CULTURE OF THE ENTOMOPATHOGENIC NEMATODE Steinernema carpocapsae (WEISER) ON ARTIFICIAL MEDIA

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ABSTRACT
In this study entomopathogenic nematode Steinernema carpocapsae (Weiser) (Rhabditida: Steinernematidae) and symbiotic bacteria Xenorhabdus nematophilus were reared on four different culture media. Media number 1 contained 45% of dog food, 50% of water and 5% of lard. Media number 2 contained 6% of powdered fish, 85.56% of water and 8.44% of symbiotic bacteria. Media number 3 contained 8.75% of silkworm pupa, 85.16% of water and 6.09% of lard and media number 4 in vivo of dog food, 50% of water and 5% of lard. Media number 2 contained 6% of powdered fish, 85.56% of water and 8.44% of symbiotic bacteria. Media number 3 contained 8.75% of silkworm pupa, 85.16% of water and 6.09% of lard and media number 4 in vivo of dog food, 50% of water and 5% of lard. Media number 2 contained 6% of powdered fish, 85.56% of water and 8.44% of symbiotic bacteria.

INTRODUCTION
Entomopathogenic nematode has emerged as excellent candidates for biological control of insect pest. Attributes making the nematode ideal biological insecticides include their broad host range, high virulence, safety for non target organisms and high efficacy in favorable habitats (Mahar et al., 2004).

Nematodes of the genera Steinernema and Heterorhabditis have long been recognized as effective insect control agents and have attracted considerable interest as a bio-pesticide. The portals of entry for nematode infective juveniles are through natural openings, i.e. mouth, anus and spiracle (Gaugler and Kaya, 1990). Infective juvenile (IJ) nematode of Steinernema carpocapsae carries the symbiotic bacteria, Xenorhabdus nematophilus, in its gut. The bacteria are released and reproduced within the insect hemocoel. The insect die within 24-48 hours because of bacterimia. The nematodes feed and develop 2-3 generations in insect cadaver before the new infective juveniles (IJs) leave for their new hosts (Mahar et al., 2004).

Production of entomopathogenic nematodes has evolved from the first large scale in vitro solid medium production (Tangchitsomkid et al., 1999; Glaser et al., 1940). Bedding (1981) developed a semi-solid culture method whereby nematodes were reared on an animal protein and lipid based medium coated onto a polyether polyurethane sponge matrix to give a large surface area for passive aeration. Development of production technology, distribution and sales of entomopathogenic nematode containing products is occurring now at a rapid pace. In many countries, commercial productions have proved successful in insect control. In Thailand nematode are reared using medium contain dogfood or in vivo culture on Spodoptera litura.

The objective of this study was to use dried fish meal and silkworm pupae which are local materials as alternative source of medium in nematode rearing.

MATERIALS AND METHODS
Nematode culture
In vivo culture the nematode S. carpocapsae was reared by modifying the method of Lindegrad et al. (1993). After the 9cm Petri dish lined with Whatman no.1 filter paper was sterilized in autoclave at 121°C, 15 lbs/inch² it was cool downed for 20 minutes at room temperature. Suspension of IJ S. carpocapsae (500 IJs/ml) was dropped thoroughly over the filter paper. Ten of the 5-6th instars S. litura were placed on the dish and incubated at 30±5°C for 48 hours. S. litura cadavers were transferred to the White's water trap where the IJs of S. carpocapsae were isolated. IJs nematodes were three times gravity washed with sterile, distilled water and surface sterilized with 0.1% Thimerosol in accordance with Dunphy and Webster (1985).

Bacterial culture
S. carpocapsae infected with IJ S. carpocapsae were surface sterilized with mixture of 0.1% sodiumhypoclorite and 1-2 drop of tween 80 for 15 minutes. Sterilized S. litura was cut to open with sterile needle and scissor. A loopful of insect haemolymph was streaked on NBTA medium [Akhurst, 19980; 2.3% (w/v) nutrient agar, 0.025% (w/v) bromothymolblue, 0.004% (w/v) triphenyl-tetrazolium chorde (TTC)]. The agar
The media flasks were autoclaved at 121 °C and 15 lbs for 15 min. After the flask was cooled, 5 ml of YS broth was inoculated into each flask for 24 hrs. The bacterial cultures from such conical flasks were transferred into modified nutrient broth solution. The concentration of bacterial cells in the broth was estimated using spectrophotometer and the bacterial suspension was then placed in shaking incubator at 23 °C for 20 days. The sponge pieces absorbed into a 500 ml nutrient broth solution. The bacterial suspension was then placed in shaking incubator for 32 hours. The concentration of bacterial cells in the broth was estimated using spectrophotometer and the concentrations of bacterial cells were adjusted for use in experiments.

Culture medium
Four culture media (i.e., three artificial media and one in vivo) as follow were tried in rearing of the S. carpopcapsae (5 replications for each media).
Medium I contained 45% dog food, 50% water and 5% lard. (Somsook and Nanta, 1992)
Medium II contained 6% powdered fish, 85.56% water and 8.44% lard.
Medium III contained 8.75% ground silkworm pupa, 85.16% water and 6.09% lard.
Medium IV (in vivo culture) the nematodes were reared on the 5-6th instars larvae of S. litura.

Nematode culture and yield
In vitro culture the artificial media of each treatment was mixed evenly with a blender. The sponge, which served as a carrier material was cut into small pieces of 1 x 1 x 1cm size and each medium was absorbed into a crumbled sponge pieces at the ratio of 1.5g : 55g (sponge: medium) before it was put into a 500 ml nutrient broth solution. The media flasks were autoclaved at 121 °C and 15 lbs for 15 min. After the fask was cooled, 5 ml of YS broth containing the bacteria symbiont X. nematophilus was inoculated into each flask for 24 hrs. The bacterial cultures were incubated at 23 °C for 20 days. The sponge pieces from such conical flasks were transferred into modified White trap. Separate at the base were harvested daily in 0.1% formalin. The total number of nematodes produced per conical flask was counted and the data recorded was subjected to analysis of variance. The yield of infective juveniles was expressed as the number per gram of medium.

In vivo culture, the 5-6th instar S. litura was used as nematode hosts. For rearing, exposure dish (petri dish: 15×90 mm) was used with Whatman qualitative #1 filter paper placed in it. S. carpopcapsae suspension (500 IJs/ ml) was dropped thoroughly over the filter paper. Ten of S. litura larvae placed on filter paper and incubated for two days. After insect mortality occurs (within 24-48 hrs.), the cadavers are transferred on to white’s water trap plates for further incubation at 30 °C for 7 days. The IJs were collected and killed in 60 C water after that their number were counted.

Nematode body length and width
Body size of the IJ nematodes from each media was measured under stereomicroscope. The body length was the distance from the anterior end to the tail and the body width was the maximum width of the body. Thirty infective juveniles were measured for each medium at each cultured time.

Virulence of nematode
The virulence of nematode IJs from different cultural media was evaluated using nematode penetration rate (Caroli et al., 1996) and mortality rate of their host. One ml of nematode suspension which contained 500 infective juvenile was transferred to each one of the 10 filter paper-padded petri dishes. Five of the 3rd instar S. litura larvae were placed into each petri dish. Numbers of S. litura died by nematodes from different cultured media and numbers of nematode inside their bodies were recorded after 24, 48 hours.

The nematode penetration rate was calculated using the following formula:
Percentage penetration

\[
\text{Percentage penetration} = \frac{\text{No. nematodes penetrated into insect larva}}{\text{No. added nematodes}} \times 100
\]

RESULTS AND DISCUSSIONS

Nematode yield
The nematode yields per gram from media I, II, III and IV were 3.038, 2.445, 2.989 and 5.547× 10³ infective juveniles/gram of medium, respectively. Number of nematode in medium I, II and III was not significantly different. These two media have amount lipids more than medium II; lipids are necessary for development and reproduction (Buecher and Popiel, 1989). The dog food and ground silkworm pupa were supplemented media, both of these media different supported greater nematode reproduction than did the powdered fish medium.

Nematode body length and width
The average length and width of the infective juveniles cultured in each medium are showed in Table-1. Nematode from medium II has longest and widest body. However length of nematode may not be as important as its width because in infection process nematodes must be able to get into their host body through the host natural openings.

Virulence of nematode
Nematodes reared in medium I, II, III, and IV gave 5.58, 4.30, 5.70 and 5.7 % penetration, respectively.
Nematodes reared in MII had lowest percent penetration while those reared in Medium I, III, and IV had no different percent penetration (Figure-2). This may concern with the nematode body width because nematodes from medium II had the biggest body size (Table-1) so they may faced some obstruction to penetrate through body opening of their hosts while the other whose their body width were lower could penetrated better.

Mortality rate after 24 and 48 hrs of the 3rd instar larva *S. litura* were 36, 24, 38, and 40% and 64, 76, 62, and 60% by the nematode reared from medium I, II, III, and IV, respectively (Figure-4). Again, this may concern with their body size and penetration rate. The nematodes reared in medium II had the biggest body size and lowest penetration rate this may caused them produced lowest mortality after 24 hrs. However after 48 hrs nematodes reared in medium II produced highest mortality rate (Figure-3) this probably because the bigger nematode possessed higher number of bacteria and as time passed the bacteria were able to reproduce and caused more infection.

Table-1. Comparison of body length and width of the infective juveniles of *Steinernema carpocapsae* different culture media.

<table>
<thead>
<tr>
<th>Culture medium</th>
<th>Body length (µm)</th>
<th>Body width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td>536.4 ± 31.97a</td>
<td>25.3 ± 1.24b</td>
</tr>
<tr>
<td>MII</td>
<td>579.8 ± 23.91d</td>
<td>26.2 ± 1.98c</td>
</tr>
<tr>
<td>MIII</td>
<td>572.4 ± 31.57c</td>
<td>25.4 ± 1.75b</td>
</tr>
<tr>
<td>MIV</td>
<td>554.5 ± 37.06b</td>
<td>24.9 ± 1.73a</td>
</tr>
</tbody>
</table>

Means followed by the same letter in the same column are not significantly different at L.S.D at 5% significance level.

Figure-1. Comparison of nematode yields among four cultural media. Different letters above each bar indicate significant differences ($P < 0.01$).

Figure-2. Comparison of penetration rates of infective juveniles from four different medium. Different letters above each bar indicate significant differences ($P < 0.01$).

Figure-3. Comparison of percent mortality of last instar *Spodoptera litura* larvae exposed of *Steinernema carpocapsae* infective juveniles at 24 hours.
CONCLUSIONS

MII or medium contain powdered fish produced nematode which induced highest mortality rate of *Spodoptera litura* at 48 hrs. At the same time mortality caused by nematodes reared in MIII or medium contain grounded silkworm pupa was as high as those reared in MI and MIV which are the medium normally used in Thailand. So dried powdered fish and silkworm pupae which are local material can easily be obtain everywhere in Thailand with low cost each can be used as raw material for nematode rearing medium.

REFERENCES


