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Evaluation of the Effect of Aqueous Extracts of Fresh Neem (*Azadirachta indica* A. Juss., 1830) Leaves and Kernels on the Mortality of the Sweet Potato Weevil (*Cylas puncticollis* Boheman, 1833) under Laboratory Conditions in Burkina Faso

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Evaluate the effect of neem extracts (kernels and fresh leaves) on the mortality of the sweet potato weevil *Cylas puncticollis* in the laboratory.
Place and Duration of Study: The study took place in Bobo-Dioulasso, western Burkina Faso. It lasted for 6 months.
Methodology: The experimental design was a completely randomized Fisher block in 5 treatments with 5 replicates. The collected fresh neem leaves and kernels were weighed and crushed to which 2.5 l of distilled water was added to each. After 12 hours, the mixture was filtered. Four concentrations were prepared from this filtrate: 10; 20; 30 and 40 cl/l and a control (distilled water). The various tests were carried out with Whatman paper cut into two equal parts then placed at the bottom of a Petri dish and spaced one centimeter apart. One ml of each dose of the solutions prepared was spread evenly on one of the two parts of the paper called the treated area while the other part received nothing called the untreated area. A batch of 10 adult weevils was placed in the center of the paper treated with neem extract.
Results: The results showed that after 1 hour's exposure to neem leaf extracts, a high mortality of 45% was recorded, whereas with neem kernels extracts, 30% mortality was observed after 30 minutes' exposure, then 32.5% after 1 hour.
Conclusion: Neem leaves and kernels extracts can be used to control the potato weevil, *Cylas puncticollis.* This is an environmentally-friendly control method that could be used as an alternative to chemicals.

Keywords: Neem; leaves; kernels; fresh; mortality; Cylas puncticollis.

1. INTRODUCTION

Sweet potato ranks sixth in the world as a food crop after rice, wheat, potatoes, corn and cassava, while in Africa it is fifth on this list (Anonyme, 2022). If the global annual production of sweet potatoes is more than 113 million tons, Africa's share represents 95% of this production. In Burkina Faso, as in other developing countries in Africa, sweet potato (*Ipomoea batatas* L.) is a subsistence crop (Doussoh et al., 2016). With an annual production of 113 million tons in 2017, harvested on more than 9 million hectares, sweet potatoes are the seventh largest agricultural production worldwide, after wheat, rice, corn, potatoes, barley and cassava (Triple performance, 2022). China is by far the leading producing country with 72 million tons (64%).

Sweet potato is consumed mainly in developing countries, where it is sometimes a staple food, for example in Papua New Guinea, the Solomon Islands and some East African countries (Burundi, Uganda, Rwanda). Sweet potato is also used in animal feed, particularly for pig breeding. In Burkina Faso, it occupies the first place among tuber and root plants. However, there are many authors (Andrade et al., 2009; Stathers et al., 2013) who showed that sweet potato production is facing several biotic constraints.

In Burkina Faso, sweet potato production increased from some 314,127 tons in 1980 to 4,124,120 tons in 2016 and more than 5,000,000 tons today (CPF, 2022). Indeed, in the Léraba province and during the 2021-2022 wet season, there were 178.25 ha of sweet potato crop for a production of 4,884.05 tons, or a yield of 27.4 tons per hectare (Kabre Mady, 2023). In the dry season of the same year, 141.83 ha were planted for a production of 3,297.54 tons of potatoes. During the 2022-2023 wet cropping season, the sown area was 172.89 ha for 3,781.10 tons of potatoes.

As a result, the areas planted and the quantities of potatoes produced are increasing from year to year. Indeed, authors (Souleymane et al., 2018) reported that from the survey of 200 farmers the weevil, *Cylas punticollis* is considered the leading leaf pest in Nahouri (52.94%), Kouritenga and Gourma (75 .51%) provinces. Sweet potato can also help fight against vitamin A deficiency, particularly in children and pregnant women (Mbela et al., 2021).

However, constraints that hamper sweet potato production include climatic hazards, root conservation, diseases and insect pests (Gbenou Pascal, 2020). Among the insect pests, *Cylas punticollis* B. and *Cylas brunneus* F. are the most widespread species of sweet potato weevils in Africa, causing yield losses reaching 100% particularly in the dry season and in stocks (Smit, 1997). In case of severe or light infestation, damaged tubers react to attacks by secreting a poison that makes them unpalatable to humans, due to phytoalexins produced in response to weevil feeding (Varin et al., 2009). Sustained control of the sweet potato pest is therefore important to avoid this poison, which can affect the lungs and hearts of humans and livestock (COLEACP, 2011).

To combat this pest, farmers rely on the use of chemical insecticides with a systemic effect (Somda Naamwin Irkoum Zéphirin, 2016). The use of these chemicals is increasingly criticized for their harmful effects on human and animal health and the environment. For this, the use of biopesticides such as aqueous extracts of neem constitute one of the alternative solutions to chemical insecticides in plant protection, but also a non-polluting means of control for the environment (Sanon et al., 2005; Simde et al., 2024). It is in this context that the present study was conducted (Simmonds and Blaney, 1996) in laboratory conditions in Burkina Faso. The main objective of this study was to evaluate the effect of Neem fresh leaves and kernels extracts on the mortality of the sweet potato weevil under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Presentation of the Study Area

The study was conducted at the eco-toxicology laboratory and the biological control laboratory of the Centre National de Spécialisation en Fruits et Légumes (CNS-FL) of the Institut de l'Environnement et de Recherches Agricoles (INERA) in Farako-Bâ, near Bobo-Dioulasso, western Burkina Faso. Coordinates: 11º09′23.7″ N and 4º17′10.4″ W.

2.2 Material

2.2.1 Plant material

The plant material consisted of all varieties of sweet potato and extracts of fresh neem leaves and kernels. The sweet potatoes were collected from potato farmers in Bama. The neem extracts were collected from a neem plant located at the study site. The dry leaves were obtained by drying fresh neem leaves collected in Bobo-Dioulasso.

2.2.2 Laboratory equipment and consumables

The equipement and consumables included:

- ✓ A graduated cylinder, plastic trays, 5mm muslin cloth, glass boxes, a 1000 ml graduated beaker and distilled water were used for the preparation of the different solutions;
- ✓ A mortar was used to pillar the neem extracts (fresh kernels and fresh leaves);
- ✓ A scissor was used to cut the Whatman blotting papers;
- ✓ Petri dishes, Whatman paper were used to do the repulsion test;
- ✓ A micropipette was used for pipetting the different 1000 µl solutions;
- ✓ Weighing the weight of the fresh material, as well as the fresh kernels, required a precision electronic balance.

2.2.3 Animal material

The animal material consisted of weevil adults. Adults were mass reared under ambient conditions in plastic tanks in the laboratory.

2.3 Methodology

2.3.1 Weevil breeding

The weevils were reared in the entomology laboratory in Bobo-Dioulasso at a temperature of 37°C. Tubers with *C. puncticollis* damage purchased on the market were placed in plastic bins. Breeding was carried out on all varieties of sweet potato showing *Cylas puncticollis* damage and those showing emergence holes. Old potatoes no longer containing larvae were removed from the bins and then replaced. The 10-liter bins were covered with muslin cloth to allow good ventilation while preventing these emerged insects from escaping (Sigri, 2022).

2.3.2 Preparation of different concentrations

Fresh neem leaves: The fresh neem leaves collected were weighed and 0.5 kg of these leaves were crushed using a mortar. 2.5 l of distilled water was added to the crushed leaves. The mixture was covered with muslin cloth and kept in the laboratory for 12 hours. After 12 hours, the macerate obtained was filtered using a

muslin cloth and the yield was calculated using the ratio: volume of solution of fresh neem leaves/volume of distilled water. 2.3 l of fresh neem solution were obtained; which corresponds to a yield of: 2.3l/2.5l= or 92%. Four concentrations were prepared from this filtrate: 10 cl/l; 20cl/l; 30 cl/l and 40 cl/l. These concentrations were obtained respectively with 5 cl; 10 cl; 15 cl and 20 cl of fresh neem solution, which volumes were reduced to 50 cl with distilled water.

Fresh neem kernels: To obtain the fresh neem kernel solution, 0.5kg of neem kernels picked directly from the neem plant were crushed then soaked in 2.5 l of distilled water for 12 hours. After 12 hours a filtrate of 2.51 l of neem kernel solution was obtained, i.e. a yield of 2.51 l of fresh neem kernel solution/2.5 l of distilled water = 100%. With this filtrate obtained, concentrations of 10 cl/l; 20cl/l; 30 cl/l and 40 cl/l were prepared from 5 cl; 10cl; 15 cl and 20 cl of neem kernel solution and 50 cl of distilled water.

2.3.3 Neem extract causing mortality test

The various tests were carried out using a micropipette. Whatman paper 110 mm in diameter was used to cover the bottom of the 9 cm diameter Petri dishes. This Whatman paper was cut into two equal parts then placed at the bottom of the Petri dish and spaced one centimeter (1 cm) apart. 1 ml of each dose of the solutions prepared were spread evenly on one of the two parts of the Whatman paper called the treated area while the other part received nothing called the untreated area. A batch of 10 adult weevils was then placed in the center of the Whatman paper treated with the neem extract contained in the Petri dish.

Under the same conditions, regarding the control, the Whatman paper was cut into two equal parts then placed at the bottom of the Petri dish and spaced one centimeter (1 cm) apart. 1ml of distilled water was spread evenly on one of the two parts of the Whatman paper called the treated area while the other part received nothing called the untreated area. 10 adult insects were also placed in the center of the Whatman paper treated with the neem extract contained in the Petri dish. Five replications were carried out for each concentration of the different solutions of neem extracts (fresh, dried leaves and fresh kernels). The number of dead weevils on the treated part of the Whatman paper and those on the untreated part was recorded after 30 minutes and one hour of time. The experimental design was a completely randomized Fisher block in five treatments with five replications.

2.4 Data Analysis

The Microsoft Office 2019 Excel spreadsheet was used for entering and processing the data collected and creating the various graphs. R software version 3.6.2 was used for statistical analyses.

The data collected were subjected to Shapiro's and Fligner's tests using R software to check the normality and homogeneity of the variances. Normality and homogeneity of variances. As the data did not meet these criteria, a non-parametric Kruskal-Wallis test was performed to compare the different means. In the event of significant differences between treatments, the pairwise test was used to separate the different means at the 5% probability threshold.

When the distribution of data did not follow the normal law of distribution, nonparametric Kruskal-Wallis analysis was performed to detect differences between treatments. When there was a significant difference between the treatments, the comparison of the pairwise means was carried out with the pairwise t-test at 5% threshold. The analyzes concerned the following parameters:

The mortality rate was calculated using the following formula of (Abbott, 1925):

$$MC\% = (Mo - Mt*100)/ (100 - Mt)$$

Mortality rate= (Number of dead individuals) / (total number of individuals) x100

Mortalities in treated boxes (Mo) were expressed according to Abbott's formula (1) in corrected mortalities (Mc), taking into account natural mortalities observed in control boxes (Mt).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of Fresh Neem Leaf Extracts on the Mortality of *Cylas puncticollis* after 30 minutes of Exposure

Table 1 shows a low mortality rate (5.00±0.50 and 2.5±0.15%), particularly with concentrations 30 and 10 cl/l in the treated area. No mortality was observed in the area treated with 0 cl and 40 cl as well as in the untreated areas.

Table 1. Comparison of the mortality rate of *C. puncticollis* according to the area (treated or untreated) with macerated extracts of fresh neem leaves

Mortality time	Plant extracts	Concentration	Treatment		Probability
			Treated	Untreated	
30 mn	Fresh leaves	40cl/l	0.00±0.00	0.00±0.00	0.00
30 mn	Fresh leaves	30cl/l	5.00±0.50	0.00±0.00	0.32
30 mn	Fresh leaves	20cl/l	0.00±0.00	0.00±0.00	0.00
30 mn	Fresh leaves	10cl/l	2.50±0.15	0.00±0.00	0.31
30 mn	Fresh leaves	00cl/l	0.00±0.00	0.00±0.00	0.00

In each column, the values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-t-test

Table 2. Comparison of the mortality rate of *C. puncticollis* according to the area (treated or untreated) with macerated extracts of fresh neem leaves

Mortality time	Plant extracts	Concentration	Treatment		Probability
			Treated	Untreated	
1 h	Fresh leaves	40cl/l	45.00±1.70	0.00±0.00	0.18
1 h	Fresh leaves	30cl/l	5.00±0.50	0.00±0.00	0.31
1 h	Fresh leaves	20cl/l	0.00±0.00	0.00±0.00	0.00
1 h	Fresh leaves	10cl/l	2.50±0.15	0.00±0.00	0.21
1 h	Fresh leaves	00cl/l	0.00±0.00	0.00±0.00	0.00

In each column, the values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-t-test

Table 3. Comparison of the mortality rate of *C. puncticollis* in treated and untreated) areas with macerated extracts of fresh neem kernels

Mortality time	Plant extracts	Concentration	Treatment		Probability
			Treated	Untreated	
30 mn	Fresh kernels	40cl/l	30.00±11.54	0.00±0.00	0.12
30 mn	Fresh kernels	30cl/l	0.00±0.00	0.00±0.00	0.00
30 mn	Fresh kernels	20cl/l	5.00±0.58	0.00±0.00	0.31
30 mn	Fresh kernels	10cl/l	0.00±0.00	0.00±0.00	0.00
30 mn	Fresh kernels	00cl/l	-	0.00±0.00	0.00

In each column, the values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-t-test

3.1.2 Effect of fresh neem leaf extracts on the mortality rate of *Cylas puncticollis* after 1 hour of exposure

Table 2 shows a high mortality rate of 45.00±1.70% in the area treated with neem leaf extracts at a concentration of 40 cl/l followed by 5.00±0.50% with 30 cl/l and 2.50±0.15% mortality with 10 cl/l.

No mortality was recorded with concentrations 40 cl/l and 20 cl/l as well as with the control (00 cl/l) and the different concentrations in 1 hour of exposure in the untreated area.

3.1.3 Effect of fresh neem kernel extracts on the mortality of *Cylas puncticollis* after 30 minutes of exposure

Table 3 shows that in the treated area after 30 minutes of exposure there was a high mortality rate (30.00±11.54%) with 40 cl/l; followed by 5.00±0.58% mortality with 20 cl/l. No mortality was recorded with 30 and 10 cl/L in the treated area. No mortality was recorded neither with 00 cl/l after 30 minutes of exposure. In the treated area, no mortality was observed. The analysis of variance showed that there was no significant difference between the treatments.

3.1.4 Effect of fresh neem kernel extracts on the mortality rate of *Cylas puncticollis* after 1 hour of exposure

After 1 hour of exposure, a high mortality rate of 32.5±7.50% was recorded with 40 cl/l followed by 12.50±4.50%; 5.00±0.58%; 0.00±0.00% were noted with 10, 20, 00 cl/l respectively (Table 4). There was no significant difference between these mortality rates. No mortality was recorded with 00 cl/l after 1 hour of exposure.

Table 4. Comparison of the mortality rate of *C. puncticollis* in treated and untreated with macerated extracts of fresh neem kernels

Mortality time	Plant extracts	Concentration	Treatment		Probability
			Treated	Untreated	
1h	Fresh kernels	40cl/l	32.5±7.50	0.00±0.00	0.14
1h	Fresh kernels	30cl/l	0.00±0.00	0.00±0.00	0.00
1h	Fresh kernels	20cl/l	5.00±0.58	0.00±0.00	0.31
1h	Fresh kernels	10cl/l	12.50±4.50	0.00±0.00	0.13
1h	Fresh kernels	00cl/l	-	0.00±0.00	0.00

In each column, the values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-t-test

Table 5. Comparison of the mortality rate of *C. puncticollis* in treated and untreated areas with macerated extracts of fresh neem leaves and kernels

Mortality time	Concentration	Treatment			
		Fresh kernels		Fresh leaves	
		Untreated	Treated	Untreated	Treated
30mn	40cl/l	0.00±0.00	20.00±3.10	0.00±0.00	0.00±0.00
30mn	30cl/l	0.00±0.00	0.00±0.00	0.00±0.00	5.00±0.50
30mn	20cl/l	0.00±0.00	5.00±0.50	0.00±0.00	0.00±0.00
30mn	10cl/l	0.00±0.00	0.00±0.00	0.00±0.00	2.50±0.30
30mn	00cl/l	0.00±0.00	0.00±0 ;00	0.00±0.00	0.00±0.00
Probability	0.02				

In each column, the values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-t-test

Table 6. Comparison of the mortality rates of *C. puncticollis* on treated and untreated) areas with macerated extracts of fresh neem leaves and kernels

Mortality time	Concentration	Treatment			
		Fresh kernels		Fresh kernels	
		Untreated	Treated	Untreated	Treated
1h	40cl/l	5.00±0.50b	42.00±7.00d	0.00±0.00	60.00±10.00a
1h	30cl/l	0.00±0.00a	0.00±0.00a	0.00±0.00	5.00±0.50b
1h	20cl/l	7.50±0.80b	5.77±0.60b	0.00±0.00	0.00±0.00a
1h	10cl/l	7.50±0.80b	15.00±1.27c	0.00±0.00	2.50±0.30b
1h	00cl/l	-	0.00±0.00a	0.00±0.00	0.00±0.00a

In each column, the values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-t-test

3.1.5 *Cylas puncticollis* mortality rate

Mortality Effect of fresh neem leaf and kernel extracts on the mortality of Cylas puncticollis after 30 minutes of exposure: After 30 minutes of exposure to fresh kernels, mortality rates of 20.00±3.10% and 5.00±0.50% were noted with 40 and 20 cl/l respectively in the treated area; no mortality was recorded with 00 cl/l; 10 cl/l and 30 cl/l then rate of 5.00±0.50%; 2.50±0.30% were respectively noted with concentrations of 30 cl/l and 10 cl/l in the treated area (Table 5). No mortality was recorded with any concentration of fresh leaf extracts in the untreated area with kernels and fresh leaves after 30 minutes of exposure. There was a significant difference between these mortality rates (p=0.02).

Effect of fresh neem leaf and kernel extracts after 1 hour of Cylas puncticollis exposure: After 1 hour of exposure to fresh kernels, a high mortality rate of 42.00±7.00% was noted with 40 cl/l in the area treated with neem leaf extracts followed by 15.00±1.27 % for the concentration of 10 cl/l and 5.77±0.50% for the concentration of 20 cl/l. No mortality was recorded with 00 cl/l and 30 cl/l then rates of 5.00±0.50%; 2.50±0.30% were respectively noted with concentrations of 30 cl/l and 10 cl/l in the treated area (Table 6). No mortality was recorded for the untreated area

with fresh leaves, however for the treated area, a rate of 7.50±0.80% was noted with the concentration of 20 cl/l and 10 cl/l followed by a rate of 5.00±0.50% respectively for the concentration of 40 cl/l. A high mortality rate of 60.00±10.00 was observed with the concentration of 40 cl/l of fresh leaf and low mortalities of 5.00±0.50% and 2.50±0.30% were observed with fresh leaf extracts after 1 hour of exposure with the concentration of 30 cl/l and 10 cl/l. No mortality was recorded with 00 cl/l and 20 cl/l of fresh neem leaves. These mortality rates were significantly different (p=0.02).

3.2 Discussion

Neem extracts are recognized for their chemical activity against insect pests.

The results show that after 30 minutes of exposure to the different neem leaf extracts, no mortality was observed in the untreated area. Low mortality was observed in the area treated with neem leaves. However, after 1 hour of exposure, a strong increase in mortality was recorded. This means that it took as long as 60 minutes for Azadirachtin to act on the exposed insects. These results are in agreement with those of (Simmonds and Blaney, 1996) who showed that neem acts systemically after spraying plots attacked by insects. This could be explained by the fact that the neem leaf extract containing Azadirachtin is a non-mutagenic and biodegradable terpene group alkaloid which is said to paralyze the digestive tract, i.e. insects are no longer able to digest what they have ingested: they stop eating and die. At least 12 modes of action of neem have been listed, and these extracts can act as a repellent, anti-appetant or phagodissuasive, or as a growth regulator affecting oviposition in females and moulting and larval growth in certain arthropods, ovicide, larvicide, weakening insects and inhibiting their resistance (Bélanger, 2005). Application of the extract poisons adult weevils. This explains why neem leaf extracts have led to mortality in C. puncticollis adults. This means that its use could provide significant protection for stored sweet potato tubers. Indeed, neem is a very powerful insecticide and has been used for centuries as a natural insecticide to protect crops in granaries.

In fact, all parts of neem have pesticidal properties (Boeke et al., 2004). Neem extracts are composed of a mixture of over 100 compounds responsible for insect mortality, including the main documented component, azadirachtin (Addea-Mensah, 1998), although its concentration varies from one part of the plant to another. Several studies have shown high concentrations of azadirachtin in neem leaves and seeds (Schmutterer, 1990; Singh et al., 2010).

Bélanger (2005) showed that the insecticidal properties of Neem extracts were also tested with great success in the field in Burkina Faso on green beans against the bean fly and the locust, on watermelons against the cucurbit fly, on tomato against tomato moth, aphids and whiteflies. In addition, several other studies have shown that extracts from different parts of Neem (*Azadirachta indica*) have broad-spectrum effectiveness against insect pests including insecticidal activities (Looli et al., 2022).

According to Musabyimana et al., (2001), neem leaf powder and aqueous extract were shown to prevent stem borer perforation of young banana trees in Kenya. Our results are similar to those of Minista et al., (2017), who showed that neem leaf extract induced an average mortality of 86.25% of adult weevils.

The insecticidal properties of Azadirachta indica were also tested in the field.

The insecticidal properties of *Azadirachta indica* were also tested in the field in Senegal on cotton against sucking biters, carpophagous caterpillars and leaf-eating caterpillars (Sané et al., 2018). Regarding fresh neem kernels, high mortality of the potato weevil was recorded after 30 minutes of exposure as well as after 1 hour. This means that the Azadirachtin contained in neem seeds had a rapid effect on weevils compared to that contained in fresh neem leaves. Indeed, (Simmonds and Blaney, 1996) showed that neem seeds contain 3 to 9 mg of Azadirachtin per gram of seed. These results are in agreement with (Kumar et al., 2003) who showed that Azadirachtin, the main component with insecticidal properties of neem extracts, is more concentrated in the seeds and oils extracted from the seeds.

Neem extract has been shown to contain a mixture of several compounds responsible for insect mortality (Gauvin et al., 2002). In particular, Azadirachtin a, which is more concentrated in neem seeds, is considered the main compound with insecticidal properties in neem (Tamgno and Tinkeu, 2014). Studies by

Lesueur (2006) have reported that Azadirachtin is the main limonoid responsible for the efficacy of neem extracts.

In addition, these results are in agreement with Keïta et al., (2023) who showed a high mortality rate of fruit flies (*Bactrocera dorsalis* and *Ceratitis cosyra*) exposed to the extract of *A. indica* after 72 hours.

Low toxicity of neem seed extract on weevils compared to leaves would be due to the low concentration of compounds with insecticidal properties. These results contrast with those of Gauvin et al., (2002) who showed a high toxicity of seeds compared to neem leaves on insects.

4. CONCLUSION

The tests carried out to determine the mortality potential of neem extracts ended up with interesting results: after 1 hour of exposure to neem leaf extracts, a high mortality of 45% was recorded in the area treated with neem leaves. However, 30% of mortality was recorded after 30 minutes then 32.5% after 1 hour with kernels. Extracts made from fresh neem leaves and kernels are harmless to the environment. They are selectively toxic, do not accumulate and have a short persistence in the ecosystem, we suggest that these extracts could be used in the control of *Cylas puncticollis* on sweet potato. Reducing weevil attacks on sweet potatoes reduces the risk of sanitary problems. Thus, neem leaf and kernel extracts could lead to significant protection of sweet potato tubers.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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