

1 Combined ~~E~~ffect of ~~M~~illet-~~C~~owpea ~~I~~ntercropping and ~~A~~pplication of ~~A~~queous ~~N~~eem ~~S~~eed
2 ~~E~~xtract on the ~~M~~anagement of the ~~M~~illet ~~H~~ead ~~M~~iner, *Heliocheilus albipunctella* ~~D~~e
3 Joannis (Lepidoptera: Noctuidae), in Burkina Faso
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25 Abstract

26 Pearl millet, *Pennisetum glaucum* L. R. Br. (Poales: Poaceae), the main cereal crop in the
27 Sahelian zone of Burkina Faso, is attacked by several insect pests, among which is the millet
28 head miner, *Heliocheilus albipunctella* De Joannis (Lepidoptera: Noctuidae). Damage and
29 yield losses caused by *H. albipunctella* on millet range from 30.00% to 85.00%. Control and
30 management of *H. albipunctella* currently rely on synthetic insecticides, which are harmful to
31 human and environmental health. Hence, there is a need to explore and develop alternative
32 management strategies. ~~Consequently the current research, which was held, we explored the use~~
33 ~~of millet-cowpea intercropping a very common practice in the Sahelian zone of Burkina Faso~~
34 ~~together with the application of a plant-based biopesticides made of Neem (Azadirachta indica~~
35 ~~A. Juss. (Sapindales: Meliaceae) seed kernels aqueous extracts.~~

36 Here add the study location& seasons to the abstract

37 Add also or mention short on the laboratory held experimentation

38 ~~The obtained results~~ We found that the application of neem extracts on cowpea plants at the
39 flowering stage, synchronized with the heading stage of millet, significantly reduced the
40 incidence of *H. albipunctella*. When millet was intercropped with cowpea, the application of
41 aqueous extracts of neem indirectly led to a significant reduction of about 50.00% in the number
42 of larvae per spike. Additionally, a reduction in the percentage of millet spikes attacked, a
43 decrease in mine length, and a gain in grain yield of more than 40.00% were observed. Thus,
44 ~~the findings from the application of this agricultural practice used cultivation of millet in~~
45 ~~association with cowpea, combined with the application of aqueous extract of neem could be a~~
46 promising control option against *H. albipunctella*.

47 **Keywords:** Pearl millet, Cultural control, Aqueous neem seed extract, *Heliocheilus*
48 *albipunctella*, Burkina Faso

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59 1. Introduction

60 Pearl millet, ~~,~~ is an ancestral cereal of critical importance in agriculture and nutrition for the
 61 Sahelian populations of West Africa (Gahukar & Ba, 2019; Dupuy, 2017). ~~It is cultivated~~ in the
 62 arid and semi-arid climatic conditions of the continent, millet is a vital resource for many rural
 63 communities (Shelke and Chavan, 2010). In Burkina Faso, ~~P~~earl millet is the third most
 64 produced cereal after maize and sorghum with an estimated production of 907,745.00 tons
 65 (DGESS, 2023). Its high-protein content, energy value, vitamin and mineral composition are
 66 higher than those from other cereals such as wheat and maize (Parthasarathy-Rao et al., 2006).
 67 In the Sahelian region of Burkina Faso, millet is the ~~most~~ widely cultivated crop, ~~a~~ covering
 68 almost 80.00% of areas under cultivation due to its resistance to extreme climatic conditions
 69 and the dietary habits of the population (Saidou, 2011; Gahukar and Ba, 2019). *H.*
 70 *albipunctella*, is ~~the insect~~ pest that causes enormous damage to millet spikes in many sub-
 71 Saharan African countries, particularly Burkina Faso (Ndoye, 1991; Amadou et al., 2017;
 72 Gahukar and Ba, 2019). Damage is observed every year and is caused by larvae with ~~a~~ grain
 73 yield losses of between 30.00 and 85.00% (Kaboré et al., 2017; Gahukar and Ba 2019; Oumarou
 74 et al., 2019). Depending on the agroecological zones, millet is generally grown in association
 75 with several legumes, in particular cowpea, *Vigna unguiculata* L.Walp. (Fabales: Fabaceae)
 76 (Boly et al., 2022). This type of intercropping system is practiced by farmers ~~to control~~ diseases,
 77 weeds, and pests (Lawane et al., 2010; Guo et al., 2020). Likewise, it is used to increase the
 78 yield of cereals (Zoundi et al., 2007, Trail et al., 2016; Namatsheve et al., 2020). This
 79 intercropping is sometimes used in combination with other phytosanitary treatments of cowpea,
 80 including the use of synthetic chemicals to control insect pests. However, the use of pesticides,
 81 comes with the risks it poses to human, ecosystems, the environment, and loss of biodiversity
 82 (Carpentier, 2010, Barzman et al., 2015). Beyond the economic and environmental
 83 consequences, the massive and prolonged use of synthetic insecticides has also led to the
 84 development of ~~resistance~~ in several ~~insect~~ pests (Martin et al., 2000; Siddiqui et al., 2023).
 85 Considering all these reasons, research in Burkina Faso like in most part of the world, has over
 86 the last decade being geared towards the search for more ecofriendly strategies for managing
 87 insect pests, namely; biological control through the use of parasitoids (Ba et al., 2014; Kaboré
 88 et al., 2017), bio-pesticides and cultural practices. Thus, biopesticides associated with cultural
 89 practices could constitute an effective and promising alternative in the management of insect
 90 pests. Among the common biopesticides available, extracts from neem, *Azadirachta indica* A.
 91 Juss, containing Azadirachtin (Shafiq et al., 2012; Kpindou et al., 2013) as its active ingredient

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102 has emerged as one of the most commonly used repellent due to its antifeedant effects on insects
103 (Ngom et al., 2018; Bonni et al., 2018). In addition to its repellent and insecticidal effect, it also
104 has little or no effect on non-target species and has a low impact on the environment and
105 biodiversity (Haseeb et al., 2004; Sanon et al., 2005). Indeed, it is used to control and manage
106 more than 400.00 insect pest species associated with crops (Tanzubil, 2000; Malick et al., 2008;
107 Younoussou et al., 2021). However, it has not been used indirectly or in combination with other
108 management strategies for cowpea or against *H. albipunctella*. Hence the reasons for this study
109 were to evaluate the effectiveness of cultural control specifically intercropping in association
110 with the biopesticide neem extract for the control and management of *H. albipunctella* in
111 Burkina-Faso/, With the specific aims of evaluating the indirect effect of cowpea treatment on
112 the number of larvae per spike, *H. albipunctella* incidence and the grain yield of millet. It is in
113 this logic that the present study, which aims

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114 2. Material and methods

115 2.1.Study location

116 The study was conducted in Burkina Faso, in the communes of Djibasso (alt: 1191; N:
117 13°05'34.0; W: 004°12'57.2) and Dori (alt: 932; N: 14°01'41.8; W: 000°00'34.9), during the
118 2021 rainy season. These two communes, Djibasso and Dori, are located in the Kossi and Seno
119 provinces, respectively. Pearl millet is the main cereal crop in these two provinces and covers
120 almost 78.00 and 80.00% of the cultivated area, respectively (DGESS, 2023), and it is often
121 intercropped with cowpea (Boly et al., 2022). The experimental plots were established in the
122 villages of Bouakuy, located about 10 km from Djibasso, and Hoggo Sambowel, located about
123 10.00 km from Dori (Fig. 1). The cumulative rainfall from May to December was 827.00 mm
124 in Djibasso and 557.00 mm in Dori. The relative humidity fluctuated between 53.16 - 91.44%
125 in Djibasso and between 44.25-83.00% in Dori. While average monthly temperatures fluctuated
126 between 25.00 and 32.00 °C. The vegetation is mostly covered with annual grass species, with
127 areas of woodland and shrubland in which the dominant trees are Acacia species, *Balanites*
128 *aegyptiaca* (Zygophyllales: Zygophyllaceae), *Faidherbia albida* (Fabales: Fabaceae),
129 *Combretum glutinosum* (Myrtales: Combretaceae), *Guiera senegalensis* (Myrtales:
130 Combretaceae), and *Piliostigma reticulatum* (Fabales: Fabaceae), (Lykke et al., 2004). The soil
131 in both communes is sandy (Fontès and Guinko, 1995).

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(alt: 932; N: 14°01'41.8 W: 000°00'34.9"

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133 2.2.Experimental design

139 The experimental design is composed of a divided plot (= Split Plot) made up of three sub-
 140 blocks (= Large plots). Each sub-block is composed of twelve (12.00) sub-plots (= Small plot).
 141 The sub-plots were each ~~a~~ 9.60 m × 9.60 m ~~in dimension~~. This type of design provides the
 142 possibility of evaluating two factors in the system namely, the type of cropping association
 143 (primary factor) and the phytosanitary treatment (secondary factor). The cropping system
 144 consists of four combinations (i) millet (MP), (ii) cowpea monocultures i.e. as single main crop
 145 (NP), (iii) an intercrop consisting of two rows of millet and a single row of cowpea (2M-1N)
 146 and (iv) farmers' practices (PP, one planting of cowpea between four plantings of millet). The
 147 four cropping systems form the main plot (Σ MP + NP+ 2M-1N+ PP). The phytosanitary
 148 treatment assigned to each main plot (Σ MP + NP + 2M-1N + PP) at each large plot level
 149 consisted of three combinations: (i) an aqueous neem seed extract, which contains a variety of
 150 bioactive compounds. Among these, azadirachtin (Tetranortriterpenoid limonoid, C₃₅H₄₄O₁₆)
 151 meliantriol (Triterpenoid limonoid, C₃₂H₅₀O₈), and salannin (Triterpenoid limonoid, C₃₄H₄₄O₉)
 152 play key roles in the tree's insecticidal and repellent properties (Saxena, 1989); (ii) a synthetic
 153 insecticide (Lambda-Cyhalothrin 15.00 g/l + Acetamiprid 20.00 g/l); and (iii) a no-treatment
 154 control. Each of the cropping systems was replicated three times in each of the sub-blocks. The
 155 spacings between rows and pockets in each cropping system were 0.80 m × 0.80 m for millet
 156 and 0.80 × 0.40 m for cowpea. A distance of 2 m and 6 m was allowed between two sets of
 157 cropping systems and between sub-blocks, respectively in order to prevent effects of treatments.
 158 For the millet, ~~we used~~ the local variety that the farmers use in each locality ~~was used~~, while
 159 the cowpea variety Komcallé was sown 20 days after the millet was sown, ~~mullein~~ order to
 160 synchronize the cowpea (45 days after sowing) with ~~the~~ millet heading stage. It is at this stage
 161 that *H. albipunctella* females prefer to lay their eggs on the spikes (Gahukar et al., 1986). The
 162 sub-plots were thinned to two (2.00) millet plants per pocket at the first weeding, three weeks
 163 after sowing. A microdose of fertilizer consisting of 5.00 g of NPK (14/23/14; 100Kg/ha) per
 164 pocket was applied to both millet and cowpea after weeding followed by an application of urea
 165 (46% N; 50Kg/ha) to 3.00 g / pocket of millet at the time of the millet bolting.

166 To prepare the neem aqueous extract, the neem seeds were collected under the trees, stored, and
 167 dried in the shade for 4 to 5 weeks. Next, they were ground into a fine powder, and this powder
 168 was macerated in water for 24 hours. Finally, the macerated material was filtered to obtain the
 169 extracts (Dabiré-Binso et al., 2008).

170 For the neem seeds extract treatment subplots, a formulation made up of 500.00 g per ten (10)
 171 L (w/v) of water per 400.00 m² was applied three times weekly (Dabiré-Binso et al., 2008).

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175 While the plots for treatments with synthetic insecticide, a mixture of Lambda-cyhalothrin,
176 15.00 g/l, and Acetamiprid, 20.00 g/l was applied twice at a concentration of one (1) liter/ha,
177 two weeks apart.

178 2.3.Data collection

179 Data collection started at the doughy grain stage of millet from each sub-plot and sub-block
180 until harvest (time in weeks). The number of *H. albipunctella* larvae per spike, the damage
181 caused by *H. albipunctella* (number and length of mines per spike and number of spikes
182 attacked) and the grain yield were recorded. To determine the number of larvae per spike, an
183 area of 1 m² was delimited in each sub-plot and the number of larvae is counted on each spike
184 contained in each surface and repeated four times. while the number of attacked spikes (spike
185 bearing at least one mine), the number and length of mines per spike and the grain yield, an
186 area of 9.00 m² was determined at each sub-plot. Thus, the number of attacked spikes, the
187 number, and length of mines per spike and the grain yield were determined. The length of each
188 mine was measured using a measuring tape. At harvest, the millet spikes from each area were
189 threshed and weighed. The percentage of attacked spikes (PAE) and the grain yield (GY) were
190 calculated for each sub-plot of each sub-block using equations 1 and 2:

191 $PAE (\%) = (NSA/TNS) \times 100.00$; where NSA: Number of spikes attacked per
192 delimited area and TNS: Total number of spikes. Equation (1)

193 $GY \left(\frac{kg}{ha} \right) = \left(\frac{GW}{S} \right) \times 10,000.00$; where GW: Grain weight of millet in kg per delimited area
194 and S: delimited area in m². Equation (2).

195 2.4.Data analyses

196 The data collected were analyzed using a two-way analysis of variance (Factorial ANOVA) to
197 examine the influence of the phytosanitary treatments and the cropping systems (two
198 independent variables) on the studied parameters. Post hoc tests were performed using the
199 'lsmeans' package in RStudio (Lenth, 2016) when factorial ANOVA was significant between
200 groups. The visualize the data, box plots and figure were plotted using R and Excel,
201 respectively. All statistical analyses were carried out using R (software version) and level of
202 significance set at 5% for all statistical analyses.

203 3. Results

204 3.1.Influence of cropping system and phytosanitary treatment on the parameters 205 studied

We found that phytosanitary treatment has a significant influence on the number of larvae per spike, *H. albipunctella* damage and grain yield (Table I). The average percentage of damaged and the average number of damaged per spike were influenced by the cropping system (Table I). On the other hand, the interaction between cropping system and phytosanitary treatments did not affect these parameters (Table I).

3.2. Number of *H. albipunctella* larvae per spike according to phytosanitary treatments

The average number of larvae per spike of millet varied significantly depending on the location of the treatment (Djibasso ANOVA, $F_{2,439} = 2.94$; $P = 0.003$; and Dori ANOVA, $F_{2,416} = 2.39$; $P = 0.01$; Fig 2). Regardless of the commune, it was greater in the sub-block that received no treatment (Fig 2). In Djibasso, the average number of larvae per spike obtained with neem aqueous extract (1.33 larvae/spike) was significantly lower compared to treatment with Synthetic insecticide (where to find the result ie which figure and then the statistics, see above (ANOVA, $F_{2,439} = 2.94$; $P = 0.003$). In contrast, no significant difference was observed between the neem aqueous extract treatment and Synthetic insecticide in the commune of Dori (Fig 2).

3.3. Damage by *H. albipunctella* according to phytosanitary treatments

The average percentage of millet spike attacked by *H. albipunctella* was significantly greater at the level of the control treatment compared to those of the aqueous extract of neem and Synthetic insecticide in the communes of Djibasso (ANOVA, $F_{2,26} = 6.26$; $P = 0.0006$) and Dori (ANOVA, $F_{2,26} = 6.95$; $P = 0.0003$) (Table II). In addition, treatments with aqueous neem extract and Synthetic insecticide reduced the average percentage of millet spike attacks by about 50.00% in both study sites. Regarding the average number of mines per spike, it varied significantly with location (Djibasso, ANOVA, $F_{2,404} = 8.21$; $P < 0.0001$) and Dori (ANOVA, $F_{2,90} = 1.28$; $P = 0.2666$) (Table II). In Djibasso and Dori, the average number of mines per spike was lower from neem aqueous extract treatments in comparison to those from Synthetic insecticide and the control (Table II). The mean length of mines, which reflects the extent of *H. albipunctella* damage, varied significantly at the level both communes (Djibasso ANOVA: $F_{2,404} = 8.06$; $P < 0.0001$; Dori ANOVA: $F_{2,90} = 2.33$; $P = 0.0394$; Table II). In addition, in Djibasso and Dori, the mean length of mines per spike was lower at the level in the neem aqueous extract treatment than those of the Synthetic insecticide and the control (Table II).

3.4.Effect of the cropping system on the percentage millet per spike attacked and the number of mines per spike in the commune of Djibasso

The percentage of millet spikes attacked and the number of mines per millet spike were significantly influenced by the cropping association system in the commune of Djibasso (Table III).

The highest and lowest significantly different percentages of damaged millet spike were observed on millet grown alone and on the combination of two rows of millet and one row of cowpea (ANOVA, $F_{2, 24} = 6,16$; $P=0,01$; Table III).

The average number of mines per spike was significantly higher in the farmer's practice compared with millet grown alone and the combination of two rows of millet and one row of cowpea (ANOVA, $F_{2, 132} = 6,16$; $P=0,002$; Table III).

3.5.millet grain yield by phytosanitary treatments

Grain yield of millet varied significantly at the level of commune of Djibasso (ANOVA, $F_{2,24} = 3.53$; $P= 0.0096$; and Dori: $F_{2,24} = 2.34$; $P = 0.00473$; Fig 3). In contrast, regardless of the commune, millet grain yield was statistically similar between the neem aqueous extract and Synthetic insecticide treatments (Fig 3). The phytosanitary treatment, in both communes, made it possible to obtain more than one ton of millet per hectare compared to the control treatment which was less than one ton (Fig 3).

4. Discussion

Millet cultivation, whether in monoculture or in association with cowpea, is a recurrent practice in Burkina Faso (Zoundi et al., 2007; Boly et al., 2022). This combination is sometimes accompanied by treatment of the cowpea with a synthetic insecticide or biopesticides to control insect pests of the cowpea crop. In contrast, the indirect effect of cowpea treatment on cereal insect pests associated with cowpea is sometimes overlooked.

265 In this study, we evaluated the indirect effect of cowpea treatment when intercropped with
266 millet on the main pest of millet, *H. albipunctella*, in the field conditions. Our results show that
267 the application of the aqueous extract of neem seeds on cowpea grown in association with millet
268 at the time of heading gives similar results to those treated with the synthetic pesticide (Lambda-
269 Cyhalothrin 15 g/l + Acetamiprid 20 g/l). These findings show a significant reduction in the
270 number of *H. albipunctella* larvae per millet spike, the percentage of spikes attacked, and the
271 length of mines in treated plots compared to the control. Synchronization cowpea treatment
272 with the millet heading stage significantly reduced the activity of *H. albipunctella* females on
273 treated sub-plots. This reduction in female activity led to lower infestations of millet spikes.
274 Indeed, infestation of millet by *H. albipunctella* is conditioned by a synchronization between
275 the period of heavy outbreaks of *H. albipunctella* and the sensitive stage of millet corresponding
276 to the beginning heading stage (Vercambre, 1978; Bal, 1988; Kaboré, 2018). Likewise, *H.*
277 *albipunctella* females prefer to lay their eggs at the heading stage, precisely at the top of millet
278 spikes, with hatching occurring 3 to 4 days after oviposition (Ndoye, 1991; Gahukar, 1984).
279 The reduced infestation observed in the treated sub-plots may be attributed to the bioactive
280 compounds in neem extract, such as azadirachtin, meliantriol, and salannin, which possess
281 repellent, insecticidal, and fertility-reducing properties (Jacobson and al., 1978; Ascher, 1993;
282 Rembold, 1989). These compounds, with their repellent properties, can disrupt insect
283 communication and disorient pest insects. They also regulate insect growth by affecting egg-
284 laying behavior (Strickman, et al., 2009; Guèye et al., 2011). The efficacy of neem extracts
285 against insect pests, particularly Lepidoptera, has been widely documented (Cherry et al., 2010;
286 Boni et al., 2018; Ngom et al., 2018; Yao et al., 2022). Similarly, the repellent properties of
287 neem extract against insect pests have also been demonstrated in beekeeping. Spraying neem
288 leaf extracts around hives within a radius of five (5) meters, both before and after insect
289 colonization, resulted in a significant reduction in the number of insects colonizing the hives
290 (Gbedomon et al., 2012). Indeed, the repellent property observed with the treatment of neem
291 extract could be attributed to the volatile compounds contained in *A. indica* (Belder Den et al.,
292 1998). These volatile compounds, present in neem seed extract, can deter females from locating
293 their egg-laying sites, especially as their effect can last in the fields for 4 to 8 days depending
294 on environmental conditions and the plant species treated (Schmutterer, 1990). The volatile
295 compounds of neem are believed to act as an inhibitor, blocking the stimuli emitted by millet
296 spikes, thereby deterring female *H. albipunctella*. Similar results were observed in Mali by
297 Passerini in 1991. These authors reported a significant reduction in the number of eggs and
298 mines of *H. albipunctella* after treating millet fields with an aqueous neem powder extract

(Passerini, 1991). Moreover, volatile compounds are used in locating oviposition sites by females of *H. albipunctella* (Hall et al., 2001; Green et al., 2004). Where the females prefer to lay eggs on spikes that have only emerged at 30.00% (Ndoye, 1991) which release high amounts of volatile compounds such as borneol that attract females. Thus, the mixture of volatile compounds from neem extracts with those emitted by the spikes in the same field of millet must have confused the females, who could no longer locate the spikes of millet for oviposition. This would explain the low number of larvae observed in the treated plots. Heavy rainfall can also reduce the density of *H. albipunctella* larvae, especially 1st and 2nd instars, which walk on the spikes. Indeed, the application of neem extract in the field resulted in a considerable reduction in the population density of *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), *Earias spp.*, *Diparopsis watersi*, Roths. (Lepidoptera :Noctuidae) *Spodoptera littoralis* (Lepidoptera :Noctuidae), and *Syllepte derogata* Fabricius (Lepidoptera : Crambidae) (Misra et al., 2002; Nboyine et al., 2013; Douro et al., 2013).

We also found a significant reduction in the incidence of *H. albipunctella*, which were lower in the treated sub-plots than in the untreated ones. This reduction could also be explained by a low infestation of spikes observed in these sub-plots at the time of heading. Damage by *H. albipunctella* is mainly caused by larvae feeding on the floral organs, perforating the glumes, cutting the floral and fruit peduncles causing them to dry out (Vercambre, 1978). This result corroborates those of Ndoye (1979), who stipulates that the extent of damage caused by *H. albipunctella* on the millet crop would strongly depend on the coincidence between the flight of the adults and the period when earing begins in millet and the density of the larval population. making the effectiveness of phytosanitary treatment at the early heading stage against *H. albipunctella* very critical. Which is why the application of endosulfan, Decis ULV (dimethoate + deltamethrin) and trichlorfon (dipterex + SI 8514) at early heading were effective against the *H. albipunctella* (Vercambre, 1978; Gahukar, 1984; Guevremont, 1982). However, considering the harmful effects of these synthetic chemicals on humans, the environment, and biodiversity, it is advisable to use these products only if there is no alternative solution. Other groups of insect pests have been the subject of similar study. According to Adda et al. (2011), sowing *Hyptis suaveolens* (L) on the edges of maize fields halved the percentage of plants infested by *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae).

In terms of the cropping system, the combination of two rows of millet and one row of cowpea resulted in a significant reduction in the percentage of millet spikes that were attacked and the average number of mines per spike. This reduction can be attributed to the diversity of the

landscape, which not only disorients females in finding oviposition sites, but also provides a refuge for natural enemies. Indeed, pests may lose the ability to locate host plants when confronted with a mixture of several volatiles from non-host plants. This hypothesis supports the assertion that volatile compounds are important for the location of oviposition sites of the MEM (Hall et al., 2001; Green et al., 2004).

The results of the present study also showed the effectiveness of neem extract in increasing grain yield of millet by more than 40.00%. The increase in grain yield observed in the treated plots could be explained by a low number of *H. albipunctella* larvae. Indeed, according to Gahukar and Ba (2019), Oumarou et al. (2019), yield losses due to *H. albipunctella* larvae attacks without any external intervention in the Sahelian zone are estimated between 30.00 and 80.00%. The results of this study show that, in the context of integrated pest management, treating cowpea in association with millet using neem seed extract can protect the millet crop against *H. albipunctella*.

Conclusion

This study on the efficacy of the crop association associated with neem extract in the control of the millet head miner, *H. albipunctella* showed a particular interest in the importance of treating cowpea in association with millet in the field. Indeed, the use of aqueous neem seed extract on cowpea in combination with millet significantly reduced the infestation of millet by *H. albipunctella*. This resulted in a significant reduction in the percentage of millet ears attacked, the number and length of mines, and an increase in productivity. For these reasons, the use of this biopesticides in the treatment of cowpea in association with millet could constitute an alternative control method for the control of the millet head miner in the Sahelian zone.

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