the experiments. In fact, *B. tabaci* is a complex species whose individuals are morphologically identical, but differ in several factors, including their genome and response to insecticides. Within this species complex is the MED-Q1 biotype from the Mediterranean genetic group. It is invasive and has the ability to resist a wide range of insecticides [30]. The latter, recently detected in Côte d'Ivoire on vegetables [31], is also mainly present on cotton plants [32] (unpublished data). The low efficacy of the formulation tested on *B. tabaci* observed would then be due to tolerance of the product by the MED-Q1 biotype of *B. tabaci*. Similar results [33, 34] have shown average resistance to flonicamid in individuals from the Asia-I genetic group in India. In our case, further investigations will enable us to rule on this hypothesis.

With regard to carpophagous caterpillars, the flonicamid + indoxacarb formulation proved effective against the larvae of *H. armigera*, *Earias sp*. and *D. watersi*, at the three doses tested (187.5 ml/ha, 250 ml/ha and 375 ml/ha). As flonicamid has a low activity on carpophagous insects [2], the efficacy of the formulation tested on lepidopterans is due to indoxacarb or a synergistic effect of its combination with flonicamid. Indoxacarb is effective against the main lepidopteran pests [18, 35, 36]. This molecule belongs to the oxadiazine class. These insecticides act by inhibiting the entry of sodium ions into nerve cells, which causes paralysis and death in insects [37]. Several studies indicate that indoxacarb has no significant effect on beneficial insects [35–37]. This molecule also reduces *A. biggutila* populations by more than 50% [21, 38, 39]. Taken together, these properties make the flonicamid + indoxacarb combination an ideal alternative for controlling the main cotton pests.

Conclusion

Heavy pest pressure is one of the main constraints linking to cotton growing. This study was carried out in a context where the invasion of the jassid species *A. bigutula* has been detected, the damage to which has halved cotton yields in Côte d'Ivoire. The results show that the indoxacarb + flonicamid formulation considerably reduces the damage caused by this pest. The results also indicate that it has a good effect on the carpophagous lepidopterans *H. armirgera*, *Earias sp* and *D. watersi*. The 3 doses tested (187.5 ml/ha, 250 ml/ha and 375 ml/ha) gave more or less the same results on both Lepidoptera and sucking pests. In the light of the above, the formulation Indoxacarb 120g/l + Flonicamid 200 g/l can be recommended for the phytosanitary protection of cotton, at a dose of 250 ml/ha, against jassids, aphids, *H. armigera* larvae, *Earias spp*. and *D. watersi*.

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