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Effect of aqueous extracts of two local plants for the management of *O. mutabilis* (Coleoptera: Chrysomelidae), pest of cowpea (*Vigna unguiculata* L. Walp.), in Guinean area of Côte d'Ivoire

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Abstract
Ootheca mutabilis is a cowpea pest (*Vigna unguiculata* L. Walp) that causes significant damage to this crop. Control tests with two aqueous extracts of two local plants and Cypercal were carried out on this pest in Adzopé located in the south of Côte d' Ivoire. The experimental design used was a randomized block with three replications consisting in total of four subplots corresponding to three treatments and untreated control. The lowest mean numbers of *O. mutabilis* per plant, as well as percentages of damaged leaves and foliar damage indices were recorded on plots treated with aqueous extract of *Ricinus communis* seed capsules at 70 g/l and chemical insecticide at the stage before flowering, flowering and podding stages. During these three phenological stages, the percentage of the reduction of damaged leaves obtained after application of the aqueous extract of capsules *R. communis* seed capsules and Cypercal were higher than those obtained on the subplot treated with the extract of *Azadirachta indica* leaves at 70 g/l. The numbers of pods harvested which were 22.85 ± 0.64 and 22.95 ± 0.63 per plant obtained with seed capsule extracts at 70 g/l and Cypercal respectively were the highest. The yield that was 914.31 ± 28.16 kg/ha after application of the aqueous extract of capsules *R. communis* seed capsule was as high as that the one obtained with Cypercal (921.35 ± 28.16). Treatments with the aqueous extract of *R. communis* seed capsule improved cowpea yield more than 180% as high as with Cypercal.

Keywords: *Ootheca mutabilis*, cowpea, aqueous extract, yield, seed capsule of *Ricinus communis*

1. Introduction
Cowpea is of very vital importance to the livelihood of several millions of people especially in the central and western part of Africa [1]. It is truly a multifunctional crop, providing food for man and livestock and serving as a valuable and dependable revenue-generating commodity for farmers and grain traders [2, 3]. Cowpea, like other grain legumes improve soil fertility through biological nitrogen fixation and increases soil conservation through greater ground cover [4]. Despite its importance, cowpea remains a marginal plant in Côte d' Ivoire [5].This situation is due to competition from the organized agricultural sector (cocoa, coffee, cashew) and parasite pressure. However, the local variety commonly called "Touba" variety, adapted to the climatic conditions of the Guinean area could contribute to the diversification of the country's economic resources. However, the major entomological constraint of this area is *O. mutabilis*. According to [6, 7, 8], it is the major pest of cowpea during the stage before flowering. In Côte d' Ivoire, the methods used to control insect pests of cowpea in a peasant environment are essentially chemical. Unfortunately, their use poses the following major problems: increase in insect resistance, disappearance of insect populations, pollution of surface water and groundwater, neutralization of soil life [9, 10]. It is therefore important to look for alternative control methods able to control the population of *O. mutabilis* and for environmentally friendly agricultural production. With this in mind, we plan to evaluate the effect of aqueous extracts of two local plants (*Azadirachta indica* leaves and *Ricinus communis* seed capsules) in controlling *O. mutabilis* population's.

2. Material and methods
The plant material is the cowpea (*Vigna unguiculata* L. Walp) of a variety commonly called in Côte d'Ivoire "Touba". The cycle duration of this variety varies from 66 to 70 days.

2.1 Study area
The study was conducted in Adzopé (06°10' of north latitude and 3°87' west longitude) located in the south of Côte d'Ivoire. The subequatorial climate is characterized by four seasons [11, 12]: a long dry season from December to March. a long rainy season, from April to mid-July; a small dry season, from mid-July to mid-September; a small rainy season, from mid-September to November. The study period is extended from May to July 2016 with average temperatures oscillating between 25.7 and 28.2 ° C, relative humidity between 84 and 87.5% and a rainfall of 418.5 mm.

2.2. Preparation of aqueous extracts
The leaves of *Azadirachta indica*, and seeds capsule of *R. communis* seeds were harvested in the locality of Adzopé. These leaves and seeds capsule were dried in the shade for three to four weeks. Leaves and seeds capsules were then crushed by a blender for to obtain powder. For each plant 100 g of powder obtained by organ was diluted in 200 ml of distilled water. The powder and distilled water were then

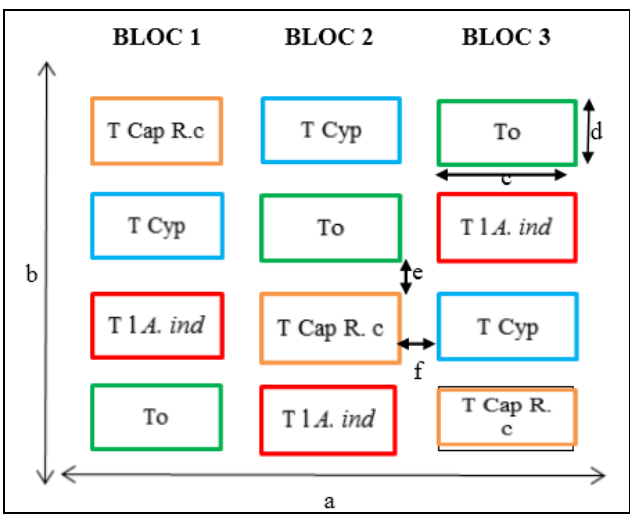
homogenized in the mixer for ten minutes. The result mixture was then filtered using muslin. Two others filtrations were made respectively with Whatman paper (3MM) and a funnel containing cotton. The product obtained in these three filtrations was put in melam plates, and concentrated by evaporation in an oven set at 50 °C for 48 hours until a dry residue. The dry residue obtained by organ has permitted to prepare the concentration of aqueous extracts. These concentrations were used to treat the plants of subplots.

2.3 Preparation of the insecticide

The chemical insecticide used is Cypercal (50 EC, AF-CHEM SOFACO, Côte d' Ivoire). It is an emulsifiable concentrate containing 50 g of active ingredient Cypermethrin ($C_{22}H_{19}Cl_2NO_3$) per liter. The recommended dose is 40 ml in 15 l for Cypercal corresponding to a concentration of 0.093 g/l.

2.4 Experimental design

The experimental design is a randomized block with three repetitions. The experimental plot (Figure 1) has an area of 492.8 m² (22.4m x 22m). It is divided into 3 blocks separated from each other of 2 m. Each block consists of 4 subplots corresponding to 3 treatments ((T Cyp, T l *A.ind* and T Cape R. c,) and control plot untreated (To) .Two consecutive subplots are separated by 2 m for to avoid contamination during treatments. In each subplot, seedlings are arranged in four rows of 5.4 m length separated from each other by an interval of 0.6 m. The agricultural practice used is the seedling planting hole with a spacing of 0.60 m between the lines. Thinning to one plant by hole was realized 15 days after sowing. Each subplot had 6 rows of 10 plants corresponding 60 plants per subplot (Figure 1).



a = 24.2 meters; b = 22 meters; c = 5.4 meters; d = 3 meters; e = f = 2 meters

a: length of the plot ; b: wide of the plot ; c : length of the subplot ; d : wide of the subplot ; e : distance between the subplots ; f : distance between the blocks;

To: Control plots, T l *A.ind*: Subplots treated with aqueous extracts of *Azadirachta indica* leaves at 70 g / l, T cap R. c: Subplots treated with aqueous extracts of *Ricinus communis* seed capsules 70 g / , T Cyp: Subplots treated with Cypercal 50 EC.

Fig 1: Experimental design

2.5 Application of treatments

For treating the plants, three hand sprayers with a capacity of one liter were used. One sprayer labeled T CYP was used to apply the Cypercal 50 EC (chemical insecticidal). The others two sprayers labeled T l *A. ind* and T Cap R.c were used to apply respectively aqueous extracts of *A. indica* leaves and seed capsules of *R. communis*. For the treatment to be homogeneous, the jets were oriented so as to cover the lower and upper faces of the leaves of each plant. The treatments with aqueous extracts were carried out on 24, 31 and 38 days after sowing (DAS) at the stage before flowering, 45 DAS at flowering stage and 52, 59 DAS during the podding stage.

2.6 Evaluation of the efficacy of aqueous extracts on adults of *O. mutabilis*

The observations were made on the central plants of each subplot [5, 13, 14]. Thus, 32 plants in inner rows (4 central lines of 8 plants) were inspected.

A survey was conducted one week before treatments (20th day after sowing) and the other surveys three days after each treatment. At each survey, adults of *O. mutabilis* were counted early in the morning (6:00 to 9:00 am) directly on the leaves of plants [6, 13, 15]. At this time of the day, the insects were generally found on the leaves and had reduced mobility.

To evaluate the damage, the number of damaged and undamaged leaves was also determined. The percentage of damaged leaves and percentage of reduction of damaged leaves has been calculated.

$$\text{Percentage of damaged leaves} = \frac{\text{Number of damaged leaves}}{\text{Total number of leaves}} \text{X } 100$$

$$\text{Pr} = \frac{\text{Nc} - \text{Nt}}{\text{Nc}} \text{X } 100$$

Pr = Percentage of reduction of damaged leaves; Nc: Number of damaged leaves in the control plot; Nt: Number of damaged leaves in the treated plot

To quantify the severity of damage, foliar damage was assessed on a visual foliar damage scale of 0 to 5 proposed by [16] where 0 = no damage; 1 = 1-5% damage; 2 = 6-25% damage; 3 = 26-50% damage; 4 = 51-75%; and 5 = 76-100% damage.

Production was evaluated through the number of pods harvested on the 32 plants. After counting, the pods were sun-dried and hand-beaten. Then after winnowing and sorting, the seeds were weighed. The grain yield and the yield gain rate were calculated from the following formula proposed by [17].

$$\text{Grain yield (Kg/ha)} = \frac{\text{Yield weight (Kg)}}{\text{corresponding area (m}^2)} \text{X } 10\,000 \text{ m}^2$$

$$\text{Yield gain rate (\%)} = \frac{\text{Yield of treated plot} - \text{Yield of control plot}}{\text{Yield of control plot}} \text{X}100$$

2.7 Statistical analysis

All data obtained were subjected to an analysis of variance using the Statistica 7.1 software. The comparison of means was performed by the test of Student-Newman-Keuls at the 5% threshold.

3. Results

3.1 Effect of aqueous extracts on the number of *O. mutabilis* per plant before and after the different treatments following the three phenological stages

Before the treatments at 20th day after sowing (20th DAS), the mean numbers of *O. mutabilis* per plant were between 3.09 ± 0.24 and 3.42 ± 0.30 on subplots. Statistical analysis revealed no significant difference between mean numbers (F = 0.15; df = 3; P = 0.92) (Figure 2). During the stage before flowering, flowering and podding stages, the mean numbers of *O. mutabilis* per plant on the untreated plot were respectively 5.07 ± 0.24; 3.91 ± 0.44 and 1.32 ± 0.24. The extract of *A. indica* leaves and *R. Communis* seed capsules permitted to record at different phenological stages lower mean numbers compared with that those of the control plot. During the stage before flowering, the numbers were 1.03 ± 0.16 and 0.77 ± 0.12; at the flowering of 0.39 ± 0.08 and 0.28 ± 0.04 and podding stage of 0.32 ± 0.03 and 0.22 ± 0.12 respectively on plots treated with the aqueous extract of seed capsules at the concentration of 70 g/l and Cypercal were the lowest. Statistical analysis showed highly significant differences between mean numbers recorded at different phenological stages after application of aqueous extracts and chemical insecticide (F = 68.65; df = 8; P = 0.00) (Figure 2).

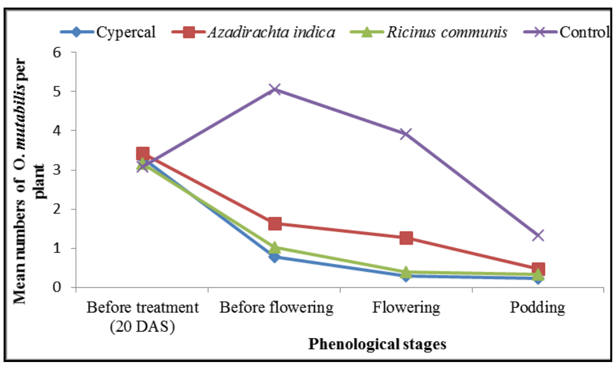


Fig 2: Evolution of the number of adults of *O. mutabilis* per plant before and after different treatments following the phenological stages

3.2 Effect of aqueous extracts on leaf damage of cowpea plants at different phenological stages

Percentage of damaged leaves

Before treatments, the percentages of damaged leaves on the subplots were between 84.11 ± 2.70 and 87.42 ± 1.36 %. Statistical analysis showed no significant difference between mean percentages (F = 0.44; df = 3; P = 0.73) (Table 1). The percentages of damaged leaves that were 92.88 ± 1.87; 95.05 ± 1.19 and 83.72 ± 2.65, recorded respectively during the stage before flowering, flowering and podding stage on untreated plot were the highest. With the chemical insecticide, the percentages were 39.57 ± 1.94; 27.03 ± 2.57 and 23.27 ± 1.13 respectively at the stage before flowering, flowering and podding stage. On the subplot treated with the aqueous extract of *R. Communis* seed capsules, the percentages obtained at different phenological stages were as low as those obtained with Cypercal. These percentages were 39.24 ± 1.30; 28.60 ± 1.18 and 24.45 ± 1.15 %. On the subplots treated with the aqueous extract of *A. indica* leaves, the percentages obtained at the three phenological stages were lower compared to those obtained with Cypercal at the different phenological stages (Table 1)

Percentage of the reduction of damaged leaves

The highest percentages of reduction of damaged leaves (55.73 ± 4,01 and 56.38 ± 0.90) during the stage before flowering, (65.74 ± 3.81 and 65.45 ± 2.74) at flowering stage and (67,27 ± 3,17 and 66,88 ± 1,92) during the podding stage were obtained respectively on subplots treated with Cypercal and the aqueous extract of *R. Communis* seed capsule. The percentages of the reduction of damaged leaves after application of aqueous extracts of *A. indica* leaves were lowest compared to those obtained on subplots treated with Cypercal and the aqueous extract of *R. Communis* seed capsule. Statistical analysis revealed significant differences in mean percentages of the reduction of damaged leaves at different phenological stages after application of aqueous extracts and chemical insecticide (F = 4.50; df = 8; P = 0.0038) (Table 1).

Table 1: Percentage of damaged leaves and percentage of reduction of damaged leaves before and after treatment of plants during the different phenological stages

Treatments		Phenological stages					
	Before treatment	Before flowering		Flowering		Podding	
	Percentage of damaged leaves	Percentage of damaged leaves	Percentage of the reduction of damaged leaves	Percentage of damaged leaves	Percentage of the reduction of damaged leaves	Percentage of damaged leaves	Percentage of the reduction of damaged leaves
Control	84.11±2.70 o	92.88±1.87 a	_	95.05±1.19 a	_	83.72±2.65 b	_
A. indica	85.77±2.19 o	45.35±1.15 c	48.88±2.56 b	38.33±0.73 d	59.52±3.80 ab	28.14±1.00 e	60.06±2.21 ab
R. communis	87.42±1.36 o	39.24±1.30 d	56.38±0.90 ab	28.60±1.18 e	65.45±2.74 a	24.45±1.15 e	66.88±1.92 a
Cypercal	85.86±1.58 o	39.57±1.94 d	55.73±4.01 ab	27.03±2.57 e	65.74±3.81 a	23.27±1.13 e	67.27±3.17 a

In the same column the means followed by the different letters are significantly different (Newman-Keuls test at the threshold of 5 %)

Foliar damage

Before the treatments, the foliar damage rating ranged from 1.84 to 1.91 (plants with less than 5% of the leaf area damaged). Statistical analysis showed no significant difference between foliar damage rating recorded on subplots before treatments (F = 0.20; df = 3; P = 0.89). During the stage before flowering, flowering and podding stages, the foliar damage ratings on the untreated plots were respectively 3.52 ± 0.18; 4.24 ± 0.14 and 4.14 ± 0.88 (more than 50% of the leaf surface of plants was consumed from flowering). The indices on subplots treated with the two aqueous extracts were less than 2 (fewer than 5% of the leaf area of plants was consumed). The smallest foliar damage rating during the different phenological stages ranging from 1.15 ± 0.09 to 0.41 ± 0.03 (less than 5% of the leaf area of plants destroyed) were recorded on subplots treated with Cypercal and the aqueous extract of *R. Communis* seed capsules at different phenological stages. Statistical analysis showed highly significant differences between foliar damage rating recorded on the subplots after treatments (F = 148.00; df= 11; P = 0.00) at the three phenological stages (Figure 3).

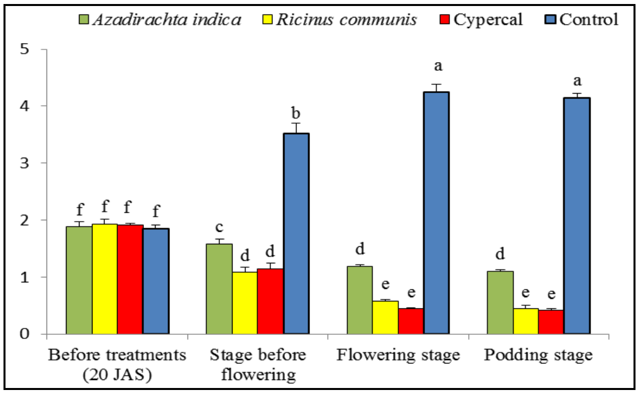


Fig 3: Foliar damage rating scale before and after treatments of the plants with the aqueous extracts of plants and Cypercal following the different phenological stages

3.3 Effect of treatments on the number of pods, yield and yield gain rate

Number of Pods

The mean number of pods harvested on the control plot was 7.86 ± 0.48 per plant. After application of the two aqueous extracts of plants, the number of pods harvested per plant was higher than that the one obtained on the control plot. The number of pods per plant harvested after treatment with the chemical insecticide was 22.95 ± 0.63. After treatment of plants with the aqueous extract of *R. communis* seed capsules at concentration of 70 g/l, the number of pods per plant (22.85 ± 0.64) was as high as that collected on the plot treated with Cypercal. Statistical analysis showed highly significant differences between the mean numbers of pods harvested per plant after spraying aqueous extracts and Cypercal (F = 119.95; df = 3; P < 0.001) (Figure 4).

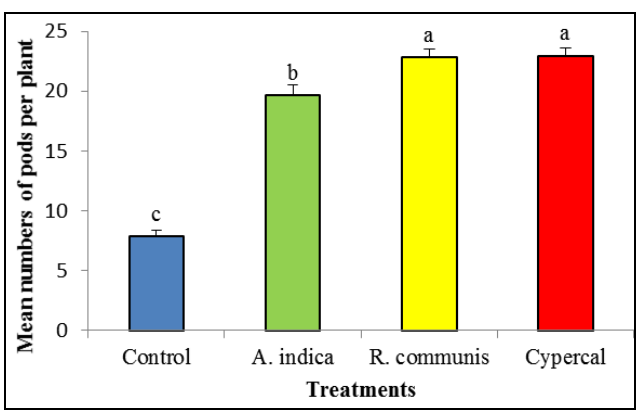


Fig 4: Number of pods per plant after application of different treatments

Yield

On the control plot, the yield was 321.76 ± 10.09 kg / ha. The two aqueous extracts permitted to obtain the higher yields than the control plot. On the subplot treated with chemical insecticide, the yield was 921.30 ± 32.41 kg / ha. The yield (731.48 ± 14.09 kg / ha) obtained after application of extract aqueous of *A. indica* leaves was lower compared to that the one recorded on the subplot treated with chemical insecticide. On the subplot treated with aqueous extract of *R. communis* seed capsule, the yield which was 914.35 ± 28.16 kg / ha was as high as the one obtained with chemical insecticide. Statistical analysis revealed highly significant differences between the yields obtained after spraying the aqueous extracts and Cypercal (F = 147.53, df= 3, P <0.001) (Figure 5).

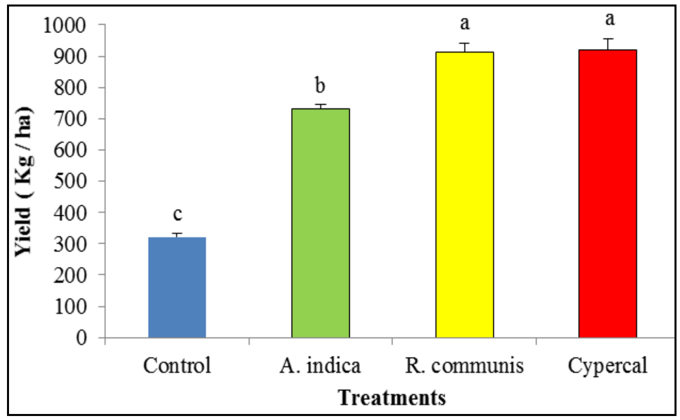


Fig 5: Yield after spraying of aqueous extracts and Cypercal

Yield gain rate

The yield gain rate obtained with the chemical insecticide was 186.55 ± 9.22%. With the extract aqueous of *R. Communis* seed capsule, the yield gain rate obtained (184.54 ± 10.06%) was as high as the one obtained in the plot treated with the chemical insecticide. The yield gain rate obtained with the extract of *A. indica* leaves was 127.61 ± 5.52 %. Statistical analysis revealed significant differences in yield gain rates obtained after spraying of aqueous extracts and Cypercal (F = 15.51; df = 2; P < 0.05) (Figure 6).

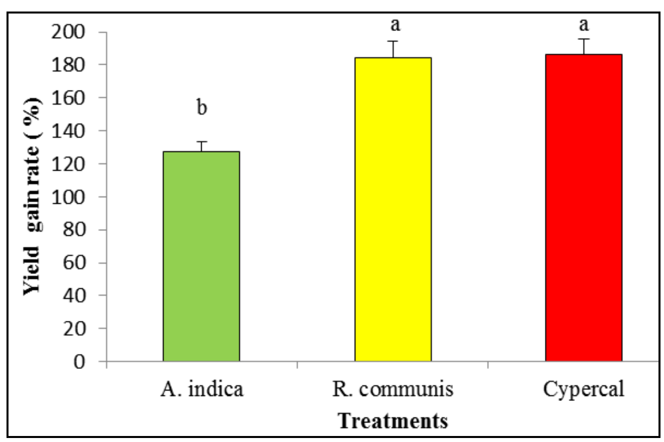


Fig 6: Yield gain rate after spraying of aqueous extracts and Cypercal

4. Discussion

During the three phenological stages, the numbers of *O. mutabilis* adults per plant recorded on subplots treated with aqueous extracts of *A. indica* leaves and *R. communis* seed capsule at 70 g/l were low than to that recorded on the untreated plot (control). The extracts reduced the infestation of subplots by the adults of *O. mutabilis*. Our results are in agreement with those of [15]. Indeed, these authors mentioned in their study on the evaluation of five spices (*Capsicum annum*, *Xylopia aethiopica*, *Piper guineense*, *Allium sativum* and *Zingiber officinale*) as biopesticides for the control of adult's *O. mutabilis*, a low number of insects on the plots treated compared to the control plot. [18] reported in their study in Nigeria on *Teilfeiria occidentalis* plants that the plots treated with neem (*Azadirachta indica)*) had low numbers of adults of *O. mutabilis* compared to the untreated plots. Small numbers of *O. mutabilis* on plots treated with the extract of *A. indica* leaves and *R. communis* seed capsules relative to that of the control would be related to the insecticidal and insect repellent properties of the substances contained in these extracts as reported by [19]. According to these authors, compounds such as quercetin, gallic acid, flavonoids and Kampferol present in the extracts of *R. Communis* are responsible for repulsion, deterrence and toxicity to pests. According to [20], active substances (Azadirachtin, Salanin, Nimbin, Nimbolin, Azadiradione) present in aqueous extracts of *A. indica* leaves have a toxic and repellent effect on insects. These compounds would have deterred adults from *O. mutabilis*, preventing them from staying on the crop for a long time or causing mortality in the population. The number of *O. mutabilis* on the plot treated with chemical insecticide was smaller than those obtained with *A. indica* leave extracts and the untreated plot. This would to be due to the active ingredient cypermethin (50g / l) present in the Cypercal 50 EC acts by contact and ingestion and has a neurotoxic activity on insects [21]. Several authors [22, 23, 24] reported the efficacy of cypermethrin on insect pests of cowpea. [25] also mentioned its effectiveness in controlling the adult population of Chrysomelidae *Podagrica uniforma* and *P. sjostedti* on okra. The numbers of *O. mutabilis* per plant obtained on plots treated with aqueous extract of *R. communis* seed capsule at the concentration of 70 g / l were as small as on the plot treated with Cypercal. Seed capsule extract at a concentration of 70 g / l controlled the population of *O. mutabilis* as much as Cypercal. Our results are similar to those of [26] who reported in their study in Nigeria on the cowpea that the leaves of *Tephrosia vogelii* had controlled the population of *O. mutabilis* as much as the chemical insecticide Decis.

The percentages of damaged leaves and foliar damage ratings on the treated plots were lower than those of the untreated plot. The reduction rates of damaged leaves ranged from 48 to 67%. Our results are different from those of [13] who reported that leaf extracts of neem (*Azadirachta indica*), Tephrosia (*Tephrosia vogelii*.) and papaya (*Carica papaya*) used did not reduce *Ootheca mutabilis* damage to the leaves during cowpea development compared to the untreated plot. This could be explained by the fact that the extracts used and the active chemical substances contained in them are different. In addition, the environmental conditions are not the same. The aqueous extract of *R. Communis* seed capsule at the concentration of 70 g/l significantly reduced leaf damage caused by *O. mutabilis* adults. This would be explained by the fact that these concentrations would contain a significant amount of active matter ricin, which effectively controlled the population of *O. mutabilis* and reduced leaf attacks. Several authors have reported that ricin induces irreversible inhibition of protein synthesis in insect cells by inactivation of ribosomes, causing cell death [27, 28, 29].

The mean numbers of pods per plant recorded on subplots treated with aqueous extracts and Cypercal were higher than that of the untreated plot. Our results rejoin those of [26, 30, 31, 15] who recorded high numbers of pods on treated plots compared to untreated plots. The low number of pods per plant obtained on the control plot could be explained by the fact that the leaves of these plants were severely damaged and defoliated (more than 50% of the surface of the leaves damaged) by the adults of *O. mutabilis* because these plants were not treated. This argument is consistent with the observations of [32] who reported that defoliation of cowpea plants at the stage before flowering and flowering stage caused a reduction in the number of pods. Grain yield of the control plot was low compared with to those of the plots treated with the aqueous extracts and the Cypercal. This could be explained by the fact that on the control plot, the plants had many damaged leaves and high foliar damage rating during the stage before flowering and flowering stage. This observation is in agreement with those of [33] who mentioned that the defoliation actions of pest, if they do not cause plants death, can reduce yield. [34] also reported that the decrease in grain yield of cowpea is due to plant defoliation by pests during its vegetative phase. Regarding the yield of the plot treated with Cypercal, many authors [35, 36, 37, 26, 30] indicated that chemical insecticides reduced pest infestation and increased yield. Grain yield and yield gain rate obtained on the plot treated with the aqueous extract of *R. communis* capsule seed at the concentration of 70 g / l were as high as those recorded on plot treated with the chemical insecticide. This is due to the fact that the extract aqueous of *R. communis* capsule seed extract at the concentration of 70 g / l controlled the population of *O. mutabilis* as well as Cypercal in the field.

5. Conclusion

This study permitted to evaluate the effect of two aqueous extracts of plants (*A. indica* leaves, and *R. communis* seed capsules) in controlling the population of *O. mutabilis*. The numbers of adults of *O. mutabilis* per plant recorded on the subplots treated with both extracts were lower than that of the untreated plot. Those recorded on subplots treated with *R. communis* seed capsules at the concentration of 70 g/l were as low as on the plot treated with Cypercal. The percentages of damaged leaves and foliar damage ratings of plants treated with *R. communis* seed capsule extract at 70 g / l and with Cypercal were lower compared to the plants treated with aqueous extracts of *A. indica* leaves. The largest numbers of pods per plant were obtained on plots treated with aqueous extracts of *R. Communis* seed capsules and the chemical insecticide. The best yields (more than 900 kg/ha) and the highest yield gain rates (over 180%) were also obtained with aqueous extracts of *R. Communis* seed capsules and Cypercal. The aqueous capsule extract of *R. Communis* could be used to effectively control the population of *O. mutabilis*. It would also provide better seed yields and healthier production. As a recommendation, application of treatments should be made earlier before the first adults of *O. mutabilis* appear in the first week after emergence of the plants.

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