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Toxicity of Insecticidal Principles from Cassava (*Manihot esculenta* Crantz) on Pseudostem Weevil (*Odoiporus longicollis* Oliver) (Coleoptera: Curculionidae) in Banana

Jithu Unni Krishnan, C.A. Jayaprakas, N.R. Lekshmi, L.S. Rajeswari and S. Leena

ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram 695 017, Kerala, India

Corresponding author: C.A. Jayaprakas, e-mail: prakashcaj@gmail.com

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Abstract

Injudicious use of chemical inputs in agriculture poses challenge to sustainable agriculture. Cassava (*Manihot esculenta* Crantz) is a climate resilient crop cultivated in tropical and subtropical countries for its tuber as food, feed, and industrial products. Nevertheless, owing to the presence of cyanoglucosides, leaves and tuber rinds are often thrown as waste or rather underutilized. Insecticidal principles from such biowaste were isolated and a formulation by name *Menma* was prepared for the management of important borer pests of fruit and tree crops. Banana pseudostem weevil (BPW), *Odoiporus longicollis* Oliver, a serious pest of banana causes 10-90 per cent yield loss due to premature crop falling. The current management strategy relies on the application of very toxic synthetic insecticides; however, no satisfactory control of the pest could be achieved. Management of this pest was achieved using *Menma* as a curative measure and the current study deals the lethality measurement of the same in the laboratory. Hydrogen cyanide is the key component imparting toxicity in *Menma* and considering it as the active ingredient the LC_{50}, LD_{50} and LE_{50} or KDT_{50} were calculated on BPW. The study imparts a clear idea regarding the respective dose and concentration of *Menma* to be recommended for the management of BPW in field.

Key words: Cassava, biopesticide, banana pseudostem weevil, bio-control, banana weevil management, mortality studies

Introduction

Cassava (*Manihot esculenta* Crantz) is the most important calorie producing crop grown as a staple or subsidiary food for 450-500 million people in 26 tropical and subtropical countries (FAO, 2012). After harvest large portion of the biomass such as cassava leaf (2 t ha^{-1}) and tuber rinds (15-23% of the tuber) are generally discarded as waste. Cassava leaf is a storehouse of protein and minerals, despite the anti-nutritional factors like cyano-glycosides in the plant is a challenge in its diversified use. Most of the cyano-glycosides in nature, are cyanogenic and since they have a nitrile group to the glycosidic linkage, and hydrolysis of this by specific enzymes will lead to the formation of hydrogen cyanide (Fig.1) (Yang and Tanaka, 1999).

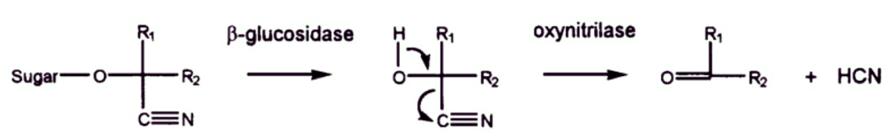

Fig.1. General plant cyanoglycoside degradation in injured plant tissue

However, leaves are often used as cattle feed after removing the toxic principle by sun drying. Literature shows that cyanogen has been used as a potential biopesticide (Price, 1985; Yong-lin Ren and Desmarchelier, 1998; Yong-lin Ren and Allen, 2001; Cortes et al., 2003; Lisbeth et al., 2003).

Banana, a food crop and as a fruit crop, enjoys the status of staple food in many of the tropical nations. Global production of banana is 102.02817 million tons, of which India contributes 29.19%, and ranks first in production (FAO, 2013). However, production of banana is severely affected by a complex of insect pests, particularly by banana pseudostem weevil (BPW) (*Odoiporus longicollis* Oliver) (Visalakshi et al., 1989; Padmanaban and Sathiamoorthy, 2001 and Bhagawati et al., 2009). Severe incidence by this pest has been reported from across the banana growing states, particularly from Karnataka, Tamil Nadu and Kerala (Reghunath et al*.,* 1992; Gailce Leo Justin et al., 2008 and Thippaiah et al*.,* 2010). Adult feeds on the pseudostem and female makes ovipositional punctures on the outer sheath of the plant (Anitha and Nair, 2004). Eggs are deposited in the air cavity, and on hatching, the grubs make longitudinal tunnels (Ravi and Palaniswami, 2002). Pseudostem of the heavily infested plant is often hollow, weak and it falls prematurely, causing 10-90 per cent yield loss depending on the stage of infestation and management efficiency (Padmanaban and Sundaraju, 1999; Gold et al., 2001 and Prasuna et al., 2008). In order to replace the chemical insecticides, various plant products have been screened against BPW (Bhagawati et al*.,* 2009), but these need further standardisation to utilise them in the field.

Current study deals with the mortality of BPW due to the treatment of '*Menma*'. The bio-efficacy of hydrogen cyanide in *Menma* against the BPW was done and calculated the lethal concentration $(LC)_{50}$, lethal dose $(LD)_{50}$ and lethal exposure $(LE)_{50}$ or knock down time $(KDT)_{50}$.

Materials and Methods

Maintenance of test insect

Adult weevils (*Odoiporus longicollis),* collected from the banana fields of Thiruvananthapuram district (8.5241° N, 76.9366° E) were maintained in the laboratory at 26±3° C and 60 ± 10% RH and 12:12 L:D. The pupae collected were maintained separately in 100 ml plastic container and its mouth was covered by muslin cloth. On emergence, the adults were transferred into1 litre plastic container and they were provided pseudostem for feeding. Adults with approximately 2 weeks, irrespective of sex, were selected for the study.

Production of biopesticide

The insecticidal principles (HCN) were isolated from cassava leaves (variety H-97) collected from the farm of ICAR-Central Tuber Crops Research Institute (ICAR-CTCRI), Thiruvananthapuram, by using the cassava biopesticide extraction unit commissioned at the biopesticide laboratory. The isolated active principles were formulated and named as *Menma* and its cyanogen content was estimated using spectrophotometer and gas chromatography.

Estimation of cyanogen in the extract

Cyanogen in the biopesticide *Menma* was estimated with the modified method of Breuer and Rumball (2007). To the 20 ml of *Menma* taken in 100 ml conical flask, added 200μl of 0.5 M NaOH (Breuer and Rumball, 2007) and by adding 0.1 ml of the newly prepared Rhodanine indicator (Breuer and Rumball, 2007), it was titrated against 0.0103 M $Ag\ NO_3$ solution until reaching the endpoint (colour change). The cyanogen in the sample was estimated using the general titration formula.

Toxicity of Menma on banana pseudostem weevil

HCN present in the *Menma* was diluted to 1 to 100 ppm (1, 2, 4, 8, 10, 20, 40, 80 and 100 ppm) and was stored in airtight containers. The test solution was poured into a plastic container of 100 ml capacity, and five test insects were transferred into the container. To avoid direct contact between the test solution and BPW, an aluminium mesh (160mesh) was fixed in the container just above this solution. Mortality of the weevil was recorded from 0 to 18 hours and LC_{50} was calculated. LD_{50} was studied with different doses of 300 ppm of *Menma* from 0 to 6 ml (0, 1, 2, 3, 4, 5 and 6 ml) and for KDT_{50} studies the insects were exposed to 1ml of *Menma* (300 ppm) for different exposure periods from 6-14 h (6, 8, 10, 12 and 14h)

Data analysis

Data transformed to log 10 were subjected to Probit analysis using SAS 9.3 statistical software. The LC_{50}, LC_{95}; $LD_{50,}$ LD_{95} and, KDT_{50} (LE_{50}) and KDT_{95} (LE_{95}) were calculated along with their respective intercepts and slopes. All the data pertained to mortality were corrected using Abbott's formula (Abbott, 1925). ANOVA was used for comparison of treatments and time of exposure.

Results and Discussion

A positive correlation between the concentration of cyanogen in the biopesticide *Menma* and the mortality of BPW was observed (Fig. 2a, 2b). In all the treatments, weevils were alive upto half-an-hour after treatment, except with 20 ppm; however with 10 ppm, mortality was significantly higher than that of all other treatments ($P<0.001$) (Table 1). Delayed mortality of weevil was observed in the batch treated with *Menma* at low concentration of cyanogen, but it was delayed. This result is in corroboration with Hensen et al. (1991) who reported that mortality of thrips in cut flowers due to the treatment of cyanogen was less in batches treated with lower concentration than the treatment with higher concentrations.

Probit analysis showed that the LC_{50} values of *Menma* at 1 (HAT) was 15.96 ppm with the range of 8.08-25.75 (intercept-0.4±0.1; slope 1.9±0.2); and the LC_{95} was 25.25 ppm with lower to upper fiducial limit 18.51-33.1 (intercept-2.7±0.4; slope 5.0±0.6). LC50 value of *Menma* in 2h experiment was 13.79 while it reduced in 10h experiment to 5.83 (Fig. 3).

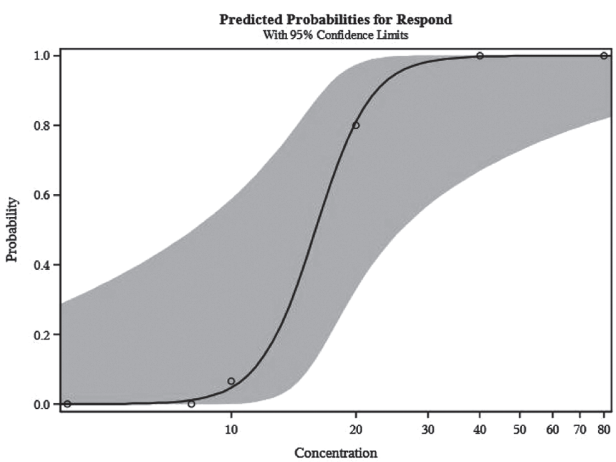

Fig.2a. Significance sketch with 95% confidence limit to mortality of *O. longicollis* for varying concentrations of *Menma* with time

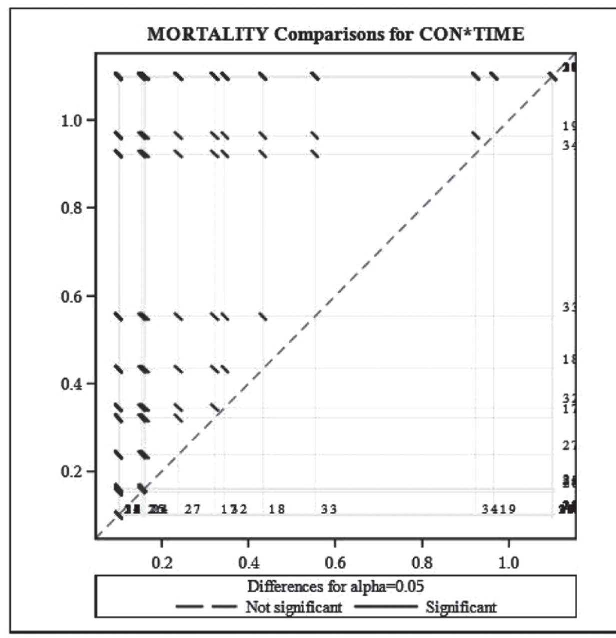

Fig.2b. Mortality curve of *O. longicollis* to varying concentrations of *Menma* with 95% confidence limits

Table 1. Mortality of banana pseudostem weevil to different concentrations of *Menma*.

Con. (ppm)	Percentage Mortality of *O. longicollis* HAT (Hours after treatment)										
	0.5	1	2	4	6	8	10	12	14	16	18
0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	6.0±6.0[d]	6.0±6.0[d]	26±11[c]	52±11[b]	100±0[a]
8	0	0	0	0	20±20[b]	32±11[b]	86±11[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]
10	0	6.0±6.0[d]	6.0±6.0[d]	26±11[c]	72±23[b]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]
20	46±11[c]	80±11[b]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]
40	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]
80	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]
100	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]	100±0[a]

Mean ± SD. Rep. 3; n= 5

Alphabets in the same column are statistically non-significant at $P<0.05$

Treatment with *Menma* (300ppm of HCN) at different doses viz. 2, 3, 4, 5 and 6 ml, a positive correlation was noticed between the dose and mortality of the test insects (Table 2). Mortality of BPW delayed at lower dose (2 to 3 ml), but at higher dose (over 4 ml) its death started as early as 10 minutes after treatment (MAT) and reached complete mortality at 4 HAT (Fig. 4a, 4b). However, no death was observed in the untreated batches. Hansen et al. (1991) observed a variation in the mortality of certain quarantine pest of Hawaii at different concentration of hydrogen cyanide fumigation.

Probit analysis showed that the LD_{50} and LD_{95} values for the *Menma* at 1 HAT were 5.26 ml (4.30-6.09 ml) (intercept -1.9±0.2; slope 4.6±0.4) and 7.45 ml (6.29-13.42 ml) (intercept -2.2±0.4; slope 5.1±0.3), respectively, but at 6 HAT the dose was 3.17 ml (2.22-3.97 ml) and 4.39 ml (3.85-7.29 ml) respectively. LD_{50} value of *Menma* in 2h experiment was 4.24 ml while it reduced to 1.92 ml in 10h experiment. (Fig. 5).

When weevils were exposed to 1ml of *Menma* (300 ppm) at different time intervals (from 6 to 14 h), it was taken 48 h to get complete mortality to the batches exposed to 6 h as against 10 HAT to the batches exposed to 14 h (Table 3). Survival of the weevils were significantly less in the batches exposed to *Menma* at 14 h than they were subjected to lower exposure ($P<0.001$) (Fig. 6a, 6b). No weevil was found alive at 48 HAT when they were exposed to 6 h, but weevil exposed to 14 h recorded death even at 0.5 HAT.

Probit analysis for KDT_{50} and KDT_{95} for *Menma* at 1 HAT was 17.57h (13.67-39.30 h) (intercept -3.2±0.6; slope 5.7±0.4) and 46.64 h (41.65-57.36 h) (intercept-2.9±0.3; slope 6.0±0.4) respectively, but at 12 HAT the KDT_{50} was 4.51h (3.53-7.01 h), and the KDT_{95} was 24.00 h (20.07-34.73) (Fig.7).

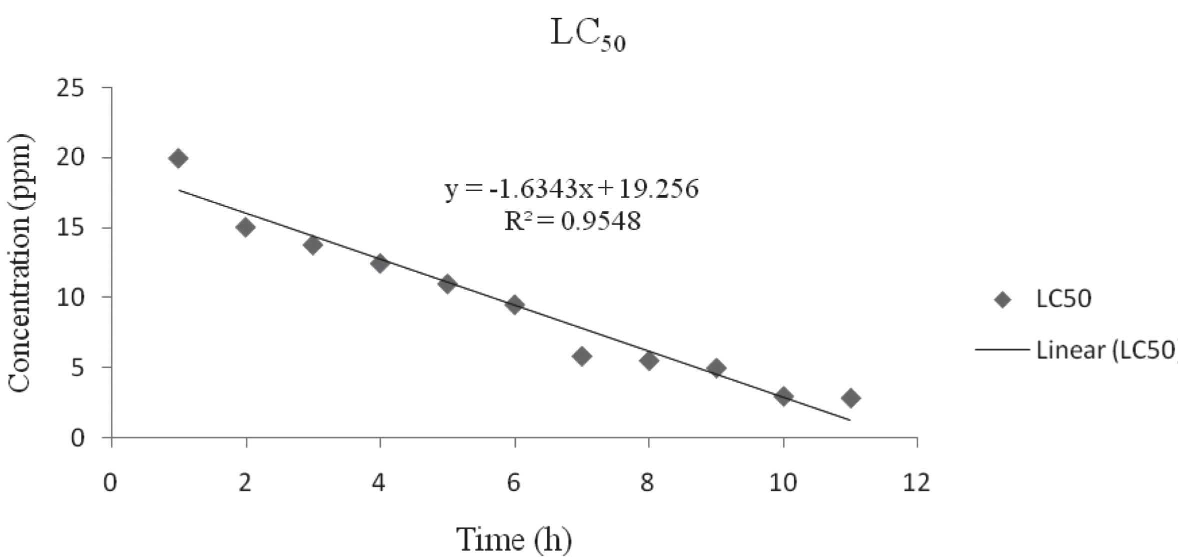

Fig.3. Scatter plot of LC_{50} values of *O. longicollis* for all different time periods observed

Table 2. Mortality of banana pseudostem weevil at different doses to *Menma*.

Dose (ml)	Percentage mortality of *O. longicollis* at different HAT (Hours After Treatment)								
	0.5	1	2	4	6	8	10	12	14
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0.3 ± 6.0^{e}	32 ± 11^{d}	52 ± 11^{c}	72 ± 11^{b}	100 ± 0^{a}
3	0	0	0	6.0 ± 6.0^{e}	26 ± 11^{d}	52 ± 11^{c}	86 ± 11^{b}	100 ± 0^{a}	100 ± 0^{a}
4	0	6.0 ± 6.0^{e}	46 ± 11^{d}	52 ± 11^{c}	92 ± 11^{b}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
5	0	52 ± 11^{c}	86 ± 11^{b}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}
6	52 ± 11^{d}	60 ± 11^{c}	86 ± 11^{b}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}	100 ± 0^{a}

Mean ± SD. Rep. 3; n= 5

Alphabets in the same column are statistically non-significant at $P<0.05$

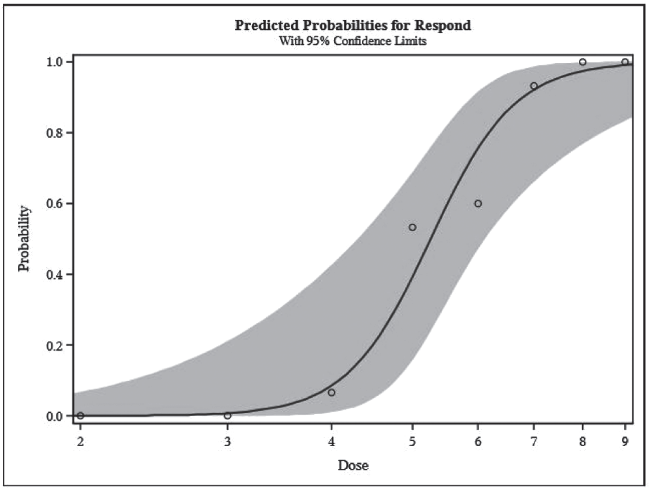

Fig.4a. Mortality curve to *O. longicollis* over varying dose (ml) of *Menma* with 95% confidence limits

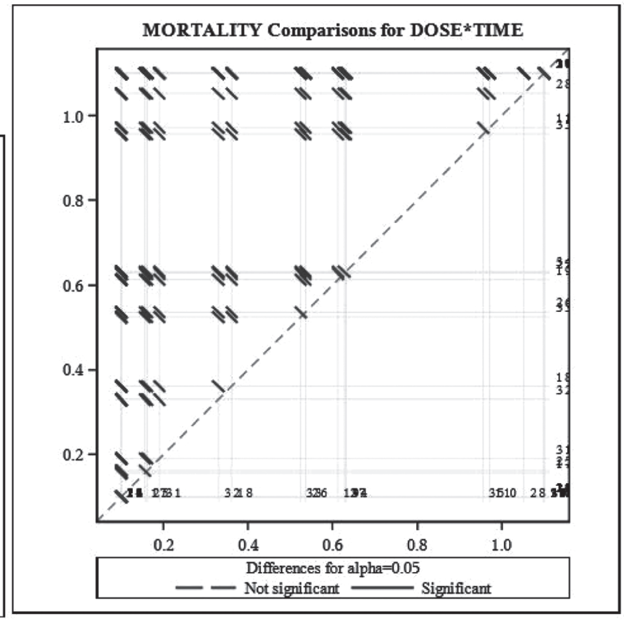

Fig.4b. Significance sketch with 95% confidence limit to mortality of *O. longicollis* for varying dose and time with *Menma*.

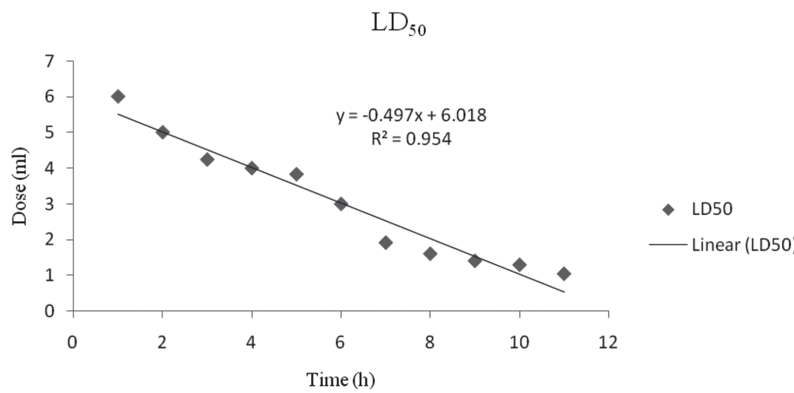

Fig.5. Scatter plot indicating the LD_{50} values of *O. longicollis* for all the time periods observed

Table 3. Mortality of banana pseudostem weevil at different exposures to *Menma*.

Sub-lethal exposure (h)	Percentage mortality of *O. longicollis* at different HAT (Hours After Treatment)								
	0.5	1	2	5	8	10	12	24	48
0	0	0	0	0	0	0	0	0	0
6	0	6.0±6.0^{g}	6.0±11^{g}	32±11^{f}	46±11^{e}	52±11^{e}	80±0^{c}	92±11^{b}	100±0^{a}
8	0	12±11^{g}	26±11^{f}	40±11^{e}	52±11^{d}	60±0^{c}	86±11^{b}	100±0^{a}	100±0^{a}
10	0	6.0±6.0^{f}	32±11^{e}	46±11^{d}	60±11^{c}	66±11^{b}	100±0^{a}	100±0^{a}	100±0^{a}
12	6.0±6.0^{g}	20±0^{f}	46±11^{e}	52±11^{d}	66±11^{c}	72±11^{b}	100±0^{a}	100±0^{a}	100±0^{a}
14	12±11^{f}	40±11^{e}	60±11^{d}	72±11^{c}	86±11^{b}	100±0^{a}	100±0^{a}	100±0^{a}	100±0^{a}

Mean ± SD. Rep. 3; n= 5

Alphabets in the same column are statistically non-significant at $P<0.05$

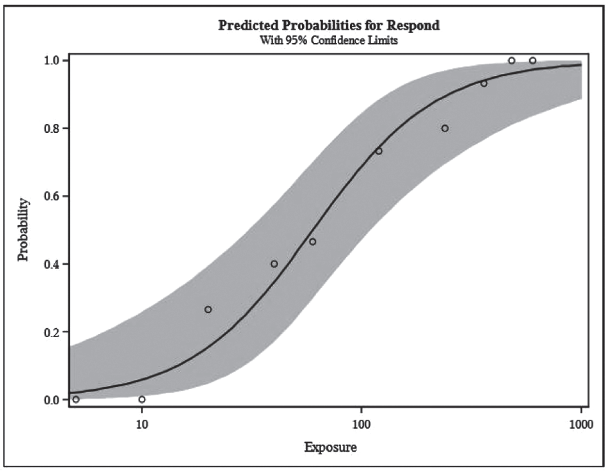

Fig.6a. Two-way ANOVA significance sketch with 95% confidence limit to mortality of *O. longicollis* for varying exposures and time of *Menma*

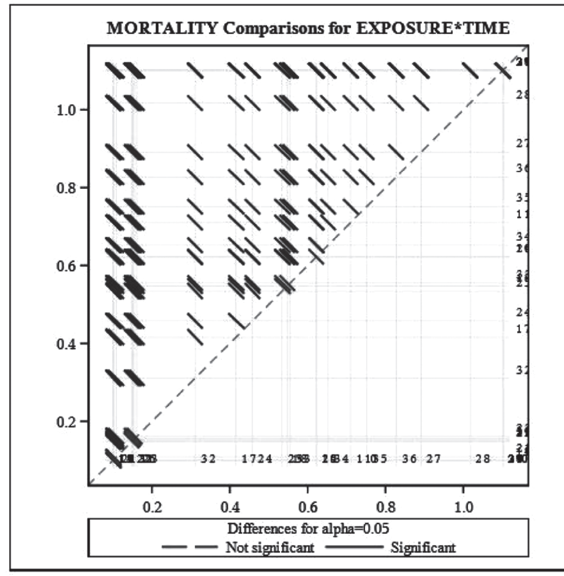

Fig.6b. Mortality curve of *O. longicollis* to varying exposures to *Menma* at 95% confidence limits

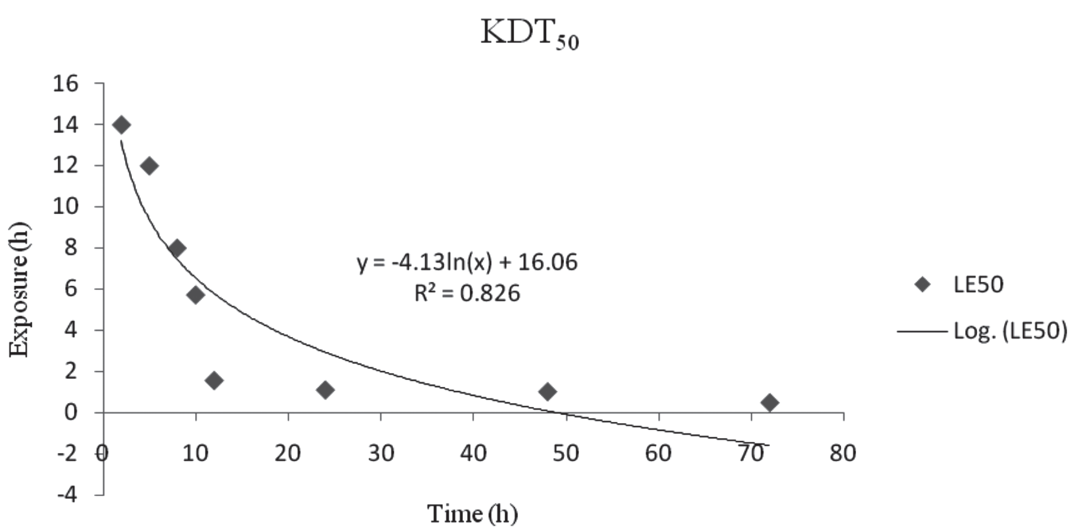

Fig.7. Scatter plot indicating the KDT_{50} values of *O. longicollis* at different time periods

Conclusion

Use of plant products to protect pests associated with horticultural crops is an age-old practice. The present study focused on the use of underutilised plant parts such as leaf and tuber rind of cassava for the production of biopesticides against borer pests of fruits and tree crops. The efficacy of the biopesticide, *Menma* against BSW was established. The study revealed the lethal status of cyano-glycosides present in the extract *Menma* over BPW. Mortality of the treated insects was noticed in 12h due to the treatment with *Menma* at concentration of hydrogen cyanide at 8ppm or 3ml doss of 300ppm or by giving a sub-lethal exposure in 1ml of *Menma* with 300ppm for 10h.

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