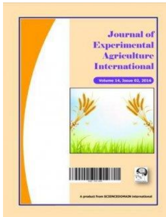

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Biopesticide Potentialities of Eagle Fern (*Pteridium aquilinum*) and Ricin (*Ricinus communis*) in the Protection of Vegetables Crops

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CM and ND designed the study, carried out laboratory and field experiments, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SK, ZD and JPOB contributed in data analysis and edited the manuscript. Author EN contributed in data analysis and oversaw the entire experimental work and edited the manuscript. All the authors approved the final version of the manuscript and its submission to the Journal of Experimental Agriculture International.

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ABSTRACT

Introduction: Pesticides commonly used in crop protection are serious causes of ecological and sanitary disorders.
Aims: This present work investigates the biopesticide properties of *Pteridium auquilinum* and *Ricinus communis* in the protection of three vegetable crops *Lactuca sativa*, *Solanum nigrum* and *Raphanus sativus*.

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Place and Duration of Study: The experimental tests were conducted on the site of BIONATURE (Bafoussam, Cameroon) from 24 June to 30 August 2017.
Methodology: Four types of preparations were made and bioefficacy tests consisted of contact treatments. Direct observations on the physical aspect of the plant, agronomic measurements, and pathology monitoring were carried out.
Results: The results show that the fermentation of the fern is complete after 5 days versus 8 days for the castor. The aqueous extracts of fern have insect repellent, insecticidal and fungicidal properties. Diluted maceration of fern (88.75%) was more efficient than the pure maceration (68%).
Conclusion: Manure and castor oil have insecticidal and repellent properties. The monitoring of pathologies after treatment reveals that castor oil is more effective than fern manure in control of gray rots and Tip burn attacks.

Keywords: Biopesticide properties; eagle fern; castor; maceration; manure.

1. INTRODUCTION

Agriculture is one of the main sectors of activity that contributes to the socio-economic development of populations [1]. The sector employs more than 40% of the world's workforce, more than 52% in Africa and Asia [2]. [3], purposely state that agricultural development is at the heart of food security strategies and the reduction of malnutrition rates, and in turn the reduction of poverty in most developing countries. For [4,5], the socio-economic development of the world's inter tropical regions is closely linked to the capacity of the countries concerned to promote the agricultural sector. In this sector, market gardening by vitamins and minerals they provide to the body occupy an important place for human nutrition. Defined as a highly specialized agriculture, market gardening is one of the most productive farming systems in Africa [6]. At the global level, indigenous and exotic vegetables considered as food sovereignty play a key role in most nutrition, food security and poverty programs [7]. In Africa, vegetables are an important component of daily diets, and important sources of income, particularly in urban and peri-urban areas [8]. These crops provide cheap proteins, vitamins and other essentials for health and well-being. However, the production of these vegetables is limited by multiple abiotic and biotic constraints that affect their yields and their commercialization (alteration of the appearance and organoleptic quality of products) [9,10,11]. Thus, to improve yields and respond to the ever-increasing markets demand, the use of synthetic pesticides by producers is almost systematic [11,12]. As a result, repeated use of synthetic pesticides has raised many concerns about human health and the environment while promoting the development of resistant strains of pests [11,13,14,15,16,17].

The disadvantages associated with the use of these pesticides militate in favor of the development of effective alternatives to chemical control among which: the combination of certain agricultural practices like the rotation of cultures, the physical protection (insect nets) [18], the use of plants producing active substances with biopesticide properties that can significantly reduce pest pressure and the need for synthetic pesticides [19,20,21,22,23,24]. Various references the literature describe the insecticidal and fungicidal efficacy of pyrethrum, nicotine, rotenone and various plants including conifers and their prospects for producing biological preparations [25,26]. The insecticidal and phytostimulant and biofertilizing properties of plant extracts have been proven by many authors. The aqueous extract from *Azadirachta indica* A. Juss can be cited. against *Heliothis armigera* (Hubner) and *Plutella xylostella* (Curtis) [27], extract from Cestrum par L'Her., Melia azedarach A. Juss., Nerium oleander L., and *Inula viscosa* (L. W. Greuter on the Desert Locust *Schistocerca gregaria.* Forskal, [28,29], the essential oils of juniper, false pepper and sagebrush on *Ryzoperta dominica* L., [30], extracts from *Pteridium aquilinum* L. on woolly apple aphids [31,32] extracts and oil of *Ricinus communis* L. on *Plutella xylostella* L., and *Callosobruchus chinensis* L. [33,34]. In the present study, the biopesticide effects of eagle fern and castor extracts were assessed on the main pests of three market garden crops.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The preparations used in the framework of this work were made from fresh and dry plants of eagle fern to the fields of BIONATURE which is a reference center of the application of botany and modern phytotherapy oriented towards the development of medicine by plants and the protection of the environment based in the city of

Bafoussam (Cameroon) and castor seeds purchased in the same locality.

2.2 Synthesis and Preparations of the Extracts of *P. aquilinum* and *R. communis*

To achieve the synthesis and extraction of fern extracts, we worked with dry leaves and fresh leaves. The preparations made were decoctions, infusions and macerations (simple and prolonged) (Table 1). The production of aqueous extract from fern was obtained by the combined action of cutting and extraction whereas that of castor oil was obtained by the combined action of pressing-extraction (oil) and cutting-extraction for the liquid manure.

2.3 Bioefficiency Test

Three devices were required for this study: pot trials, medicinal garden trials, and field trials (Fig. 1).

In pot experiments, the experimental setup consisted of delineating eighty pots of black radish (totally attacked by both fungi and insects) on which treatments based on macerated extracts pure and diluted to 20% fern were applied every 7 days. Each pot represents a repetition. The observations concerned the physical appearance of the plant after treatment.

In Medicinal Garden, we have in accordance with Fig. 1 (aii), used four devices were used, spaced from each other by 2 m. The first device noted P1 consisted of 30 feet of black radish plants distributed over an area of 3.9 mx 2.1 m that is a total area of 8.19 m^2 for a density of 3.78 plants/m^2 on which applications of fermented fern extracts diluted to 20% were made. The second device (P2) was to delimit on a surface similar to that of the device P1, 25 attacked black radish plants on which were made applications of pure macerated extracts from fern. The third device named P3 was an area of 5 m long and 0.5 m wide with 5 large stands of Comfrey with the modality of mixing the fermented extract of fern diluted to 10% and soap. The fourth device (P4) consisted of fruit trees (Dat-palm, Pawpaw, Avocado and Mango) attacked by both fungi, insects and mollusks' on which were applied infusions, decoctions and a mixture of fermented extracts fern and castor. Each plant was a repetition and the applications of different modalities were done weekly on all devices. The observations concerned the physical appearance of the plant after treatment.

In experimental field, the experimental device is that of a split plot, with six plots, each subdivided into two experimental units (Uxa and Uxb), representing a modality each. Each experimental unit measures 20 cm x 10 cm, with 25 plants each, let be 50 plants per modality. The Uxa units will not receive any treatment before transplanting the plants while the Uxb units will be covered with cake as follows: Uxb_1, Uxb_5 are covered with castor cake and Uxb_2 and Uxb_6 by fern cake. Each plant is a repetition. The treatments were carried out after transplanting on all the experimental plots every 7 days.

The following parameters were concerned in appreciating the physical appearance of the plant during treatment: the height growth of stems and number of leaves on black nightshade, the number of leaves and the evolution of fungal diseases (Mildew and Gray mold) and non-parasitic diseases (marginal necrosis) on lettuce, the lifting time that corresponds to the time between transplanting and the appearance of the first leaves, and the germination rate, which was evaluated using the formula: TG = number of feet raised / number of transplanted feet * 100.

Table 1. Summary of the different preparations made on the bracken fern and castor

Common name	Scientific name	Parts used	Types of preparations	Addiction
Fern	*Pteridium aquilinum*	- Leaf - Stem - Root	Infusion, L, S, R, LS, LR, LRS Concoction, S, R, SR Maceration, L, S, R, LS, SR, LR, LRS Fermentations, L, S, R, LS, SR, LR, LRS Strict Fermentation, L	Gr S
Castor	*Ricinus communis*	-Seed - Leaf	Heat Extraction, S Fermentation, L	S S, Gr

Legend: L = Leaf; R = Root; S = Stem; LR = Leaf + Root; LS = Leaf + Stem; RS = Root + Stem; LRS = Leaf + Stem + Root; Gr = Green clay; Se= Seed; S = soap

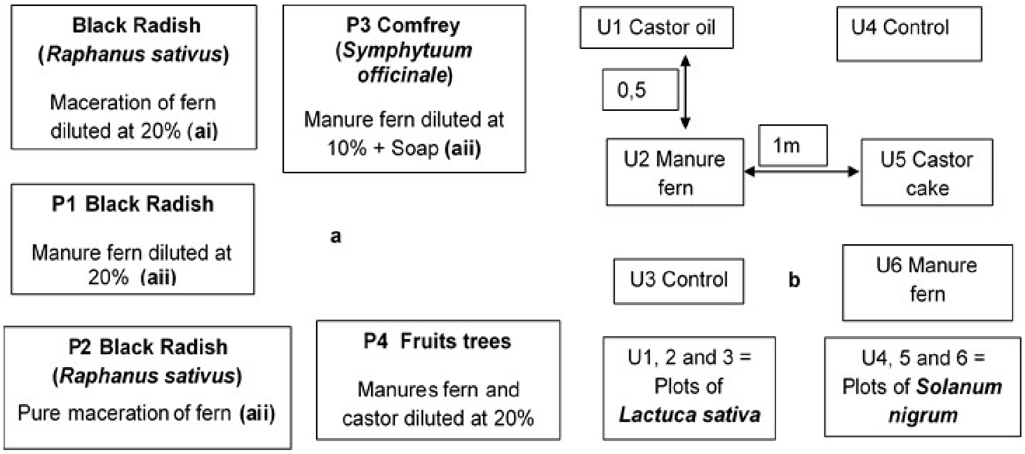

Fig. 1. Experimental design

ai: Pots; aii: Medicinal garden; b: Field trials

2.4 Data Analysis

All data collected were reported to the plant unit and analyzed using the R Software to obtain the averages that were used to make the curves presented in Figs. 2 and 3. The test of the analysis of variances (ANOVA) was used to determine the significant differences at the 5% threshold between the different treatments performed on nightshade and the averages were separated using the Welch F test. The Krushall-Wallis test was required to determine the significant differences between the different treatments performed on lettuce and the evolution of phytopathologies.

3. RESULTS

3.1 Preparation of Fern and Castor Extract

The results obtained after the extractions reveal that preparations of simple and prolonged maceration of ferns last less than those of castor oil. Indeed, the simple maceration and fermented fern extract are obtained after 4 and 5 days at a temperature of 22°C, respectively. Similarly, the simple maceration of castor oil is obtained after 6 days while obtaining the fermented extract is looped after 8 days. An extended time of 2 days for simple maceration and 3 days for prolonged maceration is therefore needed.

The amounts of extracts obtained at the end of these processes vary according to the part of the plant used and the state of the material. Indeed, 4 l and 4.4l were obtained after decoction of leaves and stems of fern respectively dry matter and fresh material. In contrast, prolonged macerations (purines) of dry leaves lead to a greater amount of extract than that obtained with fresh leaves (Table 2).

These results are different from those obtained by [35] during their work which obtained a prolonged maceration of nettle after 15 days at 20°C. In fact a fermentation time three times less than the nettle fermentation. This difference in result could be due to the variation of temperature which is of the order of 2°C or the high nitrogen content of the fern that would combine to increase the fermentation rate.

3.2 Bioefficiency Tests

3.2.1 Potted trials

They focused on the treatment based on fern macerations diluted to 20% according to the experimental device presented in Fig. 1ai. This preparation was effective against the crucifer flea beetle caused by *Phyllotreta spp* (Plate 1) at a recovery rate of 88.75% for a Time of Recovery Rate of 50% of plants attacks (TRR_{50}) recorded between the fifth and sixth day (Fig. 2).

3.2.2 Essays in medicinal garden

3.2.2.1 Black radish

Treatments based on pure fern macerate extracts on black radish have shown insecticidal efficacy in controlling *Phyllotreta spp*. Attacks and the recovery of *Phyllotreta spp*. (Plate 2: a & b) a recovery rate of 68% for a TRR_{50} of 07 days (Fig. 3).

Table 2. Summaries of the quantities of extracts obtained according to the quantities of plant material and the part of the plant concerned

Amount of plant material (Kg)	Amount of water (liter)	Parts of the plants used	State of the plant	Type of preparation	Yield (liter)
1	10	Leaves	Fresh	Prolonged maceration	8.6
0.4	10		Dried		7.8
0.4	10	Leaves+ Stems	Dried		7.1
0.4	10		Fresh		8.8
0.4	10		Dried	Concoction	8.0
1	10	Leaves + Roots		Infusion	3.8
1	1l	Leaves	Fresh	Simple maceration	7.8
0.35		Seeds		Roasting	0.2

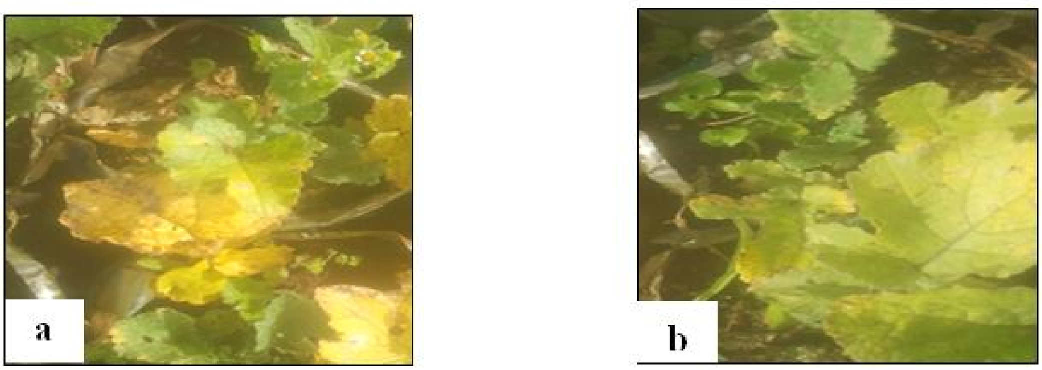

Plate 1. (a) Illustration of the leaves of an attacked radish plant and (b) Illustration of the leaves of the said plant restored after treatment with maceration fern diluted at 20%

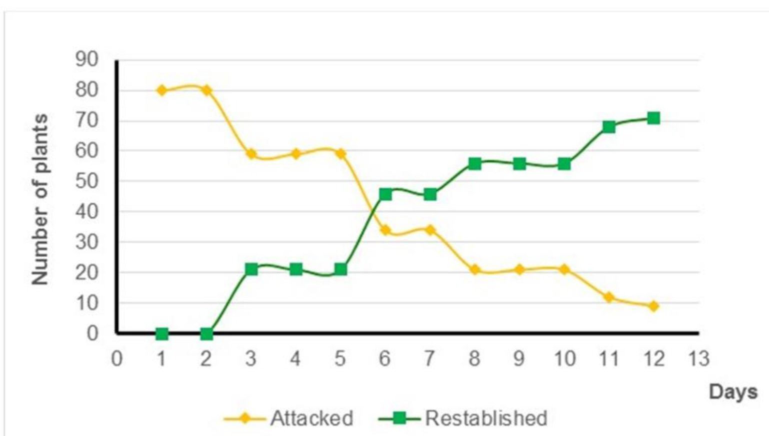

Fig. 2. Evolution over time of radish plants after treatment with diluted maceration of fern diluted at 20%

Similarly, fermented fern extracts diluted to 20% showed efficacy in the control of flea beetle and radish growth respectively at 4^{th} Days After Treatment (DAT) and 12 DAT (Plate 3: a to d), that is a success rate of 83.33% for a TRR_{50} of 06 days (Fig. 4). However, those diluted to 10% were only phytostimulants).

3.2.2.2 Comfrey

The mixture of manure of fern with a wetting agent which in our case is soap showed fungal efficacy as early as 01 DST with an 80% recovery rate on comfrey (Plate 4 a and b). These results are different from those of the previous tests but similar to those of [35]. Indeed, the fern manure associated with the soap develops fungal properties when the humidity does not act as a limiting factor.

3.2.2.3 Fruits trees

Decoction and fern infusion applications, as well as mixing 20% fern and castor manure on fruit trees, induced foot regeneration for a TRR_{50} observed at 4th DAT but no insecticidal or fungicidal effect (Plate 5: a and b). This result is in line with the work of [35]; they claim that some manure mixtures may have an effect other than if they were taken separately.

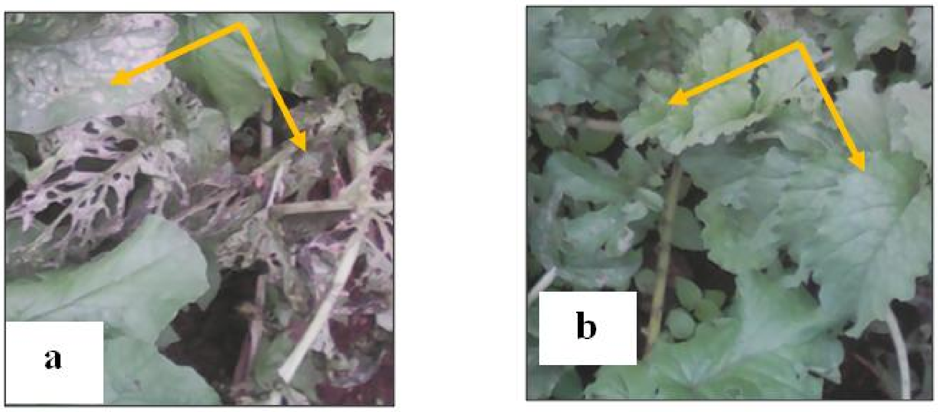

Plate 2. a: Illustration of the leaves of an attacked radish plant; b: illustration of the leaves of the plant restores after treatment with pure macerated of fern extract

Fig. 3. Evolution over time of radish plants after treatment with pure maceration of fern

Table 3. Summary of emergence time and germination rate in each experimental unit

Plots	**U1**		**U2**		**U3**	**U4**	**U5**		**U6**	
Experimental unit	U_{1a}	U_{1b}	U_{2a}	U_{2b}	U_3	U_4	U_{5a}	U_{5b}	U_{6a}	U_{6b}
Germination rate (%)	80	88	68	72	70	72	88	100	92	98
Lifting time (Days)			**5 days**					**4 days**		
Loss before T1	10		7		3	1	16		17	

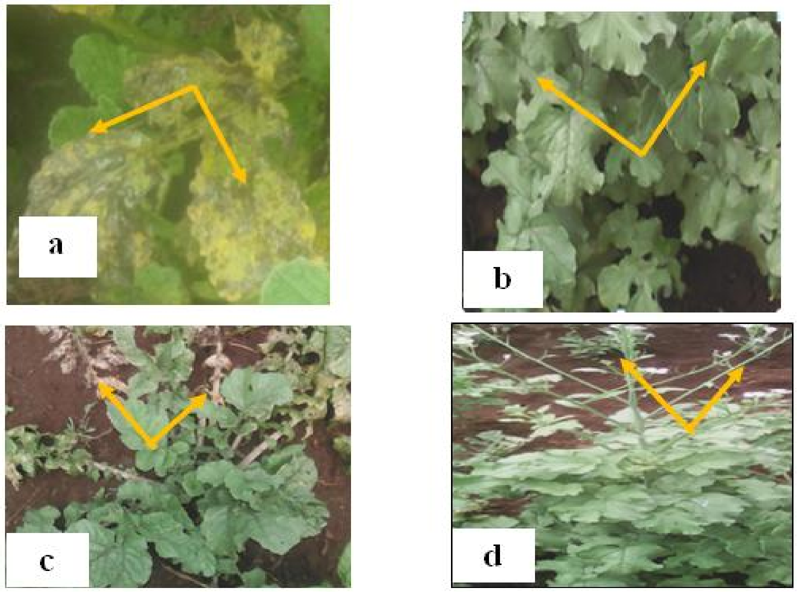

Plate 3. a: Illustration of the leaves of an attacked radish plant; b: Illustration of the leaves of the same plant 04 days after treatment (DAT) with the fermented extract of diluted fern 20%; c: Illustration of the leaves 07 DAT; d: Illustration of the leaves 12 DAT

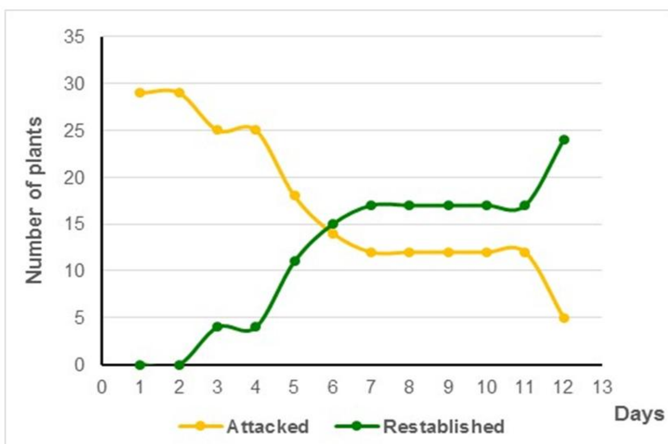

Fig. 4. Evolution over time of radish plants after treatment with fermented extract fern diluted to 20%

Our results showed a biopesticide effect depending on the nature of the plant, the stage of maturation, the type of preparations and the dilution of the different formulations applied to the infested plants. These results are supported by those of [36] which stated that after their work that the duration of the fermentation influences the quality of the manure in the sense that the temperature of 18°C and a fermentation time of 24 hours the nettle purse only expressed its insecticidal and fungal properties. However, for a maturation period of 7 or 15 days under a temperature of 30°C and 18°C respectively, this same extract had rather phytostimulant growth and biofertilizing properties. Furthermore, several authors [37,38,39] say that phytopreparations (maceration, infusion, decoction or fermented extract) can be considered as contact phyto-insecticides. [31,32,40,41] confirms the bioefficacy of these preparations when, following their work, they attribute insecticidal, manure and maceration properties to fern and fungicidal and phytostimulant properties to the decoction and to the manure associated with the soap.

The bioactive molecules of *R. communis* (manure, oil and mulch) have insecticidal and fungicidal effects observable respectively at 24 hours and 02 days depending on the biotic stress considered. These results are corroborated by [34] who respectively demonstrated that aqueous extracts of castor oil acted on *Alternaria solani* by inhibiting its average growth. [42] during their work demonstrated that castor oil diluted at 5 and 10% have an effect on the larval stage of *Plutella xylostella* by reducing the emergence of adults. [32,43] argue that mulching of soils by castor cake induces a phytostimulant effect because these cakes have a nutritional mode of action that induces a stimulation of the metabolic action and synthesis of cultures.

3.2.3 Experimental field

3.2.3.1 Germination rate and emergence time after transplanting

The results showed that, all plots had a high germination rate, with an average germination rate of 83.4 ± 3.66%. However, there was no significant difference between the germination rate of the mulched and un-mulched plots (t = 2.05, P-value=0.075) (Table 3).

3.2.3.2 Follow-up of pathologies after applying the formulations on lettuce

The analysis of the follow-up of the evolution of the pathologies on the lettuce reveals that the different formulations used have positive effects vis-a-vis the control of the pathologies observed. However, rots have proved more difficult to control than attacks of mildew or marginal necrosis (Tip burn). In fact, 100% of the plants attacked by Tip burn and Mildew were completely restored against only 50% when controlling rot. However, in U3 (control plot) several dead feet were recorded as well as a relatively weak foliar growth compared to the treated plots (U1 and U2) (Fig. 5).

3.2.3.3 Downy mildew (caused by Bremia lactucae)

The evolution of late blight pathology between plots U1 and U2 revealed that mildew was three

Plate 4. a: Comfrey leaves attacked by fungi; b: Comfrey leaves restored after application of manure fern diluted at 10% and soap

Plate 5. a: Foot of a fruit trees before application of fern and castor mixture at 50%; b: Regeneration of the foot of the trees after treatment

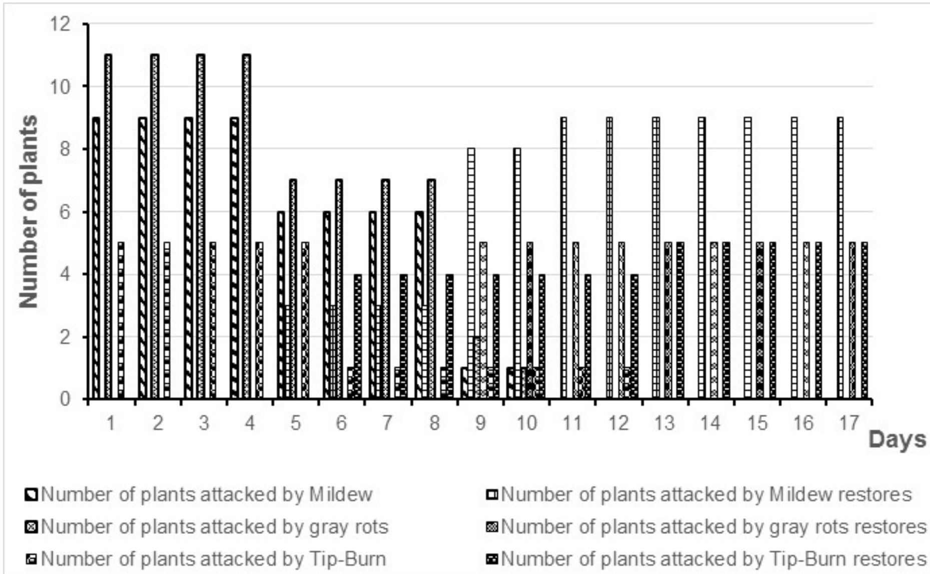

Fig. 5. Follow-up of pathologies as a function of time after applying the formulations on lettuce

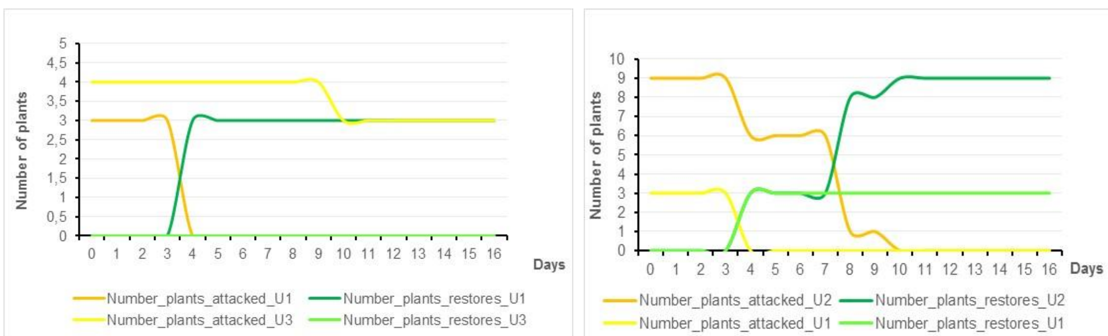

Fig. 6. Follow-up of downy mildew as a function of time after applying the formulations on lettuce (a) Control with castor Oil, (b) Control with manure

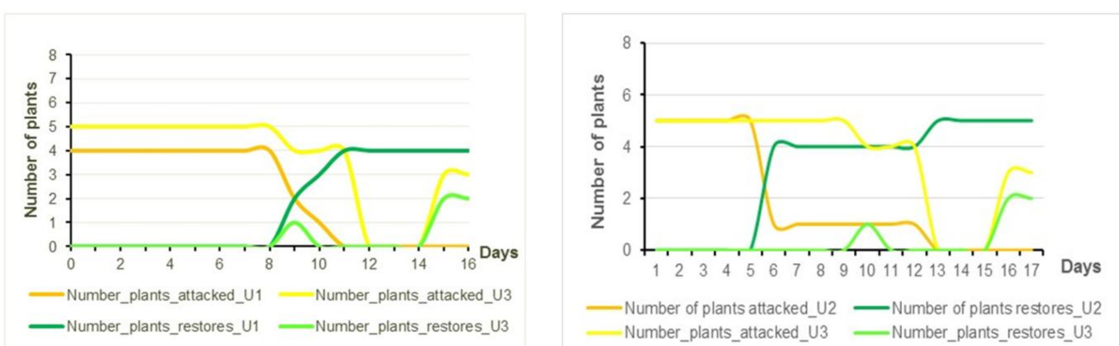

Fig. 7. Follow-up of tip-burn as a function of time after applying the formulations on lettuce (a) Control with castor oil, (b) Control with manure

times more prevalent in unit 2 than unit 1, respectively 9 feet and 3 feet attacked. The effect of castor oil (CO) treatment was found to be 04 DAT versus 08 DAT for fern manure (MF). In addition, attacks due to *B. lactucae* completely cease four days after application of while in the presence of fern mai continue until the 10th day. Howeve cases, 100% of the attacked pla restored after treatment against 0% in t plot (Fig. 6 a and b).

3.2.3.4 Marginal necrosis (Tip burn)

It has been observed that this phytopathology attacks with substantially the same frequency all the plots. The analysis of the data revealed that 100% of the attacked plants were fully recovered after treatment with fern manure and castor oil against 60% in the control plot. In addition, attacks on the fern manure plot to cease altogether 05 days after the second application (i.e. 12 days after transplanting) while in the castor oil treated plot, although plants are re-established, attacked are still observable 16 days after transplanting. In the control plot, re-established plants are observed at 9 days and 14 days (Fig. 7).

3.2.3.5 Gray rot (caused by Botrytis cinerea)

Speaking of the evolution of gray rots between U1 and U2 plots, the analysis of the data reveals that the effects of castor oil are visible 03 DAT against 08 DAT for fern manure with a recovery rate respectively of 50% and 45.45% (Fig. 8a and b).

3.2.3.6 Evaluation of the number of leaves and the height of the stem after treatment on black nightshade

The results obtained and the direct observations showed that the leaf height as well as the average number of leaves vary slightly from the units treated but considerably in comparison with the control unit (U4) (Table 4). In addition, the unit treated with castor cake (U5) has a success rate of harvest of 81% against 76% for fern manure (U6) and 6.45% for U4.

In the control of phytopathologies of vegetable crops, the bioactive molecules of the fern manure

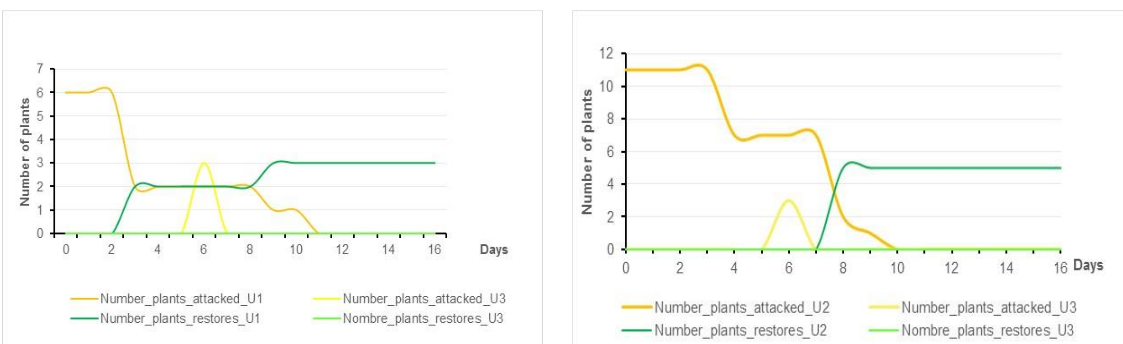

Fig. 8. Follow-up of Gray rots as a function of time after applying the formulations on lettuce (a) Control with castor oil, (b) Control with manure

Table 4. Average number of leaves of *L. sativa* and *S. nigrum* and average height of stems of *S. nigrum* according to the treatments carried out on the plots

Cultivated species	Experimental unit/treatment	Germination rate (%)	Average numbers of leaves ± SD	Average height of the stems ± SD	Value of the statistical test performed
L. sativa	U1 (HR)	84	5.53 ± 2.75^a		X^2 or Z=1,90
	U2 (PF)	70	5.95 ± 2.30^a	-	*P*-value= .39
	U3 (Te)	70	5.34 ± 2.47^a		Df=2
S. nigrum	U4 (Te)	72	5.24 ± 2.67^a	12.20 ± 2.32^a	F(1.49) =9.59
	U5 (TR)	94	3.69 ± 2.02^b	13.52 ± 2.80^b	*P*-
	U6 (PF)	96	5.73 ± 2.01^b	13.75 ± 3.02^b	value=0.0001***

*Average numbers ± S.E; Mean in the same column for the same cultivated species followed by the same letter do not differ significantly at P< 0.05; ^{ns} P> 0.05, *P< 0.05, **P< 0.001, *** P< 0.0001* have a relatively slow effect in comparison with those of castor oil respectively 08 Days after Treatment (DAT) against 03 DAT in the control of rot and 08 DAT versus 03 DAT in Mildew control. However, fern extract has a relatively fast effect in the control of Tip-burns of either 05 DAT versus 08 DAT for castor oil. Hence castor oil has a relatively low remanence time in the control of Tip-burn but long in the control of rots and mildew. In fact, treatments with castor oil 10 Days After Transplanting (DATr), 12 DATr and 21 DATr proved interesting respectively in the control of late blight (100%), rots (50%) and marginal necrosis (100%). While, the treatment of 10 DATr fern manure does not appear interesting in the monitoring of rots and mildew. On the other hand, it is rather the treatment with 14 DATr which shows an effectiveness of the bioactive molecules 100% and 45,45% respectively for the mildew and the gray rots for each of the pathologies, whereas the treatment 11 DATr induces a recovery of 100% of plants attacked by marginal necrosis (Tip-Burn). The efficacy of castor oil treatments for gray rot may be due to the presence of carbon, phosphorus, potassium or low nitrogen. [44] corroborate these results when they state that the high rate of nitrogen increases plant growth and foliage density but at the same time sensitivity to *B. cinerea*. [45] found a positive linear correlation between the C / N ratio of leaves and the susceptibility of tomato plants to *B. cinerea*. Several authors including [46]) found that fertilization of cucumber plants with 7: 3: 7% NPK with low nitrate content reduced the incidence of gray rot or castor extracts by 27-33%. Castor oil has 2% phosphoric anhydride and 1.5% potassium oxide.

The low recovery percentages found on decayed plants indicate that rots are the most difficult pathologies to manage by our extracts. This is due to the fact that they are very often the consequence of a problem in the cultural management or soil. [47] reinforces this reflection when he notes at the end of his work that the susceptibility of tomatoes to rots increased with the decrease of the level of nitrogen in the soil.Similarly, concerning mildew we find that castor oil can permanently eliminate the mushroom plots after 11 DATr against 14 DATr for the liquid manure. This could be explained by the fact that the best control of late blight is done with very low nitrogen fertilizer. However, considering that it is the second application of fern manure that induces control, we can say that castor is less rich in nitrogen compounds than fern manure. These results are similar to those of [48] which states that oilcakes and essential oils are more effective than aqueous extracts of plants.

However, against marginal necrosis it is difficult to decide because re-established plants were registered on the untreated plot. This is because marginal necrosis is a characteristic of dry season lettuce and reflects the inability of plants to move enough water and nutrients to rapidly growing leaf tissues enclosed in lettuce hearts. The probable hypothesis is that the peak is induced by a number of factors including growth rate, climate function, water and nutrient availability, calcium intake and any constraints on plant that results in unbalanced growth. Otherwise a substantial intake of water and a small amount of calcium can help to reduce the pathology. However, the application of the treatments makes it possible to control rot because marginal necrosis can serve as a starting point for bacteria or *Botrytis*.

4. CONCLUSION

The aqueous extracts from the bracken fern present bioactive molecules at the origin of their biopesticide activities. Indeed, the pure and diluted macerations of this plant induces insecticidal effects in the control of the crucifer flea beetle. Similarly, its fermented extract (manure) has a biofertilizing activity (diluted at 10% or in combination with castor manure), Insecticide activities (diluted at 30%) and fungicide activities (in combination with black soap or castor manure).Castor manure has bioinsecticidal properties. However, the bioactive molecules of castor manure are more effective than those of fern manure in controlling flea beetle. Castor oil is more excellent and efficient in the control of Downy Mildew and Gray Rot. On the other hand, the fern and castor cakes used as mulch have phytostimulant properties.

Our findings showed that eagle fern which is in Cameroon, could be used as a promising alternative tool for biological control of crucifer flea beetles. Castor could be considered as a mixture of fern extract from the form of applications of purines such as phytostimulant or fungicide.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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