Serial Dilution of Nettle Caterpillar Viruses Applied as Bioinsecticide against Setothosea Asigna van Eecke (Lepidoptera:Limacodidae) the Important Pest of Oil Palm

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***Abstract*—*Setothosea asigna* van Eecke also called as nettle caterpillars is an important defoliator of oil palm which under severe infestations, its might cause significant decrease of fruit production. The serious damage caused by the caterpillar has become a reasonable reason for oil palm grower to use insecticides to control the pest, which inevitably cause serious damages to the environment. Microbial insecticide, especially the one developed from indigenous entomopathogen, should be considered as better alternative to control the insect. In this research, viruses infecting *Setora nitens* and *Setothosea asigna*, was transmitted through their body sap to second instar of *S. asigna.* The virus was serially diluted from 10^{-1} to 10^{-8} and sprayed to oil palm leaves used to feed 30 larvae of *S. asigna*. The results showed that *S. nitens* and *S. asigna* viruses could infect larvae of *S. asigna* and developed viral infection in the larvae. Serial dilution resulted different end point dilution for both viruses, 10^{-6} for *S.nitens* and 10^{-8} for *S. asigna.* This finding suggested that infected larvae of *S. nitens* and *S. asigna* are very practical to be used as bioinsecticide to control nettle caterpillars.**

***Index Terms*—oil palm, setora nitens, setothosea asigna, entomopathogenic virus, sap dilution.**

I. Introduction

One of the most important constraint in the cultivation of oil palm is an insect pest, *Setothosea asigna* (Lepidoptera: Limacodidae) feeds on the leaves of either young or old palms. The caterpillar, together with *Setora nitens, Darna trima* and *D. bradleyi*, are also called as nettle caterpillars [1], and are considered as most important defoliators of oil palm plantations in southeast Asia [2]-[5]. Under severe infestations, larvae of .*S asigna* consume all foliage and leave only the mid-rib of the frond, which might cause significant decrease of fruit production [6]. The damaging nature of the insect is mainly supported by its long life cycle, high productivity, and high leaf consumption. The life cycle of the insect ranges from 86 to 109 days, with shorter life cycle during dry season [7]. *S. asigna* infestation might reach population density up to 200 larvae per frond, far above the population threshold of *S. asigna*, 5 caterpillar per frond [8] Under, the such high infestation outbreak, oil palm leaf surfaces are rapidly defoliated and at times reaching up to 50% defoliation, leading to more than 78% loss of production in the first year and 40% in the second year.

The serious damage caused by *S. asigna* and the potential yield losses seems to be an acceptable reason for oil palm growers to use insecticides to control the pest which might cause serious damages to the environment [9]-[12], and the growers are under immense pressure to reduce the use of chemical pesticides, but at the same time the control of pests is becoming increasingly difficult due to pesticide resistance. Alternative control methods are needed urgently as part of Integrated Pest Management [13], [14]. Biological control appears to be the best alternative to replace or reduce the use pesticides. Biological control is the use by man of a living organism to help manage the population density of a pest organism [15], [16].

The change from chemical to biological control substantially contributes to the conservation of natural resources, and results in a considerable reduction of environmental pollution. It eliminates human exposure to toxic pesticides, improves sustainability of production systems, and enhances biodiversity. Nettle caterpillar *S. asigna* was reported to have numerous natural enemies such as ichnemonids, braconids, tachinids, reduuvids and a pentatonid [17]. The interest to replace chemical control with biological control has been more increasing since the introduction of using virus infecting the insect as biological control agent. The viruses are the naturally occurring pathogens that infect some important lepidopteran insects. Their high pathogenicity, narrow host range, and complete

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safety to vertebrates and plants, make them ideal candidates for biological control of these insects. The pathogenicity of the viruses has been characterized mostly by their median lethal dose (LD50), an estimate of infectivity of the virus to its host [18]. The use of the virus as pesticides can be applied using conventional techniques and do not create the problem associated with residues. Despite their potential, viral insecticides are employed much less than they could be in crops and forests, due to difficulty in virus stability and, most importantly, slower speed of action than that achieved with chemical pesticides [19]. The use of infected caterpillars as the source of virus inoculant has been widely practiced by Indonesian oil palm plantations with various concentration. Therefore, serial dilution experiment was conducted to study the appropriate concentration of infected caterpillar sap used to reduced the infestation of nettle caterpillars, especially *Setothosea asigna*.

II. Materials and Methods

A. Source of Viruses

Nettle caterpillars, *Setora nitens* and *Setothosea asigna*, suffering from viral infection were collected from oil palm plantations in South Sumatra and were kept frozen before being processed for the experiment. Infected *S. nitens* were used separately as the source of *Setora nitens* virus, and infected *S. asigna* were used as the source of *Setothosea asigna* virus.

B. Collection of Healthy Caterpillars

Healthy caterpillars of *S. asigna* was collected from oil palm plantation heavily infested by the caterpillars. Caterpillars of same instar were removed from oil palm fronds and kept to refresh for 3 days in an insect cage and young oil palm plants was placed inside for them to feed on.

C. Preparation of Serial Dilution of Virus Inoculants

Entomopathogenic viruses were extracted from two species of nettle caterpillars, *S. nitens* and *S. asigna* and were inoculated separately to *S. asigna* caterpillars. The naturally infected nettle caterpillars were collected from highly infested plantation in South Sumatra and were frozen prior to the extraction of their sap. Sap extraction and tenfold dilution was done according to the following order.

- The frozen caterpillars were thawed and homogenized before being finely filtered to separate solid remains from the sap [20]. The filtered sap was used as virus stock.
- Dispensed 90ml diluting pure water in Erlenmeyer labeled 10^{-1} to 10^{-8} and kept the Erlenmeyer in ice.
- To make tenfold dilutions of the virus inoculant, 10 ml of virus inoculant was diluted in 90ml of diluent to get the initial dilution i.e. 10^{-1}. Subsequently, 10ml of previous virus dilution was transferred to next dilution by using a fresh pipette, to achieve serial tenfold dilutions.

D. Inoculation of S. asigna

Thirty 3rd instar of *S. asigna* were placed in 8 plastic boxes with aeration window, labeled 10^{-1} to 10^{-8}. Oil palm leaf segments sprayed with diluted virus stock of different dilution levels were placed in the plastic box accordingly to match the label, and let the *S. asigna* caterpillars feed on the leaves. Observation was made at an interval of 12 hours.

E. Calculation of LC_{50} endpoint Dilution

In the calculation of LC_{50} or 50% endpoint dilution, the first step was to find the dilution produced mortality higher than 50% and the dilution produced mortality below 50%, because the 50% endpoint dilution obviously lied between these dilutions. The next, was calculating the proportionate distance (PD) of the 50% endpoint from these dilutions by using the following formula.

$$PD = \frac{(\%\ mortality\ at\ dilution\ next\ above\ 50\%) - 50\%}{\left(\begin{matrix}\%\ mortality\ at\ next\\ dilution\ above\ 50\%\end{matrix}\right) - \left(\begin{matrix}\%\ mortality\ at\ next\\ dilution\ below 50\%\end{matrix}\right)}$$

Then 50% endpoint dilution (LD_{50}) was calculated by applying the following equation:

$$\left(\begin{matrix}\text{Negative } logaritm\\ of\ LD50\end{matrix}\right) = \left(\begin{matrix}\text{Negative } logaritm\ of\ the\ next\\ dilution\ above\ 50\%\ mortality + PD\end{matrix}\right) x\ dilution\ factor$$

where dilution factor was the logarithm of the dilution step employed (log 10 = 1).

III. Results and Discussions

A. Pathogenicity of Nettle Caterpillars Viruses

The inoculation of virus extracted from naturally infected *S. nitens* and .*S asigna* both resulted in the development of disease characteristic to viral infection. The symptoms appeared were similar to those found in naturally infected caterpillars such as moribundity, flaccidity and browning [21]. The different between two sources of virus inoculants was apparent in term of lethal concentration and lethal time (Table I-Table IV).

Virus from *S. nitens* was found to be less virulent than that isolated from *S. asigna* as indicated by lower mortality and longer lethal time.

TABLE I. Mortality of *Setothosea asigna* Inoculated With *Setothosea asigna* Virus, 48 Hours After Inoculation

Virus dilution	Died	Survived	Accumulated values		Mortality	
			Died	Survived	Ratio	Percentage
10^{-1}	15	15	74	15	74/89	83.14%
10^{-2}	11	19	59	34	59/93	63.44%
10^{-3}	10	20	48	54	48/102	47.05%
10^{-4}	10	20	38	74	38/112	33.92%
10^{-5}	9	21	28	95	28/123	22.76%
10^{-6}	6	24	19	119	19/138	13.76%
10^{-7}	4	26	13	145	13/158	8.22%
10^{-8}	9	21	9	166	9/175	5.14%

Accumulated values for the total number of died or survived caterpillars were obtained by adding in the direction of lowest to the highest values.

Data presented in above table showed that inoculating larvae of *S. asigna* with virus from the same species could produce 50% mortality 48 hours after inoculation, suggested that the virus could shortly achieve its LC_{50} when the inoculation was made at low dilution. On the other hand, inoculating *S. asigna* using virus from *S. nitens* could only produced 50% mortality 96 hours after inoculation (Table II), suggested that there was a slow infection process involving *S. nitens* virus in the larvae of *S. asigna* even though the inoculation was made at high concentration of the virus.

TABLE II. MORTALITY OF *SETOTHOSEA ASIGNA* INOCULATED WITH *SETORA NITENS* VIRUS, 96 HOURS AFTER INOCULATION

Virus dilution	Died	Survived	Accumulated values		Mortality	
			Died	Survived	Ratio	Percentage
10^{-1}	22	8	64	8	64/72	88.88%
10^{-2}	15	15	56	23	56/79	70.88%
10^{-3}	10	20	41	43	41/84	48.81%
10^{-4}	10	20	31	63	31/94	32.97%
10^{-5}	9	21	21	84	21/105	20.00%
10^{-6}	8	22	12	106	12/128	9.37%
10^{-7}	4	26	4	132	4/136	2.94%
10^{-8}	0	30	0	162	0/162	0.00%

Accumulated values for the total number of died or survived caterpillars were obtained by adding in the direction of lowest to the highest values.

The differences in mortality ratio and LC_{50} produced by the infection by *S. asigna* and *S. nitens* viruses as shown in Table I and Table II indicated that virus from naturally infected nettle caterpillar was more virulent when being inoculated to larvae from the same species. Both viruses were identified as members of *Tetraviridae* [21].

Based on the data presented in Table I and use the calculation of LD_{50} as described above, the LD_{50} end point of virus from *S. asigna* was $10^{-2.82}$, while LD_{50} for virus from *S. asigna* was $10^{-2.94}$, when being inoculated to *S. asigna*. However, the former was a value of LC_{50} reached in 48 hours after inoculation, while the later was achieved in 96 hours. At 48 hours after inoculation, LC_{50} of *S. nitens* virus had not been reached by the virus (data not shown). It was clear that the virus needed longer time to establish high inoculation frequency. The longer time required for infection process indicated that *S. nitens* virus was less virulent compared to *S. asigna* virus. The low virulence of *S. nitens* virus might be caused by host factor which was different from the original host. As found in most oil palm plantations surveyed in South Sumatra, the infestation of *S. asigna* was more frequent and covered larger areas. Therefore, *S. asigna* naturally infected by entomopathogenic virus was more abundant than *S. nitens*. Whether there was cross inoculation between the two species was not clearly understood. Another factor which possibly affected the virulence of *S. nitens* virus was the titre of the virus itself. If the number of *S. nitens* virus particles was lower than that of *S. asigna* virus, the dilution process would be influential to the differential of the virulence of both viruses.

TABLE III. MORTALITY OF *SETOTHOSEA ASIGNA* INOCULATED WITH *SETOTHOSEA ASIGNA* VIRUS, 96 HOURS AFTER INOCULATION

Virus dilution	Died	Survived	Accumulated values		Mortality	
			Died	Survived	Ratio	Percentage
10^{-1}	27	3	152	3	152/155	98.06%
10^{-2}	24	6	125	9	125/134	93.28%
10^{-3}	21	9	101	18	101/119	84.87%
10^{-4}	20	10	80	28	80/108	74.07%
10^{-5}	16	14	60	42	60/102	58.82%
10^{-6}	14	16	44	58	44/102	43.13%
10^{-7}	16	14	30	72	30/102	29.41%
10^{-8}	14	16	16	88	16/104	15.38%

Accumulated values for the total number of died or survived caterpillars were obtained by adding in the direction of lowest to the highest values.

The Table III showed that the mortality of *S. asigna* inoculated with *S. asigna* virus produced high mortality almost in all dilution level. LC_{50} endpoint was $10^{-5.78}$ which showed the high virulent of the virus. The observation done in 160 hours after inoculation revealed that all levels of dilution has produced more than 90% mortality (data not shown). The result also suggested that the dilution end point of *S. asigna* virus had not been achieved at the dilution of 10^{-8}. The situation was different from the result *S. nitens* virus inoculation which showed continues increase of mortality until 132 hours after inoculation (Table IV).

TABLE IV. MORTALITY OF *SETOTHOSEA ASIGNA* INOCULATED WITH *SETORA NITENS* VIRUS, 96 HOURS AFTER INOCULATION

Virus dilution	Died	Survived	Accumulated values		Mortality	
			Died	Survived	Ratio	Percentage
10^{-1}	28	2	111	2	111/113	98.23%
10^{-2}	24	6	83	8	83/91	91.20%
10^{-3}	24	6	59	14	59/73	80.82%
10^{-4}	10	20	35	34	35/69	50.72%
10^{-5}	14	16	25	50	25/75	33.33%
10^{-6}	7	23	11	73	11/84	13.09%
10^{-7}	4	26	4	99	4/103	3.85%
10^{-8}	0	30	0	129	0/162	0.00%

Accumulated values for the total number of died or survived caterpillars were obtained by adding in the direction of lowest to the highest values.

The results of observation conducted 132 hours after inoculation of *S. nitens* virus on *S. asigna* showed that the virus was infective and could produced high mortality even though needed longer time than *S. asigna* virus to produced the similar level of infection. The LC_{50} of the virus at 132 hours after inoculation was $10^{-4.04}$. All observation made at interval of 12 hours (data not shown) also showed a consistent data that at dilution level of 10^{-8} the *S. nitens* virus could not produced infection symptom in *S. asigna* caterpillars. The dilution end point of the virus when being inoculated to *S. asigna* was 10^{-7} whre the virus still had ability to infect the host even though with very low

frequency, and the virus had lost its ability to infect at 10^{-8}, one fold step above the dilution end point.

The effect of serial dilution on lethal time was also apparent. The LT_{50} was decreasing as the virus concentration decreasing due to serial dilution (Fig. 1). Shortest time showing 50% mortality (LT_{50}) of *S. asigna* virus was different from that of *S. nitens* virus which the former showed lower LT_{50} at all level of dilution.

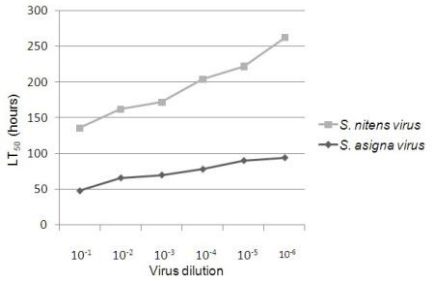

Figure 1. LT_{50} of *Setothosea asigna* and *Setora nitens* viruses inoculated to *S. asigna* at different levels of virus dilution

LT_{50} of *S. asigna* and *S. nitens* viruses showed similar patern, increased steadily from the lowest to highest dilution. However, as clearly shown in fig. 1, *S. nitens* virus took longer time to produced 50% mortality in all levels of virus dilution. The different of graph lines between *S. asigna* and *S. nitens* viruses had given stronger indication of higher virulence of *S. asigna* virus. Furthermore, as shown in the figure, LT_{50} was graphed from 10^{-1} to 10^{-6} even though the serial dilution was made up to 10^{-8}, because dilution levels of 10^{-7} and 10^{-8} had stopped *S. nitens* virus to produced high frequency of infection on *S. asigna* and never reached 50% mortality. Therefore, even though *S. asigna* virus could cause 50% mortality at dilution level of 10^{-7} and 10^{-8} (graph not shown), the comparison of LT_{50} between both viruses could only be made up to dilution level of 10^{-6}.

IV. Conclusion

S. asigna virus showed better virulence than *S. nitens* virus indicated by lower LC_{50}, shorter LT_{50} and higher level of virus dilution. The ability of *S. nitens* virus to infect *S.asigna* caterpillars, suggested that there was a closed relationship between both viruses as supported by the similarities of symptoms produced by both viruses in their host.

The ability of both virus to retain their ability to infect their host at high level of virus dilution, 10^{-8} for *S. asigna* virus and 10^{-8} for *S. asigna* virus, showed that both viruses are very potential biological control agents which could effectively work against each other species. The use of clean water as diluent in the serial dilution has contributed to the promising aspect of the viruses, the effective, applicative and inexpensive biological control agents of nettle caterpillars, important pest of oil palm.

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