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Potential Use of Entomopathogenic Virus Native to Sumatra Island as Biological Control Agent of Setora nitens L. (Lepidoptera: Limacodidae), the Main Pest of Oilpalm

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Abstract—Slug caterpillars Setora nitens, have been appearing to be more serious insect pest of oil palm as it might cause frond damages up to 90%. Many effort had been made to control the caterpillars using insecticides but the insect are still existing and causing significant damages to the palm. Microbial insecticide, especially the one developed from indigenous entomopathogenic virus, is a promising method of controlling the insect since its toxicity to non target animals and humans is extremely low. A conventional way of controlling S. nitens using crude sap of infected larva was applied in several oil palm plantations in Sumatra Island, but various improvements are required to make the method more effective, efficient, widely acceptable and scientifically justified. A research on the potential use of entomopathogenic virus native to Sumatra Island as biological control agent of slug caterpillar was conducted to comprehend the pathogenicity of virus and virulence of the entomopathogenic virus and to reveal the morphological identity of particle. The result showed that the use of virus infecting caterpillars to control the insect was quite successful in term of increasing the number of infected caterpillars and reducing the rate of population development in the field. The use of homogenized infected caterpillars to orally infect healthy S. nitens caterpillars resulted in the symptoms characteristics to viral infections appeared in all treated caterpillars with various extent of symptom developments. Some caterpillars could spine coconuts but failed to release adult moth. Purification of the virus particles from infected caterpillars resulted in the appearance of white band in the sucrose gradient indicated the presence of viral RNA. Electron microscopic observation showed that the white band in the sucrose gradient contained spherical shape of virus particles justifying that the agent infecting S. nitens caterpillars is a virus which still need further analysis for its complete identification.

Keywords—Entomopathogenic Virus; Oilpalm; Setora Nitens.

I. INTRODUCTION

The cultivation of oil palm in Indonesia was started in Sumatra before finally spread to other big islands such as Kalimantan, Sulawesi and Papua. Major palm oil producing provinces in Sumatra are Riau, North Sumatra, South Sumatra, Jambi and Lampung. There had been major challenges in the development of oil palm plantation in Indonesia, such as good governance and investment, leadership, land policy, regional autonomy and labor.

Furthermore, oil palm growing areas have potential insect pests such as rhinoceros beetle, scale insect, bagworms, slug caterpillar, cutworm, leaf miner moths, bunch moth, leafhopper, aphids, and mealy bugs. Slug caterpillars, especially Darna trima, Setotthoea assigna and Setora nitens, have been appearing to be more serious insect pest of oil palm [1]. Many effort had been made to control the caterpillars using insecticides but the insects are still existing and causing significant damages to the palm. Furthermore, as suggested by [2], the application of insecticide may cause a lot of disadvantages described as environmental and economic costs.

One constraint to produce high quality and quantity of oil palm is a main insect pest, Setora nitens (Lepidoptera: Limacodidae), slug caterpillars on the leaves of mature and immature palms [3]. [4]. S. nitens together with Darna trima, D. bradleyi, and Setothea assigna are also called as nettle caterpillars and are important defoliators of oil palm.
planted in southeast Asia [5]. In severe infestations, larvae consume all foliage and leave only the mid-rib of the frond, which might cause significant decrease of fruit production [6]. Crop losses caused by the insect feeding on oil palm leaves have been calculated to be about 30% of the crop yield [7], [8]. The serious damage caused by S. niteus and the potential yield losses seems to be an acceptable reason for oil palm growers to use insecticides to control the pest.

Controlling the outbreaks of S. niteus, and other limacoids attacking oil palm, inevitably provoked the applications of broad spectrum insecticides which might cause serious damages to the environment [9], [10], [11], [12]. The consequences of the application of this technique was apparent: pollution, toxicity to human and domestic animal, development of resistant pest population, damage to beneficial insects and the development of new pest [13]. Furthermore, since not all chemical substances have always been used correctly and some of them are particularly highly toxic, non target insects such as natural enemies sometimes are being poisoned. Synthetic insecticide has been reported to cause resistance phenomena, very wide spread pollution and sometimes caused serious imbalances within ecosystems [2].

The demand for effective and safer pesticides under sustainable agriculture system has push the search for biological pesticide. As an organism, insects are subject to diseases caused by viruses, bacteria, and fungi. A considerable number of bioinsecticides have reached the market place, and millions of hectares are treated annually with entomopathogenic microorganisms worldwide [14]. Despite recent advances, the use of bioinsecticide is proportionally limited when compared to their chemical counterparts, even in countries where many biopesticides are produced.

The growing adoption of microbial biocontrol agents depends on factors such as: 1) development of better products, 2) development and implementation of truly integrated pest management strategies in which biological option are emphasized [15], [16], [17], [3] the capacity of biopesticide manufacturers to maintain marketing and product support teams, 4) cultural changes (acceptance by farmers of slow-acting narrow-host-range product), and 5) knowledge-based recommendations for product use [18].

Most insect pathogens, especially entomopathogenic baculovirus, tend to infect primarily insect larvae and are only effective against this stage. Some of identified baculovirus have been successfully used as biological control of insect pest. For example, baculoviruses has been used to control velvet bean caterpillar (Anticarsia gemmatalis) on soybean in Brazil [19]. The virus are often specific, safe for human and other vertebrate and have little or no environmental impact.

A major drawback of baculoviruses is the long incubation period to show effect or cause disease. Their action is relatively slow (a matter of days or weeks) when compared to most chemical insecticides (<day). However, improvement of the speed of kill of certain baculovirus has been achieved either by strain selection or by genetic engineering of Spodoptera exigua multicaudapulicapsid nucleopolyhedrovirus (ScMNPV) [20]. Genetic engineering approaches have also been used predominantly to increase the speed of action such as deletion of specific insertion of various insect-specific toxin genes [21], [22].

Another successful application of baculovirus has been the control of coconut rhinoceros beetle (Oryctes rhinoceros) in the Andaman Islands in India. Virus epidemics in populations was induced by infecting and releasing a virus with Oryctes baculovirus. Results comparing levels of damage to coconut, before and after virus introduction have been 80-90% reductions.

Recently, an unidentified virus was found infecting S. niteus and there have been an outlook to use the virus as biological control agent of the insect. Trials had been made to spray crude sap of the infected caterpillar to the caterpillar population and the result were promising. However, a few of the virus characteristics are known and there is obviously a need to study the rest characteristics of the virus, as the minimal use of the virus as biological control agent is a future consideration.

The pathogenicity and virulence of the virus had not been thoroughly understood. Molecular characterization of unidentified entomopathogenic virus is another important aspect because it will provide definitive identity of the virus.

II. MATERIALS AND METHODS

The experiment was conducted in the Laboratory of Phytophathology, Department of Plant Pests and Diseases, Faculty of Agriculture, Srinivasa University in November 2012. The Setora niteus caterpillar used for bioassay were collected from PT Salim Oil Palm Plantation, Bintan, Riau. Only fresh and healthy fifth instar caterpillar were used in the experiment.

The larvae larval stage was selected because the caterpillar of this stage are very greedy, consuming large amount of leaves as preparation for their pupal stage. 100 gram infected caterpillar were collected from sprayed field were homogenized and dissolved in 5000 water. The use of an amount of water compared to that used by oil palm grower was to gaurantee that there are enough virus in the suspension to infect healthy caterpillars.

Leaf blades from middle frond of oil palm were cut into 2 cm long segments and dipped into the suspension. The 6 leaf segments were then placed in a 40 x 60 x 30 cm box with 100 caterpillar. The leaves were replaced whenever necessary, to increase the appetite of the caterpillars.

The experiment was conducted daily to record the incubation period, infection frequency, cococon formation, disease symptoms, adult moth liberation and mortality. To purifying virus infecting S. niteus caterpillar, 200 g of frozen larvae (kept in -80°C freezer) were thawed and homogenized in 500 ml of extraction buffer containing 50 mM Tris/HCl, pH 7.5, and 0.2% 2-mercaptoethanol. The homogenate were refined by centrifugation at 10,000xg for 30 minutes, and the supernatant was the centrifuged at 100,000xg for 3 hours. The pellets were resuspended in 50 ml of TE buffer containing 50 mM Tris/HCl, and 1 mM EDTA at pH 7.5, layered on 1 TE buffer containing 30% sucrose, and then centrifuged at 100,000xg for 30 minutes.
The white band observed in the gradient was collected, centrifuged down at 100,000 g for 3 hours and then resuspended in distilled water. The tentative viral fractions were observed to verify their purity and integrity by negative stain using a transmission electron microscope. Viral particles purified by sucrose gradient centrifugation were negatively stained with 2% (w/v) sodium phosphotungstate (pH 7.2) on formvar-coated grids, and examined with a transmission electron microscope (Hitachi H-800).

III. RESULTS AND DISCUSSION

The caterpillars of S. nitens initially fed normally on oil palm leaves wetted with suspension containing the juice of infected caterpillars, but 2 days later their appetite decreased significantly and some caterpillars started to show infection symptoms. The first symptom shown by infected caterpillars was the loss of their appetite, stop feeding, and no response to touch. Infected caterpillars then moved from leaf lamina to midvein and stayed there before finally drop and their color turned to yellowish brown.

On the third day, 16 caterpillars showed initial symptom and other symptoms such as moribundity, flaccidity and collapse started to occur on the forth day (Table 1). No caterpillar was found dead until the third day, but since the forth day larval mortality start to occur until the ninth day when all caterpillars have died.

The slowly infected caterpillars could reach the pupal stage but all of them failed to further develop and died inside the cocoon, no adult moth was released from the cocoons.

The incubation period of the virus was determined by — the time period for at least 50% of the inoculated caterpillars had shown viral infection symptoms. As shown in Table 1, the incubation period is 4 days, because in the forth day 53 of 100 inoculated caterpillars have shown various symptoms of infection. This incubation period is smaller than those found in the field which is around 8 days.

The difference was quite acceptable because incubation period of a viral pathogens are dependent upon the viral concentration in the suspension applied and the environmental conditions, especially temperature.

Cocoons were spined by infected caterpillars indicated by the abnormality of the cocoon which was darker in color but no adult moth was released from the cocoon even after 4 weeks, the longest stage of normal cocoons (Table 2). When the cocoon were finally dissected, no moth had alive developing moth inside.

The pupae failed to develop and died inside the cocoon. As shown in above table, 39 caterpillars did develop into pupal stage but none of them successfully developed to become adult moth. The virulence of the virus was indicated by short incubation period and high infection frequency. All caterpillars fed on leaves segment containing the virus were infected and eventually died prematurely.

The high virulence of the virus might be due to several factors such as the viral freshness and particle concentration. The viral freshness was at its optimum because the viruses were harvested from newly infected caterpillars, the moribund caterpillars collected from the area sprayed with suspensions of infected caterpillars from the previous year preserved in a freezer commonly used to preserve food (meat and fish) because the laboratory has not been equipped with technical freezer with very low temperature (-80°C).

Even though the virus could initiate the disease after being stored for approximately one year under freezing temperature, the virulence of the virus was lower than that of newly infected caterpillars, indicated by longer incubation period and lower infection frequency. Not all caterpillar fed on sprayed leaves in the field showed viral disease development, many of them escaped from viral infection and survived until the next generations.

This was an indication that virulence of the virus decreased quite significantly after long preservation under freezing temperature. This has proven that the active agent is a virus, because only virus could retain its virulence after being frozen for long time. However, keeping under freezing temperature for long time also reduced the virulence of the virus. The very high efficacy shown by the mortality of all inoculated caterpillars showed that we can rely on the virus to control the insect, but there must be some adjustment to match the situation in the filed which are very different from those in the experimental situation. The are some important factors to be considered or adjusted to meet the highest efficacy of the viral insecticide to be made based on virus naturally infecting S. nitens. The time of keeping infected caterpillars under freezing temperature and the temperature itself is very influential to the virulence of the virus derived from the insect.

The fresher the better. The Concentration of virus particle in the suspension affect the number of virus particles ingested by each caterpillar in a time and this will affect the incubation period and disease development. The spray method or the sprayer used determines the distribution on the virus particles on the leaf surfaces treated which might cause the same effect as virus concentration.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>NUMBER OF CATERPILLARS SHOWING VIRAL INFECTION SYMPTOMS DURING THE FIRST TO FOURTH DAY AFTER INOCULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom</td>
<td>Number of caterpillar showing symptom</td>
</tr>
<tr>
<td></td>
<td>day 1</td>
</tr>
<tr>
<td>Active / no symptoms</td>
<td>100</td>
</tr>
<tr>
<td>Stop feeding</td>
<td>0</td>
</tr>
<tr>
<td>Thracody</td>
<td>0</td>
</tr>
<tr>
<td>Moribundity</td>
<td>0</td>
</tr>
<tr>
<td>Drop</td>
<td>0</td>
</tr>
<tr>
<td>Sping cocoon</td>
<td>0</td>
</tr>
<tr>
<td>Larval mortality</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>
TABLE II
NUMBER OF CATERPILLARS SHOWING VIRAL INFECTION SYMPTOMS DURING THE FIFTH TO NINTH DAY AFTER INOCULATION

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Number of caterpillar showing symptom</th>
<th>day 3</th>
<th>day 4</th>
<th>day 5</th>
<th>day 6</th>
<th>day 7</th>
<th>day 8</th>
<th>day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active / no symptoms</td>
<td>18</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drop feeding</td>
<td>17</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tumidity</td>
<td>11</td>
<td>7</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mortality</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spine cocoon</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Larval mortality</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spine liberation</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>78</td>
<td>59</td>
<td>29</td>
<td>19</td>
<td>10</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

The sucrose density gradient centrifugation of pellets suggested containing virus particles resulted six fractions, of which only three fractions showed white bands (fraction 1, 3 and 5) but only. Fraction 5 is the densest and the only one showed molecular weight from the three white bands. When all the six fractions were loaded onto polyacrylamide gel (SDS-PAGE), fraction 4, 5 and 6 showed similar molecular weight. However, since only fraction 5 showed white band, only the white band from fraction 5 was processed for transmission electron microscopy (TEM).

Figure 5. Six fractions and three white band separated by sucrose density gradient centrifugation (a) and the result of polyacrylamide gel (SDS-PAGE)

Negative staining revealed that the purified particles had a sphericle shape and no envelope and were about 20 nm in diameter. This structural traits were similar to those reported for insect picorna-like viruses and members of the Family Tetraviridae.

Figure 6. Negative staining of virus particles purified from S. nitens using by sucrose density gradient centrifugation. Photographed from different steps and different magnifications.

The negative staining clearly showed the spherical or isometric shape form of the virus particle. The absence of capsule and their diameter indicated that the virus is a member of Family Tetraviridae which most of the members are pathogenic to invertebrates. According to International Committee on Taxonomy of Virus (ICTV), virus belonging to Tetraviridae are isometric or spherical, have no capsid, infecting invertebrates and have natural genome replication.

The first three characteristics match to S. nitens virus. The genome characteristics have not been studied. Therefore, the virus infecting S. nitens can be tentatively concluded as a member of Family Tetraviridae, but no genus and species can be determined because of the lack of information on the genome.

As decided by International Committee on Taxonomy of Virus, many characteristics are required to identify a virus. The characteristics required include: biologic properties (host range, vectors, mode of transmission, antigenic properties), virion physical characteristics, structural protein characteristics, and genome molecular characteristics.

IV. CONCLUSIONS

Based on, bioassay experiment and molecular study of virus naturally infecting Setona nitens of oil palm, it can be concluded that:

1. Virus naturally infecting S. nitens is very promising to be used as biological control agent of the insect. Application of suspension of infected caterpillar just before clean water is good enough to reach reasonable level of control but there is a need to formulate the standard procedure of making suspension and spraying it.

2. Infected caterpillars kept under freezing temperature can be used as the source of virus, and the virus to a lesser extent can retain its virulence. However, the virus gradually loses its virulence when frozen for long time. Fresh harvested infected caterpillars must be used. Virulence compared to the frozen ones is greater.

3. The purification and transmission electron microscopy of virus naturally infecting S. nitens revealed that the virus is spherical and no envelope. Because the virus infects invertebrates, these three characteristics place the virus into Family Tetraviridae.

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