

THE POTENCY OF BOTANICAL PESTICIDES TO CONTROL ACARINE *Tetranychus kanzawai* KISHIDA (ACARI: TETRANYCHIDAE)

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ABSTRACT

The research is aimed to confirm the potency of several botanical pesticides to be used as natural biological control and to know the most infective stadium of *T. kanzawai* was done at Pest and Disease Laboratory of Indonesian Tropical Fruits Research Institute from May to August 2009. A factorial completely randomized design was used in this research. Two factors were observed; first, four kinds of botanical pesticides consist of four treatment levels i.e. extracts of neem, soursop, Siam weed leaves and sterilized water as control. The second was the stadia of *T. kanzawai* that consist of three levels i.e. larva, nymph and adult. The result showed that Siam weed and soursop leaves extracts caused anti-feedent behavior and decreased mobility at five days after treatment. In addition, exoskeleton of treated *T. kanzawai* darkened from white, yellowish and bright brown. Extracts of Soursop and Siam weed leaves caused very high mortality (96.67%) of *T. kanzawai* compared to neem (79.17%). However, there was no significant difference in mortality rates among the four active stadia of *T. kanzawai* with average mortality range from 65.63% to 79.38%.

Keywords: botanical pesticide, *T. kanzawai*, control

INTRODUCTION

Phytophagous mites are important agricultural pests due to their diverse host range and behaviour. Their minute size often allows them to remain undetected until populations have reached economic levels. Some species of phytophagous mites are known vector of several plant viruses. One of the most damaging phyto-

phagous mites is *Tetranychus kanzawai* Kishida that caused negative changing on quantity and quality of several agricultural plants such as papaya, citrus, avocado etc.

The application of artificial pesticides is the most common techniques that have been used by farmer to control phytophagous mites. However, unwisely used of those synthesis pesticides in term of precise in dosage, time, equipment, application and target pest were resulted negative impact on human being and environment. Monitoring toward pesticide residue was found that mostly our environment has been polluted and those residues have been found in agricultural land, drinking water, river water, well water and air including our fresh daily food especially vegetables and fruit (Untung, 1996). Beside, it also caused resistance on target pest especially on acarine pest. James and Price (2002) revealed that *Tetranychus* has been known as the easiest pest to be resistance toward pesticides.

Due to those negative effects, it is inevitably need to avoid using pesticide or if so, it is used as the last choices. Therefore, natural biological control is one of the techniques that can be used and botanical pesticides have a bright prospect to be developed. According to Castillo-Sánchez *at al.* (2010) mostly the plants were used against insects have an insectistatic effect, rather than insecticidal. This refers to the inhibition of the insect's development and behavior and it is divided into: Repellence, anti-feeding activity, growth regulation, feed deterrents, and oviposition deterrents.

Siam weed *Chromolaena odorata* (L) R.M. King & H. Rob, neem (*Azadirachta indica* A. Juss) and soursop (*Annona muricata* L.) leaves can be exploited as natural biological control of several

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pests order such as Lepidoptera, Homoptera, Hemiptera and Coleoptera through reducing feed activity, egg production, constraint of moulting, including growth and development processes and finally, it increases mortality of each growth stages (Chakraborty *et al.*, 2011; Castillo-Sánchez *et al.*, 2010; Dadang and Prijono, 2008; Leatemia and Isman, 2004; Mordue and Nisbet, 2000;). However, there is no information yet on their affectivity to control mites especially *T. kanzawai*.

Based on rationale stated above, this research aimed to confirm the potency of several botanical pesticides as natural biological control and to know the most sensitive stadium of *T. kanzawai* toward those botanical pesticides.

MATERIALS AND METHODS

The research was conducted in the Pest and Disease Laboratory of the Indonesian Tropical Fruits Research Institute, Solok, West Sumatra, Indonesia from May to August 2009. A factorial completely randomized design was used in this research. There were two factors involved, first, kind of botanical pesticides that consist of four levels and second, kinds of *T. kanzawai* stadium that consist of three levels.

The level of botanical pesticides namely: control (steril water/A0), neem leaves extract (A1), soursop leaves extract (A2), and Siam weed leaves extract (A3). The level of *T. kanzawai* stadium involved: larvae (B1), nymph (B2), and adult (B3). Hence, combination of those factors presented below:

A0 B1	A0 B2	A0 B3
A1 B1	A1 B2	A1 B3
A2 B1	A2 B2	A2 B3
A3 B1	A3 B2	A3 B3

Each treatment was replicated four times. Further, to know the significant difference among the treatments Honest Significantly Different (HSD) test was used with probability of 5%.

The activities of the research were covered:

1. Making of Botanical Pesticides

Method of Sudarmo (2009) was used to make botanical pesticides of soursop and Siam weed. Meanwhile, botanical pesticide of neem was used a bottled one that had been widely used by farmer as natural biological control of several insects pest.

The steps on how to extract soursop *A. muricata* and Siam weed *C. odorata* leaves to

make botanical pesticides are follows: 1). 100 fresh leaves of soursop or Siam weed were blended until finely, 2). Soak the fine soursop or Siam weed leaves in the 10 litter of water for 24 hours, 3). Swirl the soursop or Siam weed solution until finely mingled and then, filtered by a smooth cloth. 4). Place of the filtered soursop and Siam weed solution on sealed bottle. The solution is ready to be applied on target pest.

2. Mass Rear of Acarine for Treatment

T. kanzawai for treatment was mass reared on papaya plant that was planted in a polybag. The mite was infested on the papaya leaves that firstly collected from the field. Then, the mites together with papaya plant were put inside the screen-plastic box to avoid predator and negative effect of environment. The papaya plant was taken care through watering and fertilization including removing weed periodically.

3. Research Implementation

The implementation of the research was done through put *T. Kanzawai* on mungger cell that had been pillowed by 'wheatman' wet paper filter on the base. This method was kept the relative humidity in the hole of the mungger cell constantly high. Further, on the surface of filtered paper was put papaya leaves as arena for life of *T. kanzawai*. Before the papaya leaves was put on the base of manger cell, it was soaked for 30 minutes in botanical pesticides of neem, Siam weed and soursop. Then, the papaya leaves were taken from the solution and laid down on the tissue paper and lets them patch and dry-up. After that, *T. kanzawai* stadium larva, nymph and adult were transferred as much as 10 in each mungger cell hole by using a small paint brush (number one). Finally, the mungger cell hole was covered by glass slide.

4. Data gathering

The observation was done toward stadium larva, nymph and adult of *T. Kanzawai* mites. The parameters were observed involve:

1. Infection symptom caused by botanical pesticide such as changing in momentarily feed ability, body color and walk/mobile capability

In all replications, mites are called to do a feed activity if there is more than 70% of

observed mites perform feed activity. Mites that are conducted feed activity characterized as follows: body position with tail end up, stylets puncture to the leaves, available dorsal spot movement due to process of sucking to leave cell solution.

Infection symptom was also remarked by changing of body color. Body color of dorsal larva, nymph and adult were changed from white, yellowish and bright brown to dark brown or black.

The walk capability of mites stadium larva, nymph and adult that infected by botanical pesticides were also changed from very fast to slow, very slow and unmoved but still alive when touched by very fine drawing brush. Number of tip touch of very fine drawing brush is determining the criterion of walk capability. It determines walk very fast if mite was touched 0 – 1 then walk, fast with 2 – 3 touches, slow with 4 - 6 touches, very slow with 7 - 9 touches, and unmoved with 10 – 12 touches but still alive and the last unmoved with 12 – 14 touches due to die. The number of touch was averaged to know walk capability of each replications.

2. Percentage of mortality (Pm)

The percentage of mortality was counted based on formula below:

$$PM = \frac{\sum \text{mortality of tested mites}}{\sum \text{total numbers of tested mites}} \times 100\%$$

The mortality of mite was gotten then corrected by using Abbott's formula.

$$CPM = \frac{\%MTM - \%MC}{100 - \%MC} \times 100$$

Remarks:

CPM = corrected percentration of mortality

% MTM = percent mortality of tested mites

% MC = percent mortality of control

RESULTS AND DISCUSSION

A. Infection symptom of *T. kanzawai* Treated by Botanical Pesticides

1. Momentarily feed ability of *T. kanzawai*

The result showed that treatment of kind botanical pesticide of neem, Siam weed and

soursop leaves extract on several stages of *T. kanzawai* were significantly affected to the momentarily feed capability of tested mites (Table 1).

Table 1 informed that all of botanical pesticides were tested caused all stages of *T. kanzawai* got lost their capability to feed at the end of observation except on treatment of extract neem leaves that applied on stage of larva. However, leaves extract of Siam weed and soursop were resulted early symptom on losing feed capability of tested mites on fifth days after treatment. Even, leaves extract of Siam weed caused feed elimination of larva and adult *T. kanzawai* on days fourth after treatment. Possibly, material compound contain inside the Soursop and Siam weed that caused those degrading and losing momentarily feed capability.

According to Raintree Nutrition (2004) and Kardinan (2009), leaves extract of soursop contain acetogenin compound such as annocatalin, annohexocin, annomonocin, annomontacin, annomuricin, annonacin, coronin, corossolin, corossolone, gigantetrocin, gigantetronenin, montanancin, muracin, muricatacin, muricin, robustosin, solamin, squamocin, uvariamicin. Acetogenin compound in high concentration has special function as anti feeding, mean that treated insect doesn't has feeding enthusiasm toward their preferred food. In addition, in small concentration of leaves extract of soursop functioning as stomach poison that caused mortality. This botanical pesticides mode of action could be systemic or contact and very easy to be absorbed by host plant. Castillo-Sánchez *et al.* (2010) and Kirk *et al.* (2009) added that acetogenins serve as insecticides with slow-acting toxins, feeding deterrence and growth inhibition and are particularly effective against chewing insects. Acetogenin compounds are toxic to several pest insects such as cockroaches, Colorado potato beetle, Blowfly larvae, Mexican bean beetles and their larvae, Bean leaf beetle, mosquito larvae including two-spotted spider mites. Similar result by Kardiman (1999) showed that botanical pesticide of soursop leaves extract will kill insect pest slowly by decrease feeding enthusiasm, process of moulting, growth, reproduction capability and percentage of egg hatching. Beside, it also constrains larvae and nymph to be an adult stage, causing sterile adult, disturb and

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barrier in process of mating, limiting process of egg deposit.

Biller *et al.* (1994), reported that Siam weed leave extract contain *N*-oxides of five pyrrolizidine alkaloids (PAs): 7- and 9-angeloylretronecine, intermedine, rinderine and 3'-acetyl rinderine. PAs is functioning as stomach poison with stinking smell, bitter taste. Hence, this botanical pesticide categorized as repellent for insect pest.

2. Walk capability of *T. kanzawai*

Besides degrading feed capability of *T. kanzawai*, treatment of several kinds of botanical pesticide such neem, Siam weed and soursop were also caused infection symptom i.e. decreasing walk capability. The treatments were resulted unmoved of larva, nymph and adult *T. kanzawai* at the end of observation (Table 2). The movement was triggered by touch on dorsal part of the tested mites by using a very fine drawing brush. The numbers of touch that

caused the mites move were counted to stated parameter of walk capability.

At the end of the observation, all stages of tested mites were unmoved except control treatment (Figure 1). However, in term of early emerge symptom, the treatment of soursop and Siam weed leaves extract were resulted the most early infection symptom i.e. at fifth days after treatment especially on adult stage. Since then, all of extracted leaves were affected to walk capability of tested mites. The degrading of walk capability was probably due to active poison contain that starting to work by reducing feed enthusiasm, hence, they got lost of their energy. Finally, they were started to move slowly, very slowly, and finally unmoved but still alive or die. Isman (2006) and Alali *et al.* (1999) revealed that leaves extract of soursop contain acetogenins which mode of action identical to that of rotenone, i.e. they block energy production in mitochondria of insects, and this physiological process will cause slow motion movement or no movement response.

Tabel 1. Average number of *T. Kanzawai* that was done feed activity after application of botanical pesticide

Treatments	Observation (Day after treatment)								
	1	2	3	4	5	6	7	8	9
A0B1	6.75 f	1.50 bc	1.50 b	1.75 bc	2.00 c	1.50 c	1.75 c	1.00 d	0.50 c
A0B2	4.00 d	5.00 e	5.25 c	5.25 d	4.75 d	2.50 d	2.25 d	1.75 e	1.50 d
A0B3	5.50 e	6.75 f	6.50 d	6.00 d	4.25 d	3.50 e	2.25 d	2.00 f	1.50 d
A1B1	1.00 b	2.00 c	1.25 b	1.00 b	1.00 b	0.75 b	1.00 b	0.75 c	0.25 b
A1B2	0.75 ab	1.00 b	1.75 b	2.75 c	1.25 b	0.75 b	0.25 a	0.25 b	0.00 a
A1B3	2.50 c	3.50 d	4.75 c	2.25 c	0.25 a	0.50 b	0.25 a	0.00 a	0.00 a
A2B1	0.75 ab	0.75 ab	0.00 a	0.50 ab	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
A2B2	0.00 a	1.00 b	0.50 ab	0.50 ab	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
A2B3	0.50 ab	1.50 bc	0.75 ab	0.25 ab	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
A3B1	1.75 ab	0.50 ab	0.75 ab	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
A3B2	3.25 cd	1.75 bc	0.50 ab	0.25 ab	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
A3B3	0.75 ab	0.00 a	0.25 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a

Remarks: - Means value in each column with the same letters are not significantly different at $p = 0.05$ based on Honest Significantly Different (HSD) test. Combination treatment of botanical pesticides and stadium *T. kanzawai* were used involve Control (Sterile water / A0), Neem leaves extract(A1), Soursop leaves extract(A2), Siam weed leaves extract (A3), Larva (B1), Nymph (B2), Adult (B3)

Table 2. Average numbers of touch toward *T. Kanzawai* as parameters of infection symptom such walk capability after application of botanical pesticide

Treatments	Observation (Day after treatment)								
	1	2	3	4	5	6	7	8	9
A0B1	0.93 ab	0.75 a	1.15 a	1.18 a	1.30 a	1.38 a	1.73 a	1.80 a	2.38 a
A0B2	0.58 a	0.90 a	1.15 a	1.25 a	1.05 a	1.08 a	1.48 a	2.05 a	2.53 a
A0B3	0.73 ab	1.03 a	1.03 a	1.38 a	1.30 a	1.70 a	2.0 a	3.30 a	4.10 a
A1B1	2.28 d	2.28 c	2.35 b	3.73 b	3.95 b	4.38 b	6.18 b	8.13 b	8.55 b
A1B2	3.25 e	1.83 bc	2.28 b	2.70 b	5.03 bc	5.60 bc	6.40 b	7.0 b	7.48 b
A1B3	1.68 c	2.38 c	3.0 bc	3.68 b	5.23 bc	6.28 c	8.45 c	8.43 b	9.00 b
A2B1	1.25 b	2.18 c	2.45 b	3.48 b	4.45 b	5.95 bc	6.33 b	8.40 b	8.90 b
A2B2	0.63 a	1.80 bc	4.80 d	5.13 c	7.15 cd	7.73 cd	7.53 bc	7.83 b	8.53 b
A2B3	0.68 a	2.93 d	3.60 cd	6.43 cd	8.30 d	8.60 d	8.60 c	8.68 b	8.90 b
A3B1	1.08 b	1.75 bc	4.20 d	6.68 d	7.50 cd	8.25 d	8.68 c	8.78 b	8.88 b
A3B2	0.95 ab	1.08 ab	3.28 c	5.43 c	6.40 c	7.50 cd	8.18 c	8.55 b	8.80 b
A3B3	1.10 b	1.53 b	4.68 d	7.15 d	8.20 d	8.30 d	8.58 c	8.78 b	9.00 b

Remarks: - Means value in each column with the same letters are not significantly different at $p = 0.05$ based on Honest Significantly Different (HSD) test.
 - Combination treatment of botanical pesticides and stadium *T. kanzawai* were used involve Control (Steril water / A0), Neem leaves extract(A1), Soursop leaves extract(A2), Siam weed leaves extract (A3), Larvae (B1), Nymph (B2), Adult (B3)
 - 0-1 numbers of touch mean walk very fast
 - 2-3 numbers of touch mean walk fast
 - 4-6 numbers of touch mean walk slow
 - 7-9 numbers of touch mean walk very slow or unmoved but still alive or die

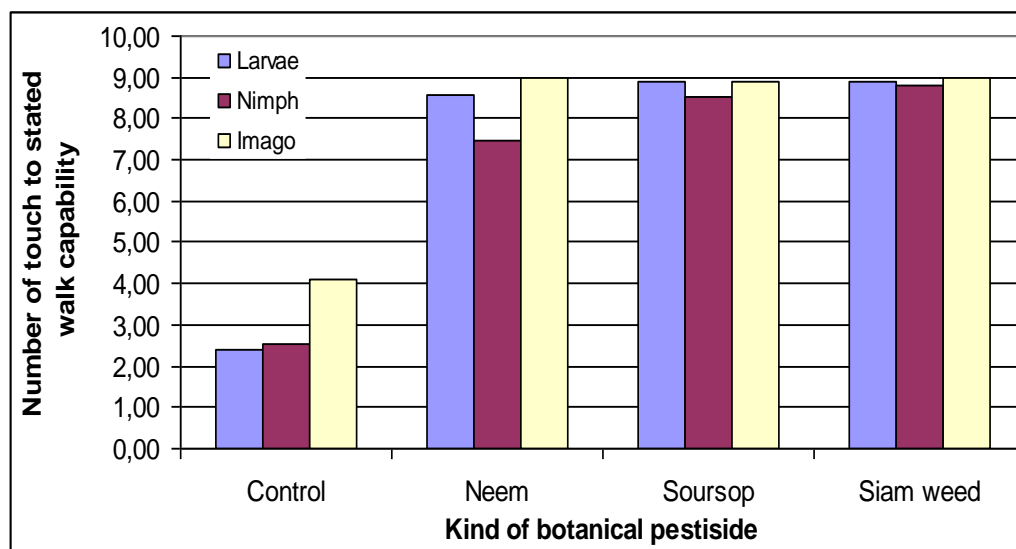


Figure 1. Walk capability of several stadia *T. kanzawai* after treated by some botanical pesticides extract at the end of observation (ninth days after treatment) based on number of touch by used of a very fine drawing brush

3. Infection symptom signed by color changes

Application of botanical pesticides caused decreasing of feed and walks capability. Furthermore, it also caused body color changes as result of infection process. The body color was changed from transparent white or yellowish green or bright reddish on stages of larva, nymph and adult, respectively, to become dark brown or black that followed by mortality (Table 3).

Table 3 showed that five days after treatment of botanical pesticides such neem, soursop and Siam weed, the adult stadium was shown a change of body color from bright reddish to dark brown to black. However, those treatments did not give infection symptom such body changes on stadium larva and nymph. Further, all of the treatments were given changes on body color after seventh, eighth and ninth days after treatment except control

treatment. Idris (2007) stated that application of bio-insecticides such cinnamon extract toward biological aspect of *Epilachna varivestis*. Mulsant showed that this bio-insecticides able to penetrate and get in to the body inside of the insect though pores body that caused damaging and disturbing physiological process on pupa. This damaging and disturbing was indicated by body color changes. Djamin and Ginting (1991) added that key character availability of imbibitions process caused by active ingredient of botanical insecticide on larva and pupa of *Hidari irava* Moor is body color changes.

4. Percentage of mortality

The result showed that there was an interaction between kind of botanical pesticide treatment and kind of *T. Kanzawai* stadium toward parameter percentage of mortality at the end of the observation (Table 4).

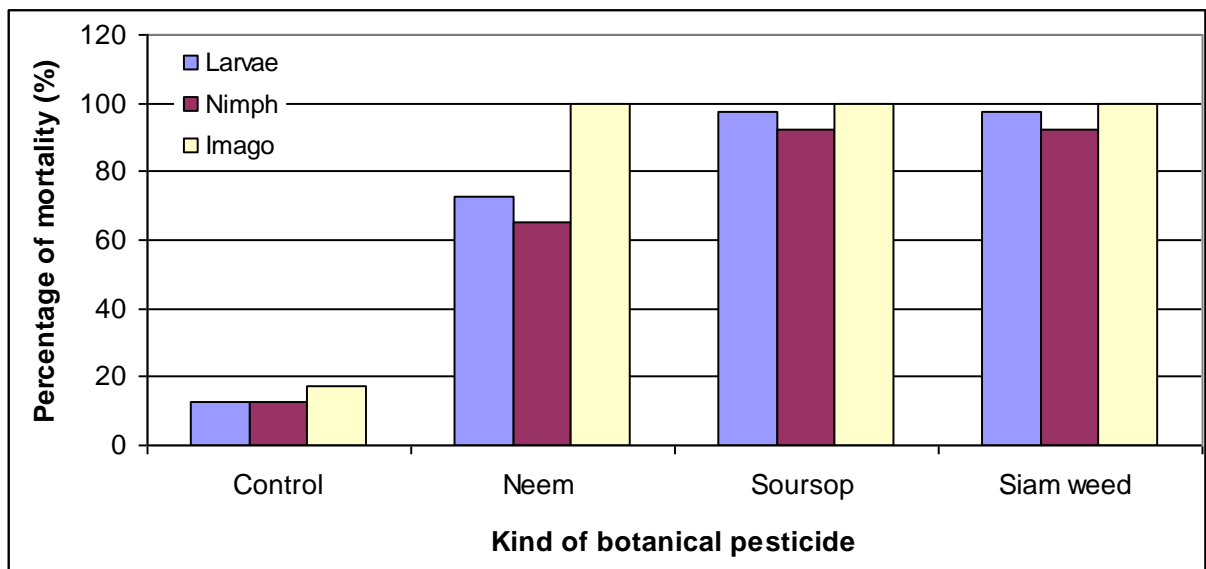


Figure 2. Percentage of mortality several stadia of *T. kanzawai* after treated with some extracts of botanical pesticides at the end of observation (ninth day after treatment)

Table 3. Body colour changes as sign of infection symptom on *T. kanzawai* as result of botanical pesticides treatment

Dat	Kind of Botanical Pesticides											
	Control			Neem			Soursop			Siam weed		
	A0B1	A0B2	A0B3	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3	A3B1	A3B2	A3B3
1	White transparent	Yellowish green	Bright reddish	White transparent	Yellowish green	Bright reddish	White transparent	Yellowish green	Bright reddish	White transparent	Yellowish green	Bright reddish
2	White transparent	Yellowish green	Bright reddish	White transparent	Yellowish green	Bright reddish	White transparent	Yellowish green	Bright reddish	White transparent	Yellowish green	Bright reddish
3	White transparent	Yellowish green	Bright reddish	White transparent	Yellowish green	Bright reddish	White transparent	Yellowish green	Bright reddish	White transparent	Bright reddish	Bright reddish
4	Yellowish green	Yellowish green	Bright reddish	Yellowish green	Bright reddish	Bright reddish	Yellowish green	Bright reddish	Bright reddish	Yellowish green	Bright reddish	Bright reddish
5	Yellowish green	Bright reddish	Bright reddish	Yellowish green	Bright reddish	Dark brown to black	Yellowish green	Bright reddish	Bright reddish	Yellowish green	Bright reddish	Bright reddish
6	Bright reddish	Bright reddish	Dark brown	Bright reddish	Bright reddish	Dark brown to black	Bright reddish	Bright reddish	Dark brown to black	Bright reddish	Bright reddish	Dark brown to black
7	Bright reddish	Bright reddish	Dark brown	Bright reddish	Bright reddish	Dark brown to black	Bright reddish	Dark brown to black	Dark brown to black	Bright reddish	Dark brown to black	Dark brown to black
8	Bright reddish	Bright reddish	Dark brown	Dark brown to black	Dark brown to black	Black	Dark brown to black	Dark brown to black	Black	Dark brown to black	Dark brown to black	Dark brown to black
9	Bright reddish	Bright reddish	Dark brown	Dark brown to black	Dark brown to black	Black	Dark brown to black	Dark brown to black	Black	Dark brown to black	Dark brown to black	Black

Remarks: - DAT : Day after treatment

- Means value in each column with the same letters are not significantly different $p = 0.05$ based on Honest Significantly Different (HSD) test.
- Combination treatment of botanical pesticides and stadium *T. kanzawai* were used involve Control (Steril water / A0), Neem leaves extract(A1), Soursop leaves extract(A2), Siam weed leaves extract (A3), Larvae (B1), Nymph (B2), Adult (B3)

Table 4. Interaction between kind of botanical pesticide treatment and kind of *T. Kanzawai* stadium toward parameter percentage of mortality at the end of the observation

Kind of botanical pesticide	Kind of <i>T. kanzawai</i> stadium			Average
	Larvae (B1)	Nymph (B2)	Adult (B3)	
Control/water (A0)	12.50 a	12.50 a	17.50 a	14.17 a
Neem leaves extract (A1)	72.50 b	65.00 b	100 c	79.17 b
Sourhop leaves extract (A2)	97.50 c	92.50 c	100 c	96.67 c
Siam weed leaves extract (A3)	97.50 c	92.50 c	100 c	96.67 c
Average	70.00 b	65.63 b	79.38 b	71.67

Remarks: Means value in each column with the same letters are not significantly different at $p = 0.05$ based on Honest Significantly Difference (HSD) test

Table 4 showed that botanical pesticide of soursop, Siam weed and neem leaves extract were caused high mortality on larva, nymph and adult stages of *T. Kanzawa* at the end of observation. Nevertheless, leaves extract of neem was given lower mortality on stadium of larvae and nymph compared to soursop and Siam weed (Figure 2). The acetogenin is one of the major constituents of the extract plants belonging to the family Annonaceae that displayed toxic effects on early larval instars of *Spodoptera frugiperda* (J.E.Smith) when incorporated to the larval diet at a dose of 50 μg per g of diet. All of the acetogenins produce more than 80% pupal mortality and adult malformations that leading to the death (Álvarez-Colom *et al.* 2007). Simanjutak *et al.* (2006) revealed that application of dusty leaves extract of soursop that mixed with bait food 6 g/ jar bottle ($\pm 157 \text{ cm}^3$) caused percentage of mortality of termite as much as 98,33%. Similar research by Dadang and Prijono (2008) confirmed that *A. muricata* leaves extract was known effective to control *C. Maculatus* whereas by concentration of 0,365% yielded percentage of mortality almost 100%.

Beside soursop leaves extract, Siam weed was also shown it potential effect to control *T. kanzawai* that reached average mortality as much as 96,67%. Not only in this case but also Siam Weed leaves extract was already known as insecticidal against several insect pests. Dutta *et al.* (2006) informed that *C. odorata* is a botanical pesticide effective against tea spider mites. Similar research by Chakraborty *et al.* (2011) showed that *C. odorata* effective to control *Helicoverpa armigera* with 20g l^{-1} and 16g l^{-1} crude extracts against 3rd instar larva reached mortality of 69.3% and 63.6% after 48 hours, respectively. In addition, Rajmohan and Logan-

kumar (2011) added that application of leaf extract *C. odorata* caused percentage of un-hatching egg of *Aedes aegypti* reached 87% in concentration of 110 ppm and caused mortality of first and fourth larval instar including pupa as much as 82, 88 and 83%, in concentration of 50, 120 and 140 ppm, respectively.

Active ingredient of neem characterized as anti-feeding and known as stomach poison including contact toxin. Application on concentration of 150, 200 and 250 g l^{-1} caused mortality of *Spodoptera litura* larva as much as 80, 86,8 and 90.4%, in 48 hours after treatment, respectively (Sutoyo and Wiriodmodjo, 1997). Tukimin (1997) added that application of neem seed extract in concentration of 25 g l^{-1} was depressed population of *Myzus persicae* up to 82,5 % after 6 days of treatment. However, even though neem leaves extract capable to cause mortality of *T. kanzawai* relatively high (79,17%), apparently leaves extract of soursop and Siam weed were the most effective botanical pesticide against *T. kanzawai*.

CONCLUSIONS AND SUGGESTIONS

Extract of Soursop and Siam weed leaves were the most potential botanical pesticides toward *T. kanzawai* that caused early infection symptom such momentarily feed and walk capability including a very high mortality (96.67%).

There was no significant different preference of mortality among kind of stadium *T. kanzawai* that treated by those botanical pesticides. All of the stadia were infected with average mortality range from 65.63% to 79.38%.

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