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Gambar sampul depan: Sebelah kiri adalah gejala serangan (galls) Crotonothrips sp. pada pucuk/daun muda pohon glodo-gan (Polyalthia longifolia); sebelah kanan atas adalah telur dan serangga dewasa Crotonothrips sp.; sebelah kanan bawah adalah gejala kerusakan akibat Crotonothrips sp. pada titik tumbuh (Foto: A. Nasrullah).

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Effectiveness of Entomopathogenic Nematode Isolated From Longicorn Borer in Controlling *Squamura celebensis* (Lepidoptera: Cossidae) on Cocoa

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ABSTRACT *Squamura celebensis* Roepeke is the most important pest among the stem borers of cocoa in Sulawesi, Indonesia. This insect destroys flower cushions and bark, and the damage may significantly reduce the productivity of cocoa. An experiment was conducted in a cocoa farm in Luwu Regency, South Sulawesi, to determine the effects of entomopathogenic nematode application on longicorn borer population and its frass. The nematode used in this trial was isolated from infected longicorn borers collected from cocoa development. The nematode used in this trial was isolated from infected longicorn borers collected from cocoa development. The nematode used in this trial was isolated from infected longicorn borers collected from cocoa development. The nematode used in this trial was isolated from infected longicorn borers collected from cocoa development. The nematode used in this trial was isolated from infected longicorn borers collected from cocoa development.

Treatments consisted of three different lengths of frass: 1-5 cm, 6-10 cm, or 11-15 cm sprayed with 1000 trees. Treatments consisted of three different lengths of frass: 1-5 cm, 6-10 cm, or 11-15 cm sprayed with 1000 trees. Treatments consisted of three different lengths of frass: 1-5 cm, 6-10 cm, or 11-15 cm sprayed with 1000 trees. Treatments consisted of three different lengths of frass: 1-5 cm, 6-10 cm, or 11-15 cm sprayed with 1000 trees. Treatments consisted of three different lengths of frass: 1-5 cm, 6-10 cm, or 11-15 cm sprayed with 1000 trees. Treatments consisted of three different lengths of frass: 1-5 cm, 6-10 cm, or 11-15 cm sprayed with 1000 trees. Treatments consisted of three different lengths of frass: 1-5 cm, 6-10 cm, or 11-15 cm sprayed with 1000 trees.

Neosteinerma Nguyen Smart, which persisted constantly on frass during the two months of observation following the nematode application, the linear development of the frass ceased after 20 days on frass of 1-5 cm, after 40 days on frass of 6-10 cm, and after 40 days on frass of 11-15 cm. While in the controls, frass continued to develop during the two-month observation period. In the nematode-treated samples, the rates of frass development on 1-5 cm, 6-10 cm, and 11-15 cm lengths were respectively 0.11 cm, 0.18 cm, and 0.18 cm per five days; and in the controls were respectively 0.21 cm, 0.24 cm, and 0.18 cm per 5 days. The decrease and cessation of frass development correlates with the rates of nematode infection. The percentage of dead larvae in stem holes were 73%, 63%, and 62% for the frass length treatments of 1-5 cm, 6-10 cm, and 11-15 cm, respectively. These field data supported the possibility of using the entomopathogenic nematode in a management strategy of *S. celebensis*.

KEYWORDS Entomopathogenic nematode, *Squamura celebensis*, cocoa, *Neosteinerma*

*Squamura celebensis* Roepeke (Lepidoptera: Cossidae) is the most important pest among the stem borers of cocoa in Sulawesi, Indonesia. This insect destroys flower cushions and bark, and the damage may significantly reduce the productivity of cocoa if population of the borer in trees is very high and a great number of trees are infested (Kbao et al. 1991). The eggs are laid on or in tree trunks or branches. After the eggs hatch, the young larvae begin their life cycle by feeding on bark, sheltering by day in a short tunnel and feeding on the cambial tissue at night, concealing the damage with a layer of frass held in silk (Kbao et al. 1991, Hajek & Bauer 2007). Pupation takes place within the tunnel and adults emerge from the tree to feed, disperse, mate, and oviposit. Completion of the life cycle takes about 3 to 4 months (Kbao et al. 1991, Hajek & Bauer 2007).

Cultural practices such as maintaining a healthy and balanced ecosystem to preserve natural enemies that kill stem borer caterpillars can be effective control measures (Buss and Foltz 2009). Chemical control has largely been ineffective. However, there is evidence that encouraging ants can provide some biological control. The stability, social organization, and foraging behavior of predatory ants enable them to react quickly to increasing prey density, and also make them uniquely able to protect crops from low-density pest (Buss and Foltz 2009, Way & Khoa 1992). Unfortunately, when borers infest severely in low shade conditions, the ants cannot develop well due to decrease of shade of cocoa canopy which does not suit them.

The control of plant pests by whole organisms continues to be an attractive method to implement in the field. Entomopathogenic nematodes are promising organisms because nematodes are capable of seeking and killing their host rapidly (Gaugler et al. 1997, Adams & Nguyen 2002, Shapiro-Ilan et al. 2002). Their use on cocoa has been initiated with *Steinerneae carpocapsae* for controlling cocoa pod borer (CPB). This nematode can persist on the pod surface in both the dry and rainy seasons and can penetrate cocoa pods and their activity against CPB increases when this nematode is applied in combination with pod sleeving. Plastic sleeves would provide high humidity on the pod surface and thus enable nematodes to persist for a longer period of time (Rosmana et al. 2009, Rosmana et al. 2010).

In this present work we evaluated the use of nematodes isolated from cocoa longicorn borer to control Lepidopteran stem borer of cocoa, *Squamura celebensis*. Due to the difficulties of rearing this cocoa pest, we applied the nematode to different lengths of frass and observed its impact on the development of this frass in the field. We also observed
the mortality of the insect in stem bore holes following nematode treatments and the persistency of the nematode on frass.

**Materials and Methods**

**Field experiment**

The research was carried out in farmers' farms in Luwu Regency South Sulawesi, which were heavily infested by the cocoa stem borer *Squamura celebensis* in an area of about 2 ha. The experiment tested the effect of nematode application on different lengths of frass: 1-5 cm, 6-10 cm, and 11-15 cm. Six treatments in the trial included controls of 1-5 cm frass, 6-10 cm frass, and more than 10 cm frass, and nematode applications on frass of 1-5 cm, 6-10 cm, and 11-15 cm. The experiment had a randomized block design and each treatment had four replications. Each treatment unit consisted of 8 frass, therefore, the total number of frass was 192. Beside these frass, the addition of 50 frass with lengths of 11-15 cm was also treated for observation of nematode persistency.

The nematode was isolated from cocoa longicorn borer, *Cerosterna* sp. (Coleoptera: Cerambycidae), found in stem holes of cocoa about 30 cm above the ground. This nematode was cultured on semi-liquid medium in vials consisting of agar, fish oil, potatoes extract, sugar, and beef liver extract. After two weeks in culture, the medium was suspended with distilled water and was filtered to get the active juveniles of the nematode. Each frass was treated by spraying once a suspension of 1000 nematodes starting at the mouth of hole until the point of frass, while the control frass was sprayed by water.

The development of frass was observed in a period of five days and if this development stopped, the frass and hole of 30 samples for each treated frass were taken apart to observe the death of *Squamura celebensis*. The persistency of nematode was evaluated during over two months by taking five samples respectively at 1, 2, 3, 5, 7, 10, 20, 30, 45, and 60 days after inoculation. The nematode was also identified for its genus.

**Analysis**

The rates of frass development in were analyzed using regression (frass development in ordinate and time in abscissa).

**Results and Discussion**

The nematode isolated from cocoa longicorn borer was identified as *Neosteinernema* Nguyen Smart. This nematode can grow and develop very well in semi-liquid medium for *Steinernema carpocapsae*. Distinguishing characters of *Neosteinernema* by comparing to members of the genus *Steinernema*, were that females have prominent phasmids, a curved tail longer than the body width at the anus, and a spiral shape in juvenile-bearing females. Males having prominent phasmids, a digitate tail tip, a characteristic spicule shape and 13-14 pairs of genital papillae, with eight pairs preanal (Nguyen & Smart, 1994). The application of this nematode can reduce or stop the development of frass of different lengths. The linear development of frass stopped after 20, 40 and 60 days on frass with initial lengths of 1-5 cm, 6-10 cm, and 11-15 cm, respectively; while in each control, frass continued to develop during the two month observation period. The rates of frass development of 1-5 cm, 6-10 cm, and 11-15 cm were respectively 0.11 cm, 0.18 cm, and 0.18 cm per five days. In controls of each treatment, the rates of frass development were 0.21 cm, 0.24 cm, and 0.21 cm per 5 days for the treatments of 1-5 cm, 6-10 cm, and 11-15 cm, respectively (Fig. 1, 2, 3). The decrease of frass development and its rate correlated to inhibition and death of insect due to nematode. The percentages of dead insects due to the nematode were 71, 63, and 62% in frass of 1-5 cm, 6-10 cm, and 11-15 cm, respectively. 5 cm. No dead insects were found in the controls.

**Figure 1.** Development of *Squamura celebensis* frass of 1-5 cm lengths following treatment by entomopathogenic nematode. NT = untreated control; T = nematode treatment.

**Figure 2.** Development of *Squamura celebensis* frass of 6-10 cm long following the treatment by entomopathogenic nematode. NT = untreated control; T = nematode treatment.
The length of frass is apparently related to the development phase of S. celebensis larvae. The younger the larvae, the more sensitive they are to infection by nematodes, as indicated by frass of 1.5 cm where the death of larvae reached 73%. The frass is a mixture of sawdust-like substance as a result of larval feeding on the cambial tissue and larvae’s waste. This frass can accumulate in large quantities in bark crevices providing a good sign of borer infestation (Hajek & Bauer 2007, Herms 2007). The nematode apparently could persist in the frass made by Squamura celebensis. Table 1 indicates that the population of nematode in frass was relatively constant during the two months observation. The passage of larvae in this frass is due to feeding on the cambial tissue at night and sheltering by day in tunnels, thus permitting the infection by nematode. This could explain the great effect of nematodes in controlling the larvae of borer. Nematodes enter hosts through natural openings such as mouth, anus, and spiracles. After entering the host’s hemocoel, they release symbiotic bacteria, which are primarily responsible for killing the host, defense against secondary invaders, and providing the nematodes with nutrition (Dowds & Peters 2002). The nematode molts and completes up to three generations within the host after which the nematode exit the cadaver to search out new hosts (Kaya & Gaugler 1993). The constant population of nematode during the two months observation can perhaps be explained by the development of nematodes in host.

With one application of one thousand individuals per frass, the nematode could effectively inhibit the development of frass and also kill Squamura celebensis. These field data showed the potential use of the entomopathogenic nematode in S. celebensis management strategy. Chemical sprays aimed to entry holes and the frass of the borer are only poorly to moderately effective; this is likely due to insufficient penetration of the chemical product into larval tunnels. In contrast, nematodes can probably infect the larvae in the frass or in the tunnel. Entomopathogenic nematodes had been observed to move within Synanthedon tipuliformis and Conopomorpha cromerellla tunnels (Miller & Bedding 1982; Rosmana et al. 2009).

### Conclusion

The application of the entomopathogenic nematode was able to suppress the linear development of the frass. The longer the size of the frass at the time of the nematode application, the longer the time needed to cease the frass development and also the higher the rate of frass development. In contrast, the percentage of dead larvae in stem holes decreased as the frass length at the time of application increased. Therefore, entomopathogenic nematode has a promising potential use in developing a management strategy of S. celebensis.

### References


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