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Study of different formulations based on *Crataeva religiosa* Forst as bioinsecticide against *Plutella xylostella* L. (Lepidoptera: Plutellidae) main cabbage pest

Toffène Diome*, Marie Cicille Ba Ghan, Cheikh Abdou Khadre Mbacké Dia, Mbacké Sembène
Genetic Team and Population Management, Department of Animal Biology, Cheikh Anta Diop University, Faculty of Sciences and Technology, Dakar, Senegal, Dakar, Sénégal

Abstract
Despite these advances, *P. xylostella* has maintained its status as a major cabbage pest, *Brassica oleracea*. Biopesticides have been shown to be effective against the moth and environmentally friendly. The objective is to test, in real environment the effect of an aqueous treatment with *Crataeva religiosa* on cabbage moth and on the weight of apples compared to different formulations and doses on plots located in the Niayes area. A block device with six treatments (aqueous extract of dry leaves at different concentrations C1, C2 and C3, aqueous extract of fresh leaves (FF) and soap extracts (S) and Crataeva extracts mixed with soap (SC = soap + Crataeva) and two controls are used due to one repeat per treatment. Results showed that untreated plots were more infested with *P. xylostella* larvae (P <0.001) and positively correlated with the number of affected leaves (r = 0.26, P <0.001), therefore indirectly related to the weight of the apples. The efficacy of treatment on the larval population of *Plutella* was more noticeable with the fresh leaf formulation followed by soap extract plus Crataeva. As for the dry leaves formulation, there was no significant difference compared to controls and no dose effect was noted.

Keywords: control, biopesticides, *brassica oleracea*

Introduction
Brassicaceae cultivation is one of the most important agricultural productions in the world (Arvanitakis, 2014)[1]. However, 80% of the horticultural sub-sector's production comes from the Niayes area (Sakho, 2013) [2] which is located in northwestern Senegal. Among the main crops that have long been cultivated there is the cabbage (*Brassica oleracea*). It's a consumer product in Senegal because it's consumed daily. In 2011, Senegal produced 50,000 tonnes of cabbage on an area of 2,444 hectares (Arvanitakis, 2014) [1]. It's the second largest producer of cabbage in West Africa after Niger. Despite the importance of this crop, its production is seriously affected by many pests, the main one is *Plutella xylostella* (Linné, 1758) (Ahmad *et al*., 2015) [3]. This species, native to the Mediterranean region (Hardy, 1938) [4], has become cosmopolitan and is found wherever cultivated and wild Brassicaceae are present (Shelton, 2001) [5]. The caterpillars of this micro-lepidoptera are defoliating and can cause up to 90% loss of production. The fight against cabbage moth was initially focused on the use of chemical substances but they soon showed their limits by encountering the amazing resistance capacity of insects. Currently, a return to biological origin insecticides, extracted from plants, is attempted. The objective of this study is to test in real-time the effect of a bioinsecticide based on *Crataeva religiosa* leaves in order to overcome the use of chemical insecticides.

Material And Methods
Experimental device
The establishment of the dry season cabbage variety "Copenhagen market" was made in collaboration with market gardeners. A 28m^2 plot divided into 14 elementary plots (PE) of 1.69m^2 each was used. The 14 PEs corresponded to 6 treatments and 2 controls. Phytosanitary treatments were carried out with aqueous extracts of *Crataeva religiosa* dry leaves at different concentrations (C1 = 200g / l, C2 = 150g / l, C3 = 100g / l), fresh leaves at 200g / l (FF) and soap extracts (S and SC). S was the water control + 25 grams of soap and SC mix water + 25 grams soap + 100g *Crataeva religiosa*. Each of the elementary plots (1.30m × 1.30m) had 3 rows of 4 cabbages spaced 0.20m apart on the lines and 0.20m between the lines.

Extraction of the biocidal substance
The plant organs used are *Crataeva religiosa* leaves. They were dried at room temperature for the dry leaf formulation. After drying, they were transformed into powder in a mortar. This powder was used for aqueous extractions of dry leaves by maceration for biological tests. The solvent used for solid-liquid extraction was tap water. A weight of 200 g of leaf powder is mixed in 1 L of tap water by maceration for 4 hours for a C1 concentration. The mixture is first filtered using a fine mesh sieve (0.05 mm × 0.02 mm) and then with chiffon. This same technique is used for C2 and C3 concentrations for 150g and 100g of shred in 1L of tap water respectively. For the fresh leaves formulation (FF), it was made by decoction in a water solvent of one liter for 200g of crushed *Crataeva religiosa*. Soap extracts were S and SC. The soap extract plus *Crataeva* (SC) followed the same procedure as the dry leaves formulation at 100g of *Crataeva* but with an addition of 25g of grated soap, S was the water control plus 25g of soap.

Phytosanitary treatment
Aqueous treatments of *C. religiosa* were applied two weeks after transplanting until the 15th day before harvest. The treatment was sprayed once a week and the readings were

taken before and after each treatment. Each concentration was used as a treatment for two elementary plots. For 6 treatments and 2 controls, 14 elementary plots were required. The control plants were not subjected to any treatment but went through all the cabbage growing processes. Watering was done with tap water.

Statistical analyses

The Microsoft Excel 2013 spreadsheet was used to classify the field data and its cross-dynamic table to generate the histograms. The analysis of the results was done with the Rstudio software. On the basis of any analysis a data normality test was performed with the Shapiro-wilks test. The effect of treatment on *Plutella xylostella* larvae and harvest weight was determined with the Kruskal wallis test. The latter being significant it ends with Wilcoxon's two-by-two comparison test. A correlation test was applied to the variables: numbers of *Plutella* larvae and the number of attacked leaves to determine their binding. In all cases, the tests will be considered significant when the probability of the p-value is lower than the threshold set at 5% (0.05).

Results

Effect of treatment on *Plutella xylostella* larvae

Analysis of the average graph of *P. xylostella* larvae according to the applied treatments reveals that (Fig. 1): Plots treated with the aqueous extract of *C. religiosa* dry leaves according to concentrations C1 (200 g / l), C2 (150g / l) and C3 (100g / l) respectively give larval means which follow a 0.24; 0.20 and 0.12 decrease. The two-by-two comparison test showed no significance between its averages. The aqueous treatment of fresh leaves at 200 g / l (FF) gives a very low average in larvae compared to the dry leaves formulation at 200 g / l (C1) and control plots (C0). A highly significant effect (P = 0.001) is noted between FF and C0 and another very significant effect (P = 0.03) between FF and C1. The soapy formulations ie the soap (S) and soap plus Crataeva (SC) control have a significant difference (P = 0.04) from their average, but statistical analyses of multiple comparisons give a large significant difference (P = 0.002) between SC and control C0. For 100g / l solutions of Crataeva (C3 and SC) the difference between larvae averages was not significant. Similarly, the comparison of the blank control (C0 = without treatment) with the 6 remaining formulations shows that the latter have a lower larval average. The two-by-two comparison test reveals a significant difference between C0-FF, C1-FF, C0-SC and S-SC. Statistical analyses generally showed a highly significant (P <0.001) effect of the treatment on the numbers of *Plutella* larvae.

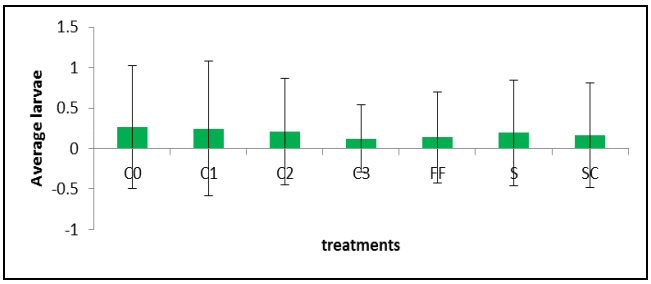

Fig 1: Average larvae of *P. xylostella* according to applied treatments

Effect of treatment on the number of attacked leaves

The histogram of the mean number of leaves attacked for each concentration showed that the control plots had more leaves attacked than the treated plots (Fig. 2). In addition, the kruskal test gives a highly significant p-value (P <0.001). Treatment with aqueous extracts of dry leaves at 200 g/l gave a significantly lower average number of attacked leaves (P <0.001). The C2 and C3 concentrations applied to the plots didn't give a significant difference between them, but the multiple comparison test revealed a highly significant effect between C2 and C0. The application of the fresh leaf formulation at 200 g/l (FF) compared to the same concentration with the dry leaves didn't reveal a significant difference (P> 0.05). On the other hand, the comparison between CO and FF shows a highly significant effect of aqueous extracts of fresh leaves on the average of the attacked leaves (P <0.001). Plants treated with 100 g/l aqueous dry leaves extract (C3 and SC) didn't give a significant difference.

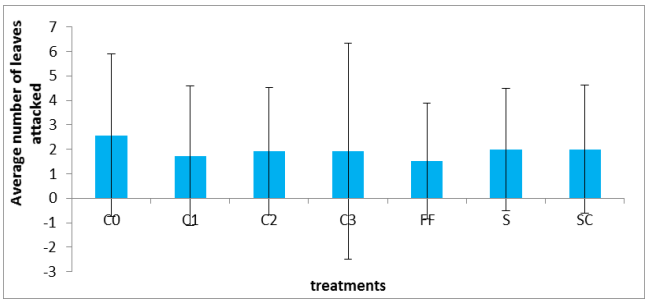

Fig 2: Average number of leaves attacked according to treatments

Correlation between *Plutella* larvae and the number of attacked leaves

The incidence of *Plutella* larvae was particularly noticeable in their ability to attack leaves. In contrast to other species such as *Hellula undalis*, *Spodoptera littoralis* and *Helicoverpa armigera* which in addition to consuming limbs have the ability to pierce the already formed cabbage. Based on this observation, a correlation test was necessary to determine the relationship between the attacked leaves and the *Plutella* larvae. This test revealed a positive correlation (r = 0.26 and P <0.001) between *Plutella* larvae and the number of leaves attacked (Fig. 3).

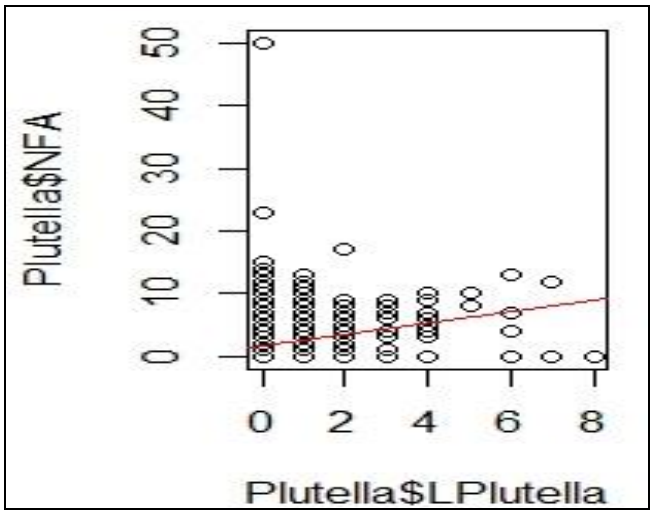

Fig 3: Relationship between the number of attacked leaves and *Plutella xylostella* larvae.

Effect of treatment on harvest weight

Out of a total of 168 transplanted plants, 138 cabbages were

harvested and 31 plants attacked by rodents, aphids, *Hellula undalis*, *Helicoverpa armigera* and *Spodoptera littoralis* larvae. The total weight of the harvest is estimated at 120.9kg. The histogram of the total harvest weight for each treatment (Fig. 4) revealed that the control plots (without treatment) had a much lower yield compared to the treated plots. The largest harvests estimated at 23.4kg and 19.9kg were from the treated plots with the highest concentration (200g / l), C1 and FF. At the same time the heaviest cabbages were obtained with its two concentrations (1.9 kg for C1 and 1.8 kg for FF). For dry leaves formulation, the higher the concentration was, the greater was the total weight of the harvest. As for the aqueous soap extract + *Crataeva* (SC) and its soap control (S) they didn't show a significant difference at the harvest. It's important to note that an inversely proportional evolution was noted between the average number of leaves attacked and the total weight of the harvest.

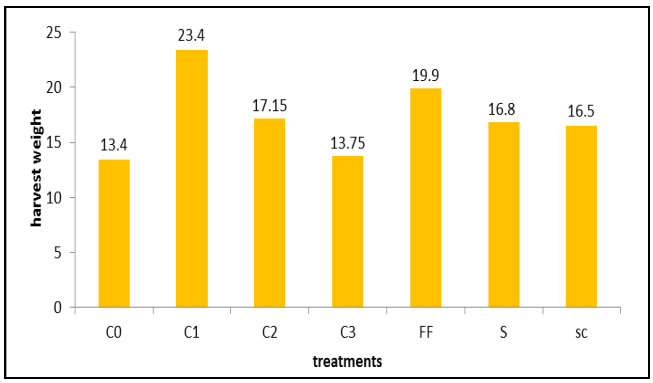

Fig 4: Total harvest weight compared to treatments

Discussion

The purpose of our study was to test the biocidal effect of aqueous extracts of *C. religiosa* on the cabbage moth *P. xylostella* in the Niayes area. After analysing the data obtained, we can discuss the probability of the effect that the substance had or didn't have on *P. xylostella* larvae as well as on the weight of the cabbage and at the same time define the most effective formulation to reduce the pest population. The frequency of occurrence of *Plutella xylostella* species is high. This justifies its presence in crop sites and its impact on other pests. This seems to confirm its status as the main cabbage pest as stated by Furlong *et al.* (2012) [6]. However, we noted that from November to March, its highest peak corresponded to the last phase of the crop, i.e the second half of February. At that time the cabbage wasn't ripe and the pest was satisfied with the most accessible leaves. A fall marked its population when cabbages start to become hard (after the 60th day) and the cover leaves start to grow. The fall in the population of *P. xylostella* caterpillars observed from the sixtieth day could be linked to the formation of mature cabbages that wouldn't be preferred as laying substrate by females, especially since the work of Sow *et al.* (2013) [8] reveals a preference for young plants by the female of *P. xylostella* during egg-laying. The evolution of the cabbage inversely proportional to the glucosinolate content in the plant could be a factor limiting egg-laying (Sow *et al*., 2013) [8]. Such a finding may be due to the development of nearby tomato and onion plants. According to Asare-Bediako *et al*. (2010) [9] and Baidoo *et al.* (2012) [10], cultural association techniques (Cabbage and / or Tomato, Onion, Chili) reduced the populations of *P. xylostella*. Our results are not consistent with those of Campos *et al*. (2003) [11], Badenes-Perez *et al.* (2014) [12] and Sow *et al*.. (2013) [8] who showed that the abundance of *P. xylostella* caterpillars (pre-imaginal stages) decreased with cabbage age. Although present in the environment, the late appearance of *P. xylostella* and its low number could be due to the long duration of crop succession that our plots have undergone (more than two years). This long crop succession is explained by the long cycle of cabbage that had deterred our market gardener and pushed him to abandon this speculation in favour of short cycle varieties such as green onion and potato. This phenomenon has been noticed in most of the Malika area.

Effect of treatment on *Plutella xylostella* larvae

Under field experimental conditions, the effectiveness of plant extracts is usually measured through the abundance of pest populations or the severity of damage (Yarou *et al*., 2017) [13]. Statistical analyses revealed that aqueous extracts of *C. religiosa* had an effect on the abundance of *P. xylostella larvae*. This can be justified by the presence of many more larvae in the untreated plots and the degrees of significance. For the dry leaf formulation, the three tested concentrations (C1, C2 and C3) don't have a significant difference in their larval average, indicating that there was no dose effect. In contrast to the work of Faye (2015) [14] which obtained a higher mortality at the highest *Crataeva* concentrations. Although no significance was noted between the SC and S concentrations, only the first has a significant difference compared to the white control. Therfore, the biocidal effect of this formulation could be accentuated by the combined action of the two components (soap and *Crataeva*) because the soap control (S = soap + water) and the C3 concentration (water + *Crataeva*) compared to the white control (C0 = water) gives no significant difference in mean number of larvae. In this case, soap may not have been used as a wetting agent as claimed by market gardeners, especially since the work of Tabone *et al*. (2011) [15] declines as a physical method the use of black soap to control pests, especially the tomato leafminer, *Tuta absoluta*. The results of the treatment with aqueous extracts of fresh leaves (FF) are more promising. Indeed, the formulation with fresh leaves reduced the pest population more significantly compared to the untreated plots and those treated with the dry leaves formulation. We can say that of its three most effective formulations, it seems to be the one with the aqueous extracts of crushed fresh leaves which have also been very effective against the cowpea weevil *Callosobrochus maculatus* Fabricius (Faye *et al*. 2012) [16]. This effectiveness of *Crataeva* leaves may be justified by its ability to retain more active substances by being fresh than dried. The results obtained on the number of leaves attacked and on the correlation test confirm the predominance of the species *P. xylostella* in the control plots (C0), which has led to greater damage to the latter. The opposite effect is noted in the plots treated with the aqueous extract of fresh leaves (FF). So in terms of the number of leaves attacked, FF plots are the least infested. In parallel, the relatively low abundance of caterpillars could be due to the applied treatments which can be explained by a biocidal effect of *C. religiosa* leaves on *P. xylostella* pest. As the biocidal effect of *Ricinus communis* L (Tounou *et al*., 2011) [17] and *Azadirachta indica* AJuss (Sow *et al*., 2012) [18] plants, the

use of aqueous extracts of *C. religiosa* could be used as an alternative to control *P. xylostella*. Studies comparing plant extracts with conventional insecticides have shown that some extracts may be as effective as synthetic insecticides (Sola *et al*., 2014) [19]. Thus, extracts of *Cassia sophera* L., *Jatropha curcas* L., and *R. communis* would be as effective as emamectin benzoate and lambda-cyhalothrin for the control of *P. xylostella* on cabbage culture (Amoabeng *et al*., 2013) [20]. Similar observations have also been reported between an *A. indica* product and dimethoate for the control of *P. xylostella* on cabbage (Sow *et al*., 2013) [8].

Effect of treatment on harvest weight

From the analysis of the agronomic data, it is noted that the harvests differ according to the treatment applied. Untreated plots had a higher level of infestation and lower yield. The inversely proportional evolution of the incidence in the number of attacked leaves and the harvest weight shows that *P. xylostella* larvae could indirectly influence the harvest weight. The higher number of infected leaves in control plots and the number of *P. xylostella* larvae would indicate the need for treatment for better yield. Similar results were obtained by Sow *et al*. (2013) [8]. According to them, agronomic parameters were strongly affected by the level of *P. xylostella* infestation. The application of a phytosanitary treatment based on *C. religiosa* seems to have an effect on crop yield, which is in agreement with the studies of Amoabeng *et al*. (2014) [21] who have shown that natural products derived from plants can also increase yields with a cost / benefit ratio comparable to that of synthetic pesticides.

Conclusion

The intensification of cabbage cultivation in tropical areas, particularly in the Niayes, has led to a significant increase in the growth of this vegetable species, to such an extent that it's now used daily in the local diet of families. For the first time in integrated pest management, the use of a phytosanitary treatment based on *C. religiosa* against *P. xylostella* has been the subject of a scientific study. In the field, the application of the aqueous extract from *Crataeva* leaves showed its greater effectiveness in reducing *P. xylostella* larvae. The results obtained show that among our formulations the most interesting in the management of larval populations of the pest is the one with fresh crushed leaves. The latter applied to cabbage plants had fewer larvae, less attacked leaves and one of the highest yields despite the incidence of cabbages destroyed by *Spodoptera littoralis* and *Hellula undalis* more accentuated in these plots. The dried leaves formulation provided the largest harvest but reduced the larval population of the pest and had a higher incidence compared to the number of affected leaves. The last formulation (soap plus *Crataeva*) also shows its effectiveness against *P. xylostella* pest. The aspect of the physical control that can result from the last formulation i.e soap plus *Crataeva* leads us not to recommend it in a purely biological control. However, this practice with fresh *Crataeva* leaves has to be deepened and considered in integrated pest management programs, especially as it's more lucrative for the farmer.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

All authors contributed to the realization of this work. They also approved this manuscript

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