



## Isolation of Bioactive Fumigants from Different Varieties of Cassava (*Manihot esculenta* Crantz) and their Toxicity on *Tribolium castaneum* Herbst and *Rhyzopertha dominica* Fabricius

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### Abstract

*Tribolium castaneum* Herbst and *Rhyzopertha dominica* Fabricius are significant pests causing both qualitative and quantitative deterioration of stored products. The reliance on synthetic fumigants to control these pests poses numerous risks to human health and the environment. Given cassava's status as a cyanogenic plant, this study screened nine cassava varieties to isolate insecticidal compounds effective against stored product pests. Both young and mature leaves were subjected to hydrogen cyanide extraction, a potential fumigant approved by the Central Insecticidal Board (CIB) of India. Extraction was performed using two methods: direct leaf crushing at room temperature and mechanical extraction with a biopesticide extraction plant at ICAR-Central Tuber Crops Research Institute. Among the varieties, *Sree Swarna* exhibited the highest cyanogen content, while *Sree Jaya* had the least. Laboratory assays demonstrated that *R. dominica* was more susceptible to the cassava-derived bio-fumigant than *T. castaneum*. These findings suggest that bio-fumigants from cassava leaves are a viable alternative to synthetic fumigants for managing stored product pests.



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### Introduction

Post-harvest management of stored products is crucial for ensuring food security for the growing global population. Numerous insect species, over a hundred, are known to infest stored products.<sup>1,2</sup>

Among these, *Tribolium castaneum* Herbst (red flour beetle) and *Rhyzopertha dominica* Fabricius (lesser grain borer) are identified as the most destructive pests in tropical and subtropical regions, leading to substantial economic losses.<sup>3,4</sup>

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Synthetic fumigants are extensively utilized in warehouses due to their effective dispersion and penetration properties. Major fumigants include carbonyl sulfide, methyl iodide, sulfuryl fluoride, methyl bromide, and phosphine.<sup>5</sup> However, the use of methyl bromide has been banned since 2005 under the Montreal Protocol due to its adverse effects on the ozone layer and ecosystem. Phosphine, derived from aluminium or magnesium phosphide, is widely employed for grain disinfestation but has led to the development of resistance in insect populations with its continuous use.<sup>6,7</sup> Consequently, there is a global effort to identify safe, economical, and viable alternatives to synthetic pesticides.

Plant-based phytochemicals are gaining attention due to their high biodegradability, low toxicity to non-target organisms, and easy accessibility. These characteristics make them suitable for protecting agricultural products from a variety of insect pests.<sup>8,9,10,11,12,13</sup> Secondary metabolites such as alkaloids, terpenoids, and phenolics, although not involved in primary physiological processes like photosynthesis or growth, have documented bioactivity against insects, nematodes, and microorganisms.<sup>14,15</sup> Hydrocyanic acid, produced through the enzymatic conversion of cyanogenic glycosides—a group of nitrile-containing plant secondary compounds—exhibits fumigant action against stored-product pests.<sup>16,17,18,19,20</sup> Despite over 300 cyanogenic plant species, including bamboo, apple seeds and cassava, the practical use of these compounds in pest management is hindered by limited material availability, extraction challenges, and low extractability.

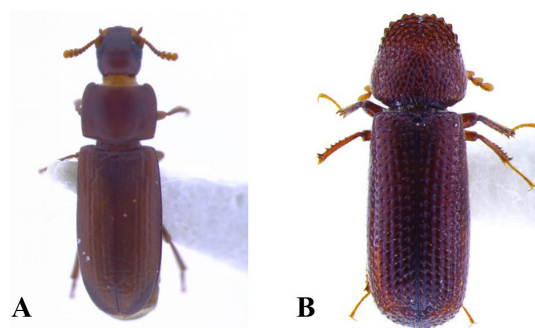
Cassava leaves, although abundant, remain underutilized. This study aims to screen nine varieties of cassava leaves for the isolation of insecticidal molecules and evaluate their efficacy against the most publicized stored grain pests *viz.* *T. castaneum* and *R. dominica*

## Materials and Methods

### Maintenance of Test Insects

*T. castaneum* and *R. dominica* (Figure 1) were collected from stock cultures and maintained in the laboratory at 32±2°C and 70±5% relative humidity. *T. castaneum* was reared on wheat flour, while

*R. dominica* was reared on black gram. Approximately 100-150 adults of each species were subcultured in 500 ml plastic containers containing their respective food sources for feeding and oviposition. The container mouths were covered with muslin cloth secured with rubber bands. Infested grains were separated and kept for adult emergence. Cohorts of 50 adult insects, two weeks old, were used for each bioassay analysis.

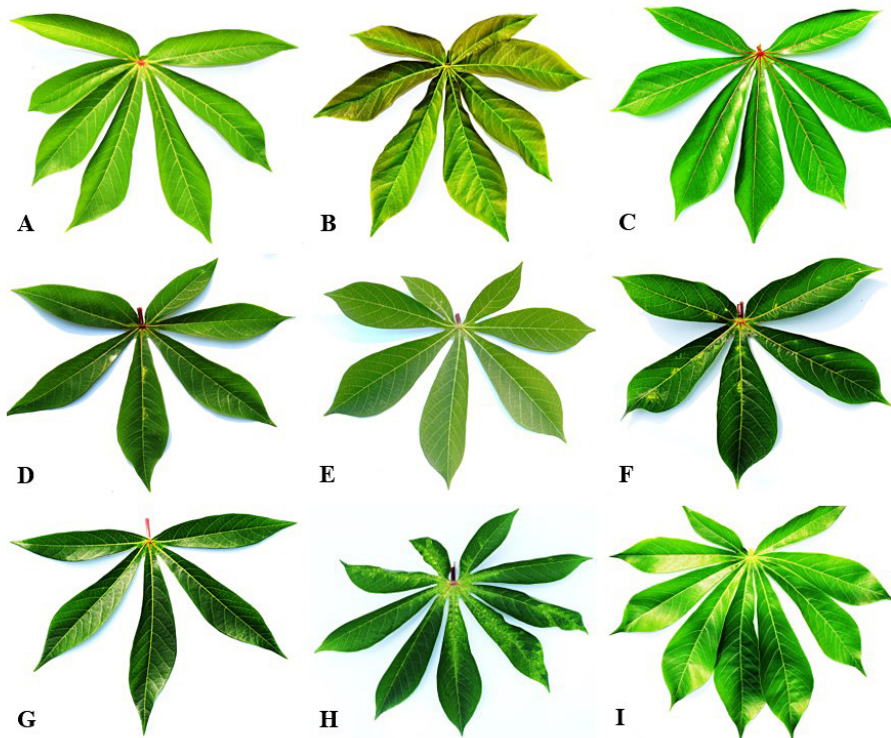


**Fig.1: Adults of A) *Tribolium castaneum* and B) *Rhyzopertha dominica***

### Isolation of Bio-Fumigant from Cassava

Tender shoots and leaves from nine cassava varieties (*Sree Swarna*, *Sree Pavithra*, *Sree Suvarna*, *Sree Prabha*, *Sree Harsha*, *Sree Sahya*, *Sree Jaya*, *M4*, and *CMR4*) (Figure 2) were collected from the ICAR-CTCRI, Kerala, for the isolation and quantification of insecticidal compounds.

**Direct leaf crushing method:** Young and mature leaves (100 g each) were chopped into approximately 0.5 cm pieces and transferred into 500 ml conical flasks. Freshly prepared alkaline picrate paper (5×1 cm) was suspended in each flask, which was then tightly sealed and kept at room temperature (31°C, RH 70%) for 24 hours. The hydrogen cyanide (HCN) content was quantified using the alkaline picrate paper method.<sup>21,22,23</sup> with modifications. The picrate paper was removed, washed with 5 ml distilled water using a micropipette, and centrifuged at 10,000 rpm for 10 minutes. The optical density (OD) of the supernatant was measured at 510 nm using a spectrophotometer (Make: PerkinElmer Lambda 25). The experiment was replicated thrice, and HCN content was calculated using the following calibration equation.<sup>21</sup>  $CN \text{ (ppm)} = 555.821 \times OD$



**Fig. 2:** Leaf morphology of cassava varieties employed in the study: A) Sree Swarna, B) Sree Pavithra, C) Sree Suvarna, D) Sree Prabha, E) Sree Harsha, F) Sree Sahya, G) Sree Jaya, H) M4, and I) CMR4



**Fig. 3:** The bio pesticide distillation unit at ICAR-CTCRI

Mechanical isolation: The distillation unit at CTCRI (Figure 3) was used for the mechanical extraction of cyanogen from cassava leaves. Young and mature cassava leaves (8 kg each) from the nine

selected varieties (*Sree Swarna*, *Sree Pavithra*, *Sree Suvarna*, *Sree Prabha*, *Sree Harsha*, *Sree Sahya*, *Sree Jaya*, *M4*, *CMR4*) were loaded into the mixer-cum-grinder unit of the biopesticide extraction plant and pulverized with a predetermined quantity of water. Cyanogen extraction followed a standardized protocol (patent pending). The liberated bio-fumigant passed through moisture-absorbing chambers and was compressed into a 4 kg portable tank. The quantity of cyanogen liberated at 80, 85, 90, 95, and 100°C was estimated using the picrate paper method.

**Detection of Cyanogen by Gas Chromatography**

The liberated HCN was converted into cyanogen chloride (CNCl) using Chloramine-T solution (0.77 g in 50 ml deionized water). The cassava bio-fumigant (CBF) was bubbled through 20 ml of 0.1 M sodium hydroxide solution for 15 minutes to convert it into sodium cyanide. After 10 minutes, 0.3 ml of Chloramine-T solution and 3 ml of n-hexane

were added as an extraction solvent for cyanogen chloride, mixed thoroughly, and left for 20 minutes to separate the active component. A standard solution was prepared with 500 µg L<sup>-1</sup> cyanogen ion (potassium cyanide) and 50 ml of 0.1 M sodium hydroxide solution. Samples (1 µl) were analysed by gas chromatography (Perkin Elmer, Clarus 580) using nitrogen as the carrier gas at a flow rate of 3.0 ml min<sup>-1</sup> in split-less mode<sup>24</sup>

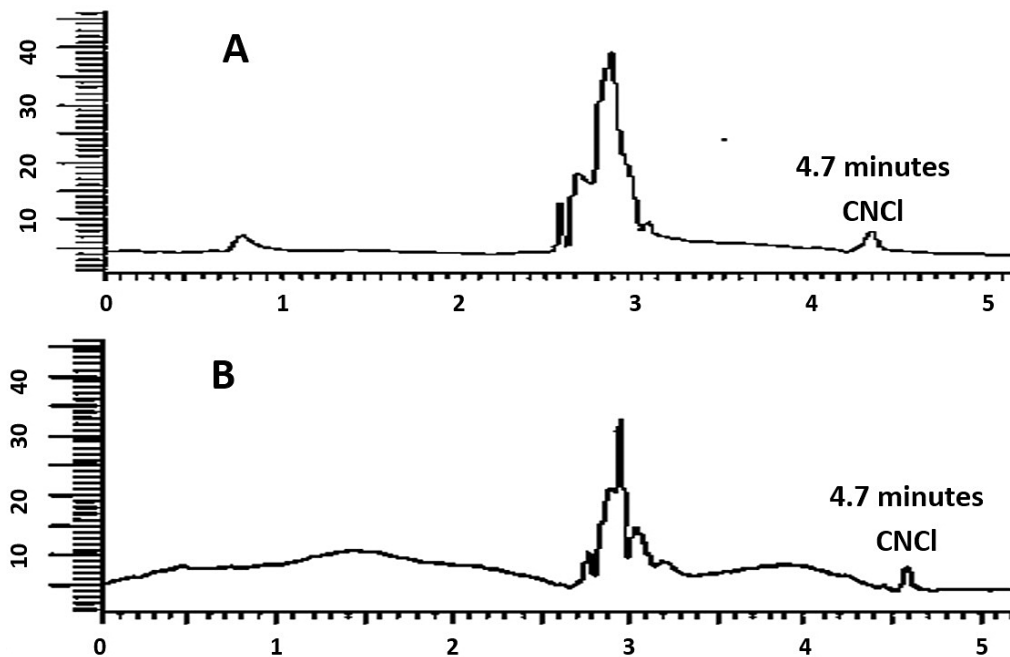
**Bioassay on the Efficacy of Active Principles:** Cohorts of 50 adults and larvae of *T. castaneum* and *R. dominica* reared in the laboratory were placed in polypropylene bags (20 × 15 cm), each containing a strip of alkaline picrate paper (5 × 1 cm). Fumigation was performed for 1-10 minutes at one-minute intervals by flushing the bags with CBF stored in portable cylinders. Control bags

were flushed with atmospheric air. The experiment was replicated thrice, and mortality was recorded from 5 to 90 minutes after treatment (MAT). HCN concentration was estimated using the picrate paper method<sup>21</sup>. Lethal concentration was calculated by probit analysis.

Statistical analysis was done using IBM SPSS Software Version 25 available from ICAR-CTCRI. The comparison studies were done using Duncan's multiple range tests- ANOVA.

**Results and Discussion**

Quantification of cyanogen in bio-fumigant from different cassava varieties: The chromatogram at a retention time of 4.7 minute ensured cyanogen is one of the active principles in CBF (Figure 4).



**Fig. 4: A. Gas chromatogram of potassium cyanide represented as cyanogen chloride; B. Gas chromatogram of cassava bio fumigant represented as cyanogen chloride.**

**Direct Leaf Crushing Method**

Cyanogen content in both young and matured leaves was significantly higher in *Sree Swarna* than the other varieties evaluated (Table 1). Concentration of cyanogen in young leaves varied from 245.8ppm in *Sree Swarna* to 87.1ppm in *Sree Jaya*; however, its difference among *Sree Suvarna*, *Sree Harsha* and

*Sree Sahya* were found not significant. There was a significant variation in the extractability of cyanogen between young and matured leaves. As in the case of matured leaves, significantly higher quantity of cyanogen was noticed in *Sree Swarna* (288.7ppm) and it was lowest in *Sree Jaya* (28.8ppm).

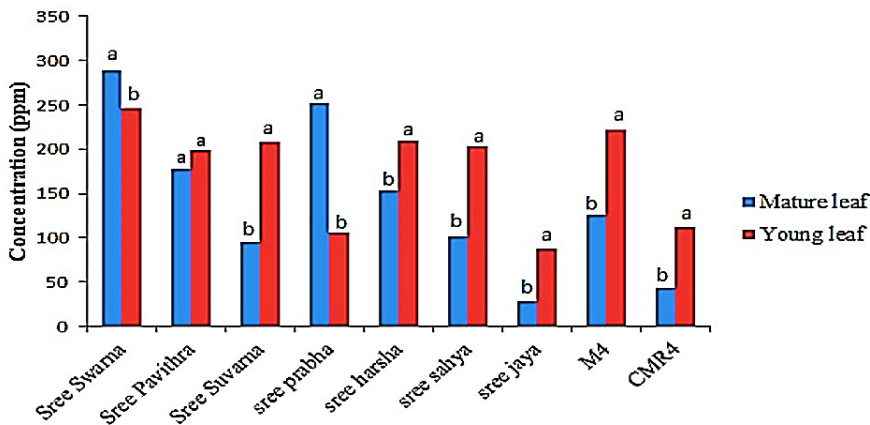
**Table 1: Concentration of HCN (ppm) in cassava leaves (Direct crushing method)**

Variety	Young leaf	Matured leaf
<i>Sree Swarna</i>	245.8±8.6 <sup>a</sup>	288.7±8.2 <sup>a</sup>
<i>Sree Pavithra</i>	198.3±10.8 <sup>c</sup>	177±4.4 <sup>c</sup>
<i>Sree Suvarna</i>	208.1±7.4 <sup>b<sup>c</sup></sup>	95.4±7.1 <sup>f</sup>
<i>Sree Prabha</i>	106.4±6.6 <sup>de</sup>	252.5±11 <sup>b</sup>
<i>Sree Harsha</i>	209.2±9.2 <sup>bc</sup>	153.4±6.1 <sup>d</sup>
<i>Sree Sahya</i>	203.2±17.3 <sup>bc</sup>	100.8±7.7 <sup>f</sup>
<i>Sree Jaya</i>	87.1±4.9 <sup>e</sup>	28.8±7.1 <sup>h</sup>
M4	221.1±18.7 <sup>b</sup>	125.2±8.6 <sup>e</sup>
CMR4	112.8±12.6 <sup>d</sup>	44±6.5 <sup>g</sup>
p-Value	<.0001	<.0001
CV (%)	6.63	4.93
SE(d)	9.569	5.662
LSD at 5%	20.286	12.003

$\bar{X} \pm SD$ , Duncan's multiple range tests; letters of the same alphabet in the same column are statistically not significant; Replication: 03 nos./ variety.

The young leaves of *Sree Suvarna*, *Sree Harsha*, *Sree Sahya*, *Sree Jaya*, M4 and CMR4 had

significantly higher level of cyanogen content than their matured leaves (Figure 5); whereas, it was higher in matured leaves of *Sree Swarna* and *Sree Prabha*, but no significant difference was noticed between young and matured leaves of the variety *Sree Pavithra*.

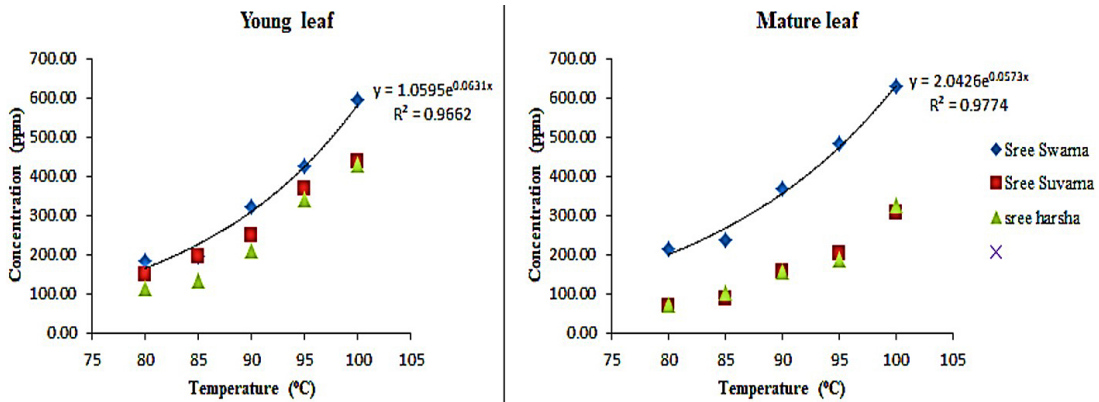


**Fig. 5: Cyanogen content of young and matured leaves of all the selected varieties of Cassava**

**Mechanical Isolation**

Irrespective of variety, a positive trend between temperature and the amount of cyanogen liberated was noticed (Figure 6). The alkaline picrate test

revealed that liberation of cyanogen starts at 80 °C and reaches maximum at 100 °C. After this temperature we observe a uniform yield.



**Fig. 6: Concentration of HCN at different temperature on young and mature leaf of Sree Swarna, Sree Suvama and Sree Harsha**

High concentration of HCN was extracted from the variety *Sree Swarna* in both young and matured leaves (Table 2), and it was least in the variety *Sree Jaya*. Extractability of cyanogen was higher in

the young leaves than in the matured leaves of the varieties *Sree Pavithra*, *Sree Suvama*, *Sree Harsha*, *Sree Sahya*, *Sree Jaya*, M4 and CMR4 (Table 2 & 3).

**Table 2: Concentration of HCN isolated from the young leaves of cassava at different temperature.**

Temperature (°C)					
Variety	80	85	90	95	100
<i>Sree Swarna</i>	182±8 <sup>a</sup>	194±5.6 <sup>a</sup>	320.3±8.1 <sup>a</sup>	425.4±20.3 <sup>a</sup>	595.3±7.5 <sup>a</sup>
<i>Sree Pavithra</i>	117.2±6 <sup>cd</sup>	133.7±5.7 <sup>c</sup>	199.1±13.2 <sup>d</sup>	276.1±15.5 <sup>e</sup>	331.7±12.3 <sup>e</sup>
<i>Sree Suvama</i>	147.5±8 <sup>b</sup>	195.9±5.7 <sup>a</sup>	250.1±23.2 <sup>c</sup>	366.9±10.1 <sup>bc</sup>	436.5±8.6 <sup>b</sup>
<i>Sree Prabha</i>	57.1±9 <sup>f</sup>	81.7±7.1 <sup>e</sup>	147.6±5.7 <sup>e</sup>	219.9±9.3 <sup>f</sup>	233.1±7 <sup>g</sup>
<i>Sree Harsha</i>	112.7±10 <sup>d</sup>	134±11 <sup>c</sup>	211.5±9.2 <sup>d</sup>	340.2±10.5 <sup>d</sup>	431.4±4.9 <sup>bc</sup>
<i>Sree Sahya</i>	129±7 <sup>c</sup>	135.5±8.8 <sup>c</sup>	313.6±10 <sup>ab</sup>	370.2±4.8 <sup>b</sup>	414.2±13 <sup>c</sup>
<i>Sree Jaya</i>	39.33±9.8 <sup>g</sup>	58.2±3.1 <sup>f</sup>	62.7±6.8 <sup>f</sup>	107.8±5.9 <sup>g</sup>	129.2±2.4 <sup>h</sup>
M4	118.3±5.6 <sup>cd</sup>	157.1±6.6 <sup>b</sup>	297.1±9.7 <sup>b</sup>	347.8±7.4 <sup>cd</sup>	354.3±11.6 <sup>d</sup>
CMR4	87.4±4.5 <sup>e</sup>	118.9±2.7 <sup>d</sup>	127.8±5.6 <sup>e</sup>	277±22.1 <sup>e</sup>	269.6±15.5 <sup>f</sup>
p-Value	<.0001	<.0001	<.0001	<.0001	<.0001
CV (%)	7.43	4.72	5.37	3.85	2.88
SE(d)	6.678	5.177	9.41	9.551	8.345
LSD at 5%	14.157	10.975	19.948	20.247	17.691

$\bar{X}$ ±SD, Duncan's multiple range tests; Letters of the same alphabet in the same column are statistically not significant; Replication: 03 nos./ variety.

**Table 3: Concentration of HCN isolated from the matured leaves of cassava at different temperature**

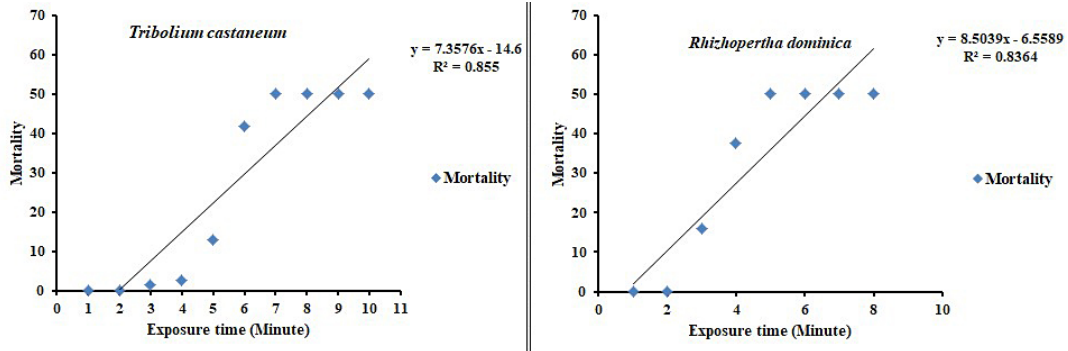
Temperature (°C)					
Variety	80	85	90	95	100
Sree Swarna	213.9±12 <sup>a</sup>	237.78±7.8 <sup>a</sup>	366.5±10.7 <sup>a</sup>	483.2±17.5 <sup>a</sup>	629.1±18.4 <sup>a</sup>
Sree Pavithra	87.8±7.8 <sup>c</sup>	91±4.7 <sup>de</sup>	117.2±6.6 <sup>d</sup>	217.1±16.1 <sup>d</sup>	225.8±22.8 <sup>e</sup>
Sree Suvarna	66.5±6.6 <sup>d</sup>	86.7±7.1 <sup>e</sup>	154.9±7.8 <sup>c</sup>	203.1±6.5 <sup>De</sup>	307.3±6.8 <sup>c</sup>
Sree Prabha	167.1±8.3 <sup>b</sup>	175±4.1 <sup>b</sup>	193.9±7.1 <sup>b</sup>	248.9±17.2 <sup>c</sup>	274.2±6.2 <sup>d</sup>
Sree Harsha	71.7±11.2 <sup>d</sup>	101.1±8.7 <sup>e</sup>	156.3±7.5 <sup>c</sup>	187.8±8.6 <sup>e</sup>	324.6±9.2 <sup>bc</sup>
Sree Sahya	48.5±9.3 <sup>e</sup>	63.2±3.5 <sup>f</sup>	187.2±7.1 <sup>b</sup>	312.2±13.3 <sup>b</sup>	326.9±14.2 <sup>b</sup>
Sree Jaya	14.9±5.8 <sup>f</sup>	31.2±7 <sup>g</sup>	43.6±5.1 <sup>e</sup>	51.5±2 <sup>g</sup>	56.4±3.1 <sup>g</sup>
M4	84.8±3.1 <sup>c</sup>	116.8±6.1 <sup>c</sup>	164±7 <sup>c</sup>	213.1±15.2 <sup>d</sup>	224.8±9.4 <sup>e</sup>
CMR4	21.8±1.9 <sup>f</sup>	28.7±3 <sup>g</sup>	44.3±6.8 <sup>e</sup>	110±5.8 <sup>f</sup>	124±10.6 <sup>f</sup>
p-Value	<.0001	<.0001	<.0001	<.0001	<.0001
CV (%)	8.37	5.7	4.48	5.59	3.79
SE(d)	5.903	4.821	5.809	10.287	8.567
LSD at 5%	12.513	10.22	12.314	21.808	18.162

$\bar{X}$ ±SD, Duncan's multiple range tests; Letters of the same alphabet in the same column are statistically not significant; Replication: 03 nos./ variety.

**Bioassay on the Efficacy of Active Principles**

As the *Sree Swarna* was found highest extractability of cyanogen, this variety was used for further study.

A positive correlation was noticed between mortality of the target pests and exposure time of CBF (Figure 7).



**Fig. 7: Mortality of test insects at different time of exposure**

Mortality of *T. castaneum* started at 5 Minutes after treatment (MAT) with a concentration of 28.48ppm of CBF, and it reached 100% at 49.84ppm (Table 4). When the insect was exposed to 7.12ppm of bio-fumigant the mortality was observed approximately 50% at 60 MAT.

*T. castaneum* was observed at 49.84 ppm in 5 MAT (Table 4) and it was 35.6ppm for *R. dominica* (Table 5). The LC<sub>50</sub> values for *T. castaneum* and *R. dominica* were calculated as 35.96 and 22.59ppm respectively (Table 6).

*R. dominica* was found higher susceptible than *T. castaneum*, to CBF, and 100% mortality of

**Table 4: Mortality of *Tribolium castaneum* due to the exposure of cassava bio-fumigant**

Time of exposure (min)	Conc. (ppm)	Minute After Treatment (MAT)									
		5	10	20	30	40	50	60	70	80	90
1	0.18±0.0 <sup>i</sup>	-	-	-	-	-	0	0	0	0	0
2	3.56±0.2 <sup>hi</sup>	-	-	-	0	0	6 <sup>d</sup>	19.3 <sup>b</sup>	36 <sup>c</sup>	45 <sup>b</sup>	50 <sup>h</sup>
3	7.12±0.9 <sup>h</sup>	-	-	-	1.3 <sup>d</sup>	3 <sup>d</sup>	12 <sup>c</sup>	22.7 <sup>b</sup>	41 <sup>b</sup>	47 <sup>b</sup>	50 <sup>g</sup>
4	14.24±3 <sup>g</sup>	-	-	0	2.7 <sup>d</sup>	15.3 <sup>c</sup>	32.3 <sup>b</sup>	48.7 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>f</sup>
5	21.36±4.9 <sup>f</sup>	0	0	0.3 <sup>c</sup>	13 <sup>c</sup>	43 <sup>b</sup>	49.3 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>e</sup>
6	28.48±4.1 <sup>e</sup>	5 <sup>d</sup>	9.7 <sup>d</sup>	14 <sup>b</sup>	41.7 <sup>b</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>d</sup>
7	35.6±4.3 <sup>d</sup>	36.7 <sup>c</sup>	41.3 <sup>c</sup>	47.3 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>c</sup>
8	42.72±1.6 <sup>c</sup>	43.3 <sup>b</sup>	47 <sup>b</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>b</sup>
9	49.84±4.4 <sup>b</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>
p-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CV (%)	12.29	15.7	7.7	11.3	8.7	6.4	7.1	5	3.9	3.4	
SE(d)	2.609	2.372	1.249	1.956	1.843	1.626	2.03	1.616	1.377	1.256	0
LSD	5.4819	4.984	2.6245	4.109	3.8725	3.417	4.2649	3.396	2.8931	2.639	0

$\bar{X} \pm SD$ , Duncan's multiple range tests. Letters of the same alphabet in the same column are statistically not significant. Replication 03, n=50. Parenthesis shows percentage of mortality.

**Table 5: Mortality of *Rhizopertha dominica* due to the exposure of cassava bio-fumigant**

Time of exposure (min)	Conc. (ppm)	Minute After Treatment (MAT)									
		5	10	20	30	40	50	60	70	80	90
1	0.18±0.0 <sup>i</sup>	-	-	-	-	0	0	0	0	0	0
2	3.56±0.2 <sup>h</sup>	-	-	0	0	5.3 <sup>d</sup>	23.6 <sup>c</sup>	36.3 <sup>c</sup>	41.67 <sup>b</sup>	45.67 <sup>b</sup>	50 <sup>f</sup>
3	7.12±0.9 <sup>h</sup>	0	0	8.3 <sup>d</sup>	16 <sup>c</sup>	29 <sup>c</sup>	39 <sup>b</sup>	45.33 <sup>b</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>e</sup>
4	14.24±3 <sup>g</sup>	1 <sup>d</sup>	3.6 <sup>d</sup>	22.3 <sup>c</sup>	37.6 <sup>b</sup>	45.3 <sup>b</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>d</sup>
5	21.36±4.9 <sup>f</sup>	18.3 <sup>c</sup>	33.3 <sup>c</sup>	40.6 <sup>b</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>c</sup>
6	28.48±4.1 <sup>e</sup>	42 <sup>b</sup>	44.6 <sup>b</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>b</sup>
7	35.6±4.3 <sup>d</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	5 0 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>	50 <sup>a</sup>



p-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
CV (%)	12.29	10.6	7.4	10.6	5.3	6.5	10.1	6	4.5	3.3	0
SE(d)	2.609	1.749	1.387	2.409	1.382	1.861	3.23	2.059	1.567	1.167	0
LSD at 5%	5.4819	3.752	2.9755	5.168	2.9632	3.992	6.9275	4.415	3.3615	2.502	0

$\bar{X} \pm SD$ , Duncan's multiple range tests. Letters of the same alphabet in the same column are statistically not significant. Replication 03, n=50. Parenthesis shows percentage of mortality.

**Table 6: Lethal concentration of cassava bio-fumigant on *Tribolium castaneum* and *Rhizopertha dominica***

Insects	Lethal concentration (ppm)		
	LC <sub>50</sub>	LC <sub>90</sub>	LC <sub>99</sub>
<i>Tribolium castaneum</i>	35.96	41.92	47.54
<i>Rhizopertha dominica</i>	22.59	29.50	35.80

The high volatility and efficiency in penetrating stored product bulks, along with the immediate knockdown effect on targeted pests, are crucial criteria for selecting fumigants to manage stored product pests. However, the adverse effects of synthetic fumigants on human health and the environment have driven a global search for alternative strategies.<sup>12</sup> Phytochemicals, particularly those from the families Apiaceae, Lamiaceae, Lauraceae, Euphorbiaceae, and Myrtaceae, have shown efficacy against stored product pests.<sup>25,26,27,28,29,30</sup> Although over 3000 higher plant species are cyanogenic, only about 300 species are known sources of hydrogen cyanide (HCN).<sup>31</sup> The FAO (1984, 1989) has endorsed cyanogen as an effective fumigant for confined fumigation. Park & Coats in 2002 followed by Hooper in 2003 have demonstrated the effectiveness of synthetic cyanogen and hydrogen cyanide against various stored product pests, suggesting HCN as an immediate alternative to synthetic fumigants.<sup>19,32</sup>

The synthesis and accumulation of primary and secondary metabolites vary among plant species based on physiological and environmental factors. This variation affects the extractability of cyanogen within the nine cassava varieties studied. Previous research by Poulton (1990), Cardoso (2005) and Mushumbusi (2018) had reported significant differences in cyanogen levels among cassava varieties.<sup>31,33,34</sup> Nambisan (1994) found cyanogen concentrations in Indian cassava

varieties up to 1100 ppm.<sup>35</sup> Cuvaca (2015) suggested that these variations are more a function of plant physiology than growing conditions.<sup>36</sup> Ojiambo (2017) attributed differences in cyanide levels to variations in cellular structures affecting the diffusion of cyanogenic glycosides.<sup>17</sup> Tender twigs and leaves were chosen for bio-fumigant production due to their higher cyanogen content.<sup>37</sup>

Our findings show that mechanical isolation yielded higher cyanogen extractability than direct leaf crushing. The grinding process accelerates the breakdown of cyanogenic glycosides by facilitating contact between glycosides and the enzyme linamarase, catalysing hydrolysis.<sup>33</sup> Agitation in water solubilizes cyanogenic glycosides, and subsequent heating releases hydrogen cyanide.<sup>17,38</sup> Cyanogen release commenced at 55°C, with optimal linamarase activity reported at this temperature.<sup>35</sup> Pereira (2016)<sup>39</sup> found that 99.95% of cyanogen was liberated at 75°C when cassava leaves were treated with buffer and linamarase. Our study observed cyanogen recovery in the collection tank between 80 and 100°C.

Resistance to phosphine among *T. castaneum* and *R. dominica* has been well documented.<sup>7,40,41,42</sup> Our findings corroborate with Park (2004), who reported greater susceptibility of *R. dominica* to cyanogen compared to *T. castaneum*.<sup>18</sup> Aulicky (2014) and Stejskal (2016) demonstrated 100% mortality

of both pests in flour mills fumigated with HCN.<sup>43,44</sup> Continuous use of phosphine has led to resistance in several pest species, highlighting the need for alternative fumigants.<sup>6,7,40-47</sup>

### Conclusion

Cassava leaves, often discarded as waste post-harvest, represent an underutilized resource with significant potential in sustainable pest management.<sup>48,49-55</sup> This study highlights that the bio-fumigant isolated from cassava leaves is an effective alternative to synthetic fumigants for controlling stored product pests. With the increasing need for safe pest management measures, especially post-harvest, the utilization of natural bio-fumigants offers a promising solution. Cassava, primarily cultivated for its tubers, can now provide an additional income stream for farmers through the adoption of bio-fumigant extraction technology. This approach not only mitigates the environmental and health hazards associated with synthetic fumigants but also makes use of an agricultural by-product that would otherwise go to waste. The rich cyanogenic content in cassava leaves, when efficiently extracted, can serve as a potent fumigant, ensuring the protection of stored products without the adverse effects of chemical alternatives. By integrating this bio-fumigant extraction into existing cassava farming practices, farmers can enhance their economic stability while contributing to sustainable agriculture. Our study highlights the effectiveness of cyanogen as a bio-fumigant, with mechanical isolation yielding higher cyanogen extractability than direct leaf crushing. The grinding process facilitated the breakdown of cyanogenic glycosides, with optimal release occurring between 80 and 100°C. Our findings corroborate previous studies, demonstrating that *R. dominica* is more susceptible to cyanogen than *T. castaneum*, achieving 100% mortality in fumigated environments. This supports the use of cyanogen as an alternative to phosphine, particularly in light of the growing resistance of stored product pests to synthetic fumigants. This study underscores

the dual benefits of this technology: effective pest management and additional revenue for farmers, paving the way for a safer and more sustainable post-harvest storage system.

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### Conflict of Interest

The authors declare no conflict of interest by any means.

### Data Availability Statement

All the raw data concerned to the representations and analysis made out in this paper is available with the first author A.G. and this can be availed by writing a mail to joyajesh87@gmail.com.

### Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

### Authors Contribution

A.G. and J.U.K conceived the idea, contribute to the initial concept and supervision of the work; A.G. design the methodology; A.G. and J.C.A. acquisition of data, investigation; A.G. data curation, primary drafting of the article and field photo credits; A.G. lead the data analysis; A.G. contributed equally on the representation and visualization of the data; A.G. and J.C.A. worked together on the interpretation of data; A.G. and J.U.K drafting the article. All authors contributed critically to the draft within their responsibilities and gave final approval for publication.

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