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ANALYZE THE EFFECT OF BIOPESTICIDES DERIVED FROM EXTRACTS OF THE LEAVES OF NEEM, TEPHROSIA VOGELLI AND TOBACCO IN THE FIGHT AGAINST THE CABBAGE APHID BREVICORYNE BRASSICAE IN BURUNDI

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ABSTRACT

Disease and pest management is at the heart of the solutions to be provided to Burundian agriculture to address food and nutritional insecurity in Burundi. This study allowed us to analyze the effect of biopesticides derived from extracts of the leaves of neem, Tephrosia vogelli and tobacco in the fight against the cabbage aphid Brevicoryne brassicae in Burundi.The products used were dudu fenos for the positive control or T7 and the extracts of neem, Tephrosia vogelli and tobacco for treatments T1 (containing neem only), T2 (containing Tephrosia vogelli only), T3 (containing tobacco only), T4 (containing neem, and Tephrosia vogelli), T5 (containing neem and tobacco), T6 (containing neem, Tephrosia vogelli and tobacco). For the ISABU site, TO-5, T1=1.44, T2=1.22. T3=0, T4-0.56, T5-0 and T6-0 and T7=0 for the average number of Style leaves of infested plants after the third spraying. The results of the analysis of biopesticide residues gave a value higher than the control value 144.167 for treatments T3 (144.167 lower than 199.787) and T6 (144.167 lower than 168.272).

Keywords: Botanics pesticids, neem cabbage aphid, tobacco, Tepirosia vogelli.

1. INTRODUCTION

Cabbage cultivation plays an important role in maintaining food and nutritional security because the use of vegetables from the Brassicaceae family, particularly cabbages, is the first idea in nutritional education. The cultivation of cabbages plays an important role due to the high content of water, calcium and vitamin C. This vegetable has been classified by the FAO among the 20 plant crops important for human nutrition. It is the fourth most produced vegetable in the world (FAO, 1988).

In Burundi; Cabbage production is one of the main income-generating activities in the agricultural sector. The horticultural sector of Burundi would have tripled the economic weight of this sector, but this objective was not achieved due to multiple biotic and abiotic constraints (MINAGRIE, 2008).

The gray cabbage aphid; Brevicoryne brassicae Linnaeus, 1758 (Sternorrhyncha: Aphididae), is a specialist in plants of the Brassicaceae family, also called Cruciferae (GABRYS et al., 1997; PONTOPPIDAN et al., 2001). It is one of the main pests of cabbage (FATHIPOUR et al., 2006). Under severe infestation conditions, B. brassicae colonies can cause crop losses of 70-80% (RUSTAMANI et al., 1988; KHATTAK ***et al***., 2002).

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Chemical control is currently the most widely used method of combating crop pests and diseases throughout the world. Agricultural pesticides help to increase agricultural productivity, but at the same time pose potential risks to human health and the environment (OECD., 2008).

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Given the negative effects of conventional chemical insecticides on human health, fauna and flora, it is imperative to propose new alternatives that are not only environmentally friendly but also effective in controlling crop pests (REGNAULT-ROGER, 1997).

Our investigations will focus on the choice of spontaneous plants from sub-Saharan vegetation, particularly in Burundi, the analysis of the effectiveness of extracts from these plants and the possibility of using these plant extracts to improve pest control conditions. It is within this framework that we thought it would be useful to analyse the effect of biopesticides based on extracts of Neem, Tabac and Tephrosia vogelli in the control of the ashy cabbage aphid, *Brevicoryne brassicae* in Burundi, while awaiting encouraging results.

2. MATERIALS AND METHODS

2.1. Materials

The study was carried out in two provinces of Burundi over a period from October 2023 to April 2024 in the entomology laboratory of the Institute of Agronomics Sciences in Burundi (ISABU), located in the urban commune of ROHERO in the province of Bujumbura, and at the Institute of the Agricultural Technic in Burundi (ITAB KIGOZI) located on Yaranda hill in the province of Kirundo in the north of Burundi. This experiment was conducted under greenhouse conditions during the 2023-2024. A cropping season at ISABU and in the field during the B cropping season at ITAB KIGOZI.

The field equipment used consisted of a hoe for ploughing and maintenance work, a rope for aligning stakes, a tape measure for measuring distances within and between rows, a marker for writing on signs, a sprayer for applying plant protection products, a pen and a register for recording data. The seeds we used were of the CABBIS variety. In addition, neem, *Tephrosia vogelli* and tobacco leaves were used to produce a biopesticide to test its bioactivity on the ash aphid, *B,brassicae,* which attacks the cabbage crop.

The equipment used in the entomology laboratory consisted of a magnifying glass for viewing or enlarging the insect, brushes and forceps for touching different parts of the insect's body, alcohol and petri dishes. The equipment used in the phytopathology laboratory consisted of a mortar for crushing the neem leaves, Tephrosia vogelli and tobacco, a graduated cylinder for measuring the volume of water and products to be applied, an electronic balance for weighing the collected neem leaves, Tephrosia vogelli and tobacco powder, a filter for separating the juice from the crushed leaves, the powder and the residues, and a seal for preserving and storing the prepared product. The equipment used in the CNTA's analysis laboratory consisted of the CHARMII luminometer reader, which enabled us to display positive and negative readings, a centrifuge for extracting samples, a sample grinder to reduce the samples to small, easy-to-extract particles, and an incubator. All this equipment was used to determine the content of botanical insecticide residues in the cabbages harvested.

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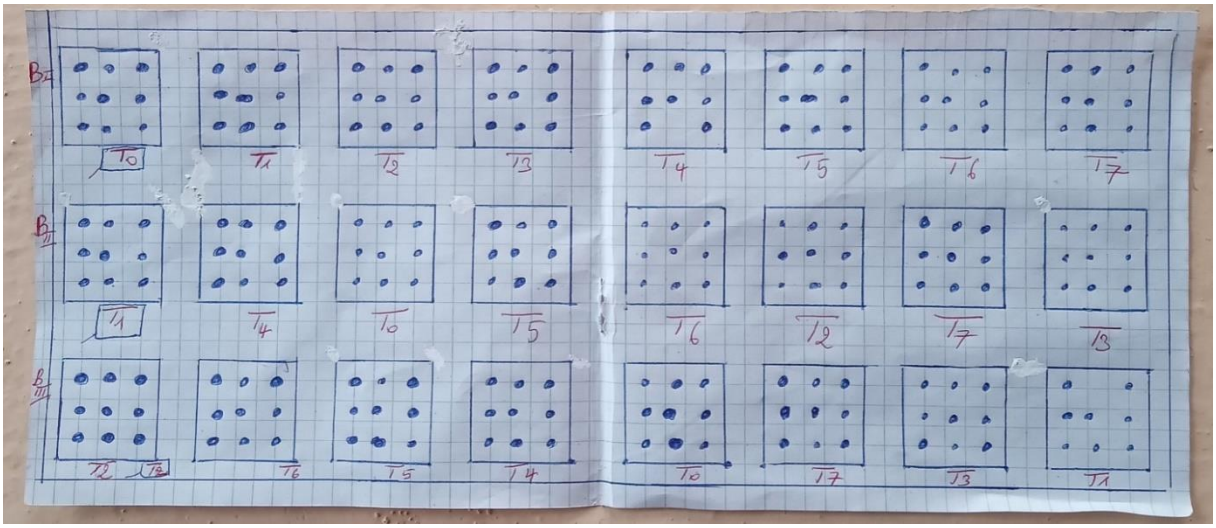
2.2 Methods

The experimental area was ploughed, well crumbled and levelled. It was subdivided into blocks and small plots (treatments) with three replicates. The overall trial design was a randomised complete block design with three replicates. Each block comprised 8 plots/treatments, for a total of 24 plots. The blocks were separated by a distance of 2 m. The experimental unit was represented by a plot with four rows 2.25 m long separated by a distance of 0.75 m. The plots were separated by a distance of 0.5 m. Each block was 7.75 m long and 2.25 m wide, i.e. an area of 17.4375 m2. The following figure shows the experimental set-up for the treatment trial. Each plot/treatment comprised 9 plants, i.e. 72 plants per block. In all, there were 216 cabbage plants.



Experimental set-up

In each plot/treatment, three plants were randomly selected using the letter N, resulting in a sample of 72 cabbage plants. The following diagram shows how the sample was selected.

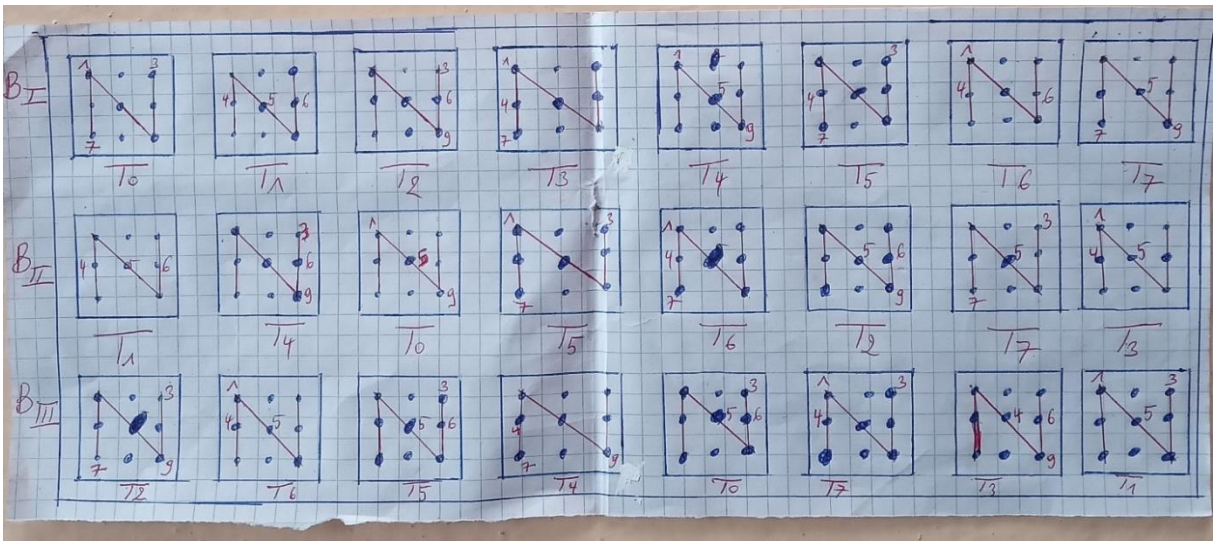


Diagram of sample selection

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Analysis of the efficacy of the biopesticides was carried out on these 72 cabbage plants. One hundred millilitres (100ml/plant) of biopesticides were applied to each plant in treatments T1 (containing neem only), T2 (containing Tephrosia vogelli), T3 (tobacco), T4 (containing neem and Tephrosia vogelli), T5 (containing neem and tobacco) and T6 (containing neem, Tephrosia vogelli and tobacco). Observations were made every 7 days on the 72 plants, i.e. 3 plants per plot/treatment mentioned above. We began our observations two weeks after planting. The pests identified during our observations concerned only the ash aphid B. brassicae. Neem and Tephrosia vogelli leaves and tobacco were used to produce botanical insecticides:

These leaves were harvested from trees planted at the Institute of Agronomic Sciences of Burundi (ISABU) in Bujumbura province and at the Institute of Agronomic Technical of Burundi (ITAB KIGOZI) in Kirundo province. The steps below show how to prepare extracts of neem and Tephrosia vogelli from tobacco:

-Grind 3kg of neem leaves, Tephrosia vogelli and 1kg of tobacco in a mortar.

-Macerate in 10 litres of water for 6 to 12 hours until the mixture is homogeneous.

-Filter and press.

We used a mixture of neem, macerated Tephrosia vogelli and soapy water at a rate of 3 litres per 10m2 to improve the product's adhesion; the residual effect lasts 6 to 10 days, which underlines the length of time the product remains on the cabbage leaves to lose its active ingredient. In general, 1.5-2litres per hectare is often used, i.e. 40ml in 20litres of water. In our trial, each plot was 5 m^2 and we had 8 treatments/block. The experimental field was subdivided into three blocks. Each product had to be used for a treatment covering an area of $5m \times 3 = 15m^2$. The dose we used was 3ml in 1.5l of water to spray $15m^2$.

Three separate spraying operations were carried out once a week. Each spraying operation was preceded by a colony count of the cabbage aphid B.brassicae in the morning or evening before and after spraying. For the colony count, we used 3 plants per biopesticide treatment and 5 leaves per plant, including 2 inner leaves and 3 outer leaves. Colonies were counted along the axes of the secondary veins. With regard to counting aphid colonies and the number of infested leaves, the first spray was applied 3 weeks after planting. The second spray was carried out after one week and the third after two weeks. Samples of cabbage harvested from treatments T3 (containing tobacco only) and T6 (containing neem, Tephrosia vogelli and tobacco) were sent to the CNTA laboratory for analysis of biopesticide residue levels.

Once the data had been collected, it was entered into Microsoft Excel. The parameters studied related to the number of infested leaves before and after spraying, the number of colonies of ash aphid before and after spraying and the analysis of Biopesticide residues in harvested cabbages. The unit of analysis was the plant for analysis of the infestation threshold. The factor used in the analysis was the type of BiopesticideT1(comprising neem only),T2(containing Tephrosia vogelli),T3(Tobacco),T4(containing neem and Tephrosia vogelli),T5(containing neem and tobacco) and T6(containing neem, Tephrosia vogelli and tobacco) in order to justify the effect of Biopesticides on food security and nutrition in the context of the control of the cabbage aphid in Burundi. The data were analysed using R software, version 4.1.2.

Description of the data (number of leaves infested before and after spraying, number of colonies of cabbage aphid before and after spraying) using descriptive statistics (number of observations, minimum, mean, standard deviation, maximum) overall and according to the type of Biopesticide. As we had quantitatively discrete variables (number of infested leaves and number

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of cabbage aphid colonies) which are not continuous, we thought it would be useful to analyse only the number of infested leaves. These data were represented using bar charts.

3.RESULTS

3.1 ISABU site

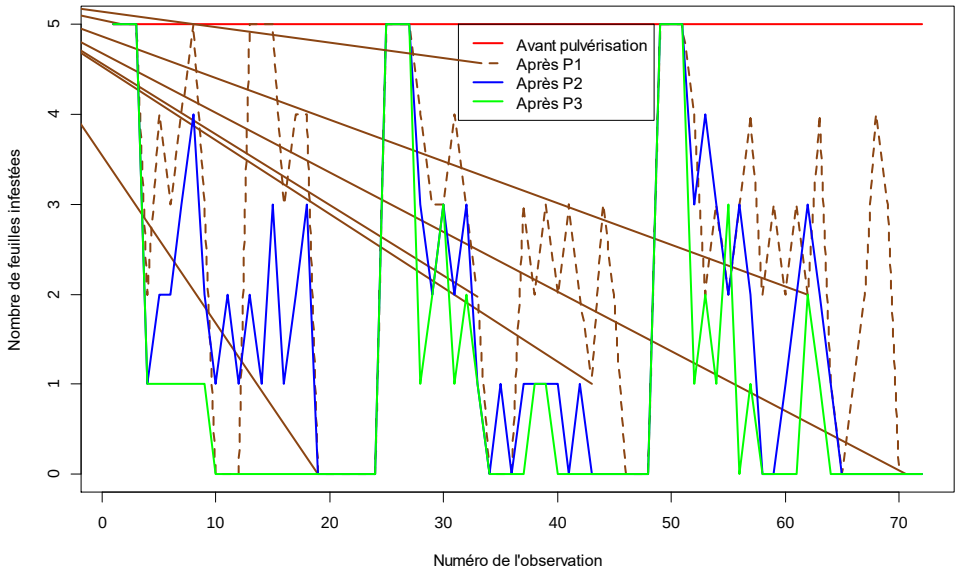
Combined descriptive statistics of the number of infested leaves according to treatment

After P1 After P2 After P3

Treatment Mean Standard deviation Mean Standard deviation Mean Standard

	After P1		After P2		After P3	
Treatment	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
T0	5	0	5	0	5	0
T1	3,11	0,78	**2,56**	0,88	**1,44**	0,73
T2	3,33	1	2,44	0,88	1,22	0,83
T3	**0,78**	1,2	0,67	0,71	**0**	0
T4	**3,56**	1,24	1,78	0,83	0,56	0,73
T5	2,22	1,39	1	1	**0**	0
T6	1,67	1,5	**0**	0	**0**	0
T7	0	0	0	0	0	0
Together	2,46	1,82	1,68	1,7	1,03	1,67

This table shows the combined descriptive statistics for the number of infested leaves according to treatment. Treatment T3 containing only tobacco was clearly the most effective with only one infested leaf after the first and second sprays (0.78, 0.67) and no infested leaves after the third spray while treatments T1 (containing only neem) and T4 (containing neem and Tephrosia vogelli) remained the least effective after the first spray (3 infested leaves for T1 and T4), after the second spray (2 infested leaves for T1 and 1 infested leaf for T4) and after the third spray (1 infested leaf for T1 and T4).



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Trend in the number of infested leaves at the ISABU site.

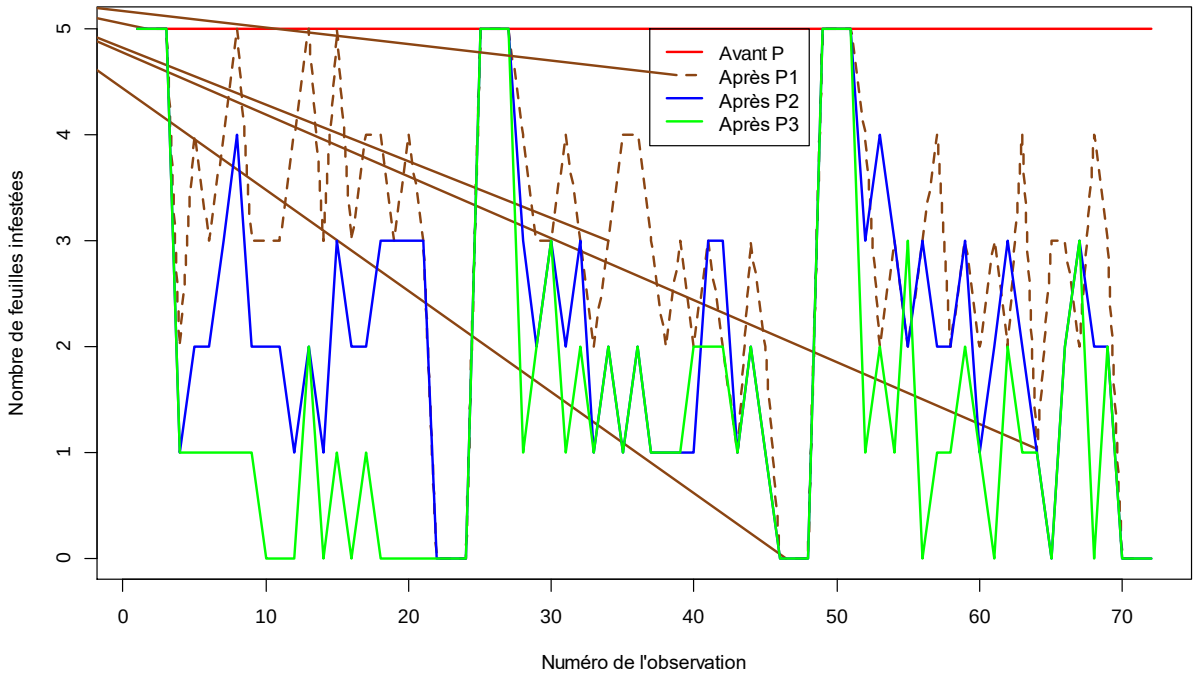
This figure shows the bar chart of the average number of infested leaves before the sprays, after the first spray (P1), after the second spray (P2) and after the third spray (P3) as a function of colour. Overall, the average number of infested leaves becomes lower after the third spray.

3.2. ITAB KIGOZI site

Combined descriptive statistics for the number of infested leaves according to treatment

	After P1		After P2		After P3	
Treatment	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
T0	5	0	5	0	5	0
T1	3,11	0,78	**2,56**	0,88	**1,44**	0,73
T2	**3,33**	1	2,44	0,88	1,22	0,83
T3	3,11	0,78	**1,78**	0,67	**1**	0,87
T4	**3,33**	1,12	**1,78**	0,83	**1**	0,71
T5	**2,78**	0,97	1,89	1,05	1,11	0,93
T6	**2,78**	0,97	2,22	0,83	**1**	1,12
T7	0	0	0	0	0	0
Together	2,93	1,51	2,21	1,48	1,47	1,57

This table shows the combined descriptive statistics for the number of infested leaves according to treatment. All treatments T1(containing only neem),T2(containing only Tephrosia vogelli),) T3(containing only tobacco), T4(containing neem and Tephrosia vogelli),T5(containing neem and tobacco) and T6(containing neem, tobacco and Tephrosia vogelli) had an average of one infested leaf after the third spray.



Trend in the number of infested leaves at the ITAB site.

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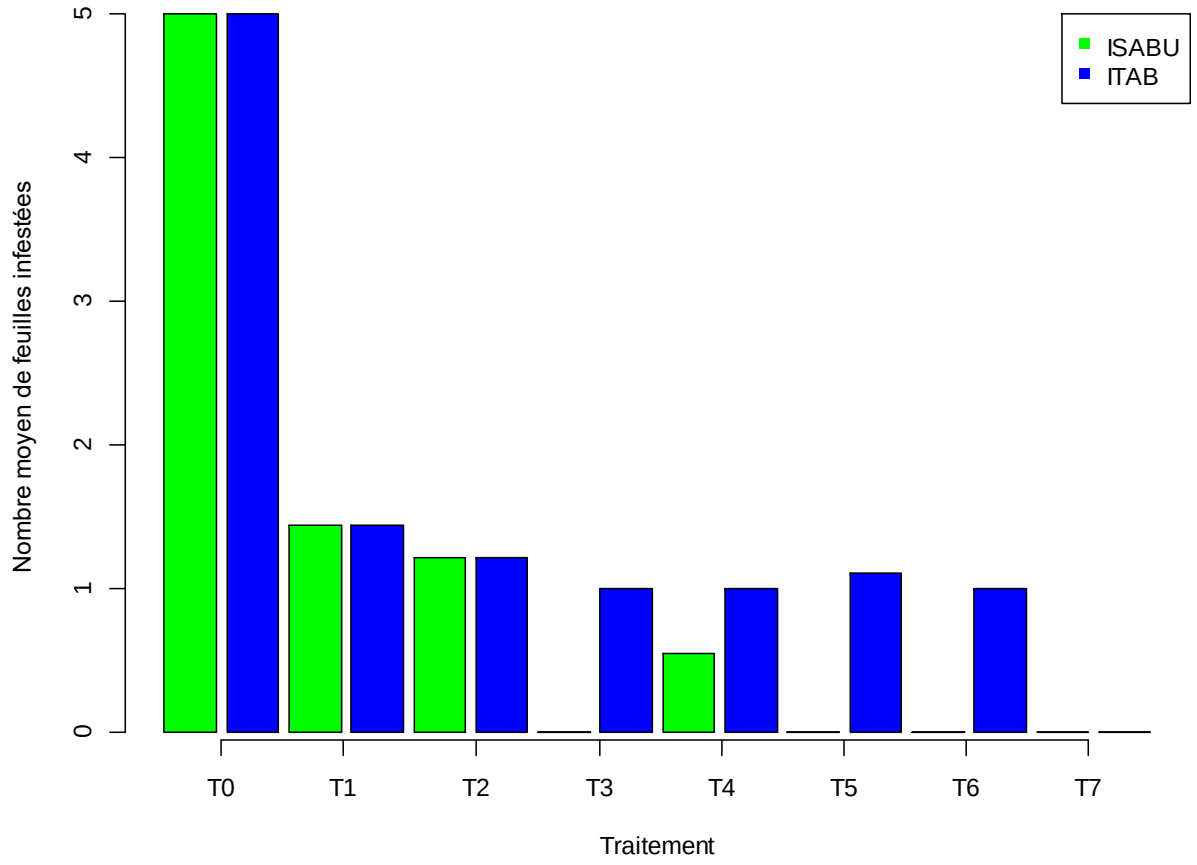
This figure shows the bar chart of the average number of infested leaves before the sprays, after the first spray (P1), after the second spray (P2) and after the third spray (P3) as a function of colour. Overall, the average number of infested leaves becomes lower after the third spray.



Bar chart of the average number of infested leaves after the third spraying according to the ISABU and ITAB KIGOZI sites.

This figure compares the ISABU and ITAB sites. After the third spray, treatments T3 (containing tobacco only), T5 (containing tobacco and neem) and T6 (containing tobacco, neem and Tephrosia vogelli) were clearly the most effective, as was T7 (containing the chemical pesticide) at the ISABU site, whereas these same treatments succeeded in bringing the number of cabbage aphid colonies to a fairly tolerable nuisance threshold at the ITAB site. For all sites, treatments T1 (containing neem only), T2 (containing Tephrosia vogelli only) and T4 (containing neem and Tephrosia vogelli) reduced the degree of attack (threshold of harmfulness).

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3.3 Results of analysis of pesticide residues in harvested cabbages

Nature of the sample	Sample No.	Analyses	Results				
Cabbage(T3)	1524/15		**Negative control (cpm)**	**Positive test (cpm)**	**Control Point (cpm)**	**Sample CPM**	**Conclusion**
		Pesticide residues	240.278	128.399	144.167	**199.787**	Not detected
Cabbage(T6)	1524/16					**168.272**	Not detected

After carrying out this analysis of pesticide residues, the results in the table below are interpreted as follows:

Negative: absence of residues because the sample result (RLU) is greater than the control point for cabbages harvested in treatments T3 (144.167 is less than 199.787) and T6 (144.167 is less than 168.272).

4. DISCUSSION

The results obtained with the three extracts used (containing neem, tobacco and Tephrosia vogelli) as Biopesticides, showed different efficiencies on leaf infestation rates. The population of this insect pest, the cabbage aphid, was abundant in the untreated control plots compared with the plots treated with neem, Tephrosia vogelli and tobacco extracts. At the ISABU site, the results showed that the number of infested leaves before spraying was equal to 5. After the first spraying, the average number of infested leaves was 1 for treatment T3 (containing tobacco) and 2 for treatments T5 (containing neem and tobacco) and T6 (containing neem, tobacco and Tephrosia vogelli). It should be noted that after the first spraying, treatment T3 At the ITAB site, after the first spray, treatments T5 (containing tobacco and neem) and T6 (containing tobacco, neem and Tephrosia vogelli) were clearly the most effective. After the second spray, treatments T3 (containing tobacco only) and T4 (containing neem and Tephrosia vogelli) were the most effective. After the third spray, treatments T3, T4 and T6 showed the same behaviour with only one infested leaf. Comparing the two sites (ISABU and ITAB KIGOZI), the results showed that after the third spray, treatments T3 (containing tobacco only), T5 (containing tobacco and neem) and T6 (containing tobacco, neem and Tephrosia vogelli) were clearly the most effective, as was T7 (containing the chemical pesticide) at the ISABU site, while these same treatments succeeded in bringing the number of cabbage aphid colonies on the leaves to a fairly tolerable nuisance threshold at the ITAB site. For both sites, treatments T1 (containing neem only), T2 (containing Tephrosia vogelli only) and T4 (containing neem and Tephrosia vogelli), the results showed that these treatments made it possible to reduce the degree of attack (nuisance threshold). All these results seem to confirm that the tobacco-based treatment (T3) or the treatment based on neem leaves, Tephrosia vogelli and tobacco (T6) is more effective than the tobacco-based treatment (T3).

Flavonoids disrupt the insect's natural motricity (REGNAULT-ROGER et al., 2004).

In a real environment in Côte d'Ivoire, another study showed the effectiveness of neem extracts in controlling caterpillars and aphids (GNAGO et al., 2010).

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In addition, the various constituents of neem, in particular neem oil, appear to be as effective as chemical pesticides in controlling crop pests (BIDIGA, 2014; SANE et al., 2018).

However, this efficacy appears to be selective against pests. For example, hot and cold extracted neem oils have an effect on eggs under laboratory conditions in Burkina Faso (TRAORE et al., 2019).

In addition, the oil in neem extracts is known for its insecticidal and ovicidal properties (FAYE, 2010).

Another study showed that extracts of neem leaves, Tephrosia vogelli and tobacco were effective in controlling cabbage aphids in Côte d'Ivoire (GNAGO et al., 2010).

In Burundi, another study showed the efficacy of neem and Tephrosia vogelli extracts in controlling caterpillars, weevils and aphids (NIYONIZIGIYE ,2017 ; NIYONZIMA,2022).

In addition, studies have reported the efficacy of neem, Tephrosia vogelli and tobacco biopesticides in controlling pests of other types of crops in addition to cabbages. These include crops such as cowpea, sorghum, chilli and cotton (AGGARWAL and BRAR, 2006; AGBOYI, 2009; GNAGO et al., 2010; SANOU, 2018; 2018; SANE et al., 2018).

Results similar to those of the present study have highlighted the fact that it is simpler for producers to use leaf extracts, which are available at all times and easier to extract than seed and oil extracts, especially as their efficacy has been demonstrated, especially hydroethanolic leaf extracts. The use of such a biopesticide should make it possible to improve cabbage yields and those of other crops, thus helping to reduce poverty and improve food security for African populations (MONDEDJI et al. , 2016).

5.CONCLUSION

The study showed that the use of biopesticides derived from tobacco leaf extracts or treatment T3 and neem leaf, *Tephrosia vogelli* and tobacco or treatment T6 is more effective than other biopesticides. These treatments gave encouraging results in reducing cabbage leaf infestation rates. The results obtained allow us to recommend to cabbage growers the use of extracts based on neem leaves, *Tephrosia vogelli* and tobacco as a less costly alternative to chemical pesticides for better control of the harmful insects that cause damage to this vegetable crop. What's more, this biopesticide preserves the environment while maintaining the ecological balance. Further studies should be carried out to improve the formulation of this biopesticide based on neem leaves, *Tephrosia vogelli* and tobacco.

However, it is not of a nature to make a definitive conclusion considering its realisation in time and space. For this reason, we would like to make a few recommendations to future researchers in the near future:

to separate the molecules of Neem, tobacco and Tephrosia vogelli extracts in order to synthesise a biological pesticide that can be sold here in Burundi and on a regional or even international scale.

- to make an inventory of the natural enemies of this pest and carry out a study of the effect of these biopesticides on plant auxiliaries (natural enemies and other beneficial organisms);
- resume similar work on other abiotic and biotic parameters and other cabbage varieties.

To the government:

 to allocate 10 to 15 of the national budget to the agricultural sector, as signed in SYRTHE in Libya and in MAPUTO in Mozambique during the 2002 and 2003 meetings in the commitments of African countries to ensure food security and African development.

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 to provide the country's research centres with the financial resources to adopt the control methods that have proved successful elsewhere, in particular those described above in the strategies for solving the problems of the cabbage aphid *Brevicoryne brassicae* ;

 Carry out other similar work in all regions of the country to identify the host plants that constitute the reservoir of the various species of the aphid family, in particular Brevicoryne brassicae, with a view to developing effective strategies adapted to the local context to combat them or reduce their threshold of harmfulness or economic loss;

 To assess the economic importance of this pest on cabbages in Burundi, as the collection of fruit samples from different cabbage varieties will make it possible to evaluate the extent of losses caused by this pest; To test other plant species that could act as biopesticides, and also to carry out a quantitative analysis of biopesticide residues in harvested produce.

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