



## 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 nematophila* 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 lard. Media number 3 contained 8.75% of silkworm pupa, 85.16% of water and 6.09% of lard and media number 4 in vivo cultures, which were the last instar larvae of the *Spodoptera litura*. Results showed that the infective juveniles nematode rearing from medium I, II, III and IV yielded  $3.04 \times 10^5$ ,  $2.445 \times 10^5$ ,  $2.989 \times 10^5$  and  $5.547 \times 10^5$  IJs/gram medium, respectively. The average body width of the infective juveniles rearing from medium I, II, III and IV were  $25.33 \pm 1.24$ ,  $26.17 \pm 1.98$ ,  $25.40 \pm 1.75$  and  $24.85 \pm 1.73 \mu\text{m}$ , respectively. The average body length of the infective juveniles rearing from medium I, II, III and IV were  $536.45 \pm 31.97 \mu\text{m}$ ,  $579.85 \pm 23.91 \mu\text{m}$ ,  $572.39 \pm 31.57 \mu\text{m}$  and  $554.57 \pm 37.06 \mu\text{m}$  respectively. The nematode penetration rate into *S. litura* larvae within 48 hours were 5.58%, 4.30%, 5.70% and 5.76%, respectively. The percent mortality of *S. litura* larvae tested with infective juveniles within 24 and 48 hours. By the nematode reared from medium I, II, III, and IV were 36%, 24%, 38% and 40% and 64, 76, 62, and 60%, respectively.

**Keywords:** *Steinernema carpocapsae*, *xenorhabdus nematophilus*, culture, artificial media.

### 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 bacteremia. 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 *vitro* culture the nematode *S. carpocapsae* was reared by modifying the method of Lindegren *et al.* (1993). After the 9cm Petri dish lined with Whatman no.1 filter paper was sterilized in autoclave at 121°C, 15 lbs/inch<sup>2</sup> 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 5-6<sup>th</sup> 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% Thimersol in accordance with Dunphy and Webster (1985).

#### Bacterial culture

*S. carpocapsae* infected with IJ *S. carpocapsae* were surface sterilized with mixture of 0.1% sodiumhypochlorite and 1-2 drop of tween 80 for 15 minutes. Steriled *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 choride (TTC)]. The agar



plates were incubated at 28°C in the dark for 24 hrs. The bacterial colonies were sub-cultured onto nutrient agar until pure colonies of uniform size and morphology were obtained. *X. nematophilus* phase I absorbs bromothymol blue, producing dark blue colonies, whereas cells of *X. nematophilus* phase II do not absorb bromothymol blue but reduce TTC and produce red colonies (Akhurst, 1980). The pathogenicity of the isolate was confirmed by Kochs' postulate. A single colony of bacteria was selected and inoculated 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. carpocapsae* (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 5-6<sup>th</sup> 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 crumbed sponge pieces at the ratio of 1.5g : 55g (sponge: medium) before it was put into a 500 ml/flask. The media flasks were autoclaved at 121°C and 15 lbs for 15 min. After the flask 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 5-6<sup>th</sup> instar *S. litura* was used as nematode hosts. For rearing, exposure dish (petri dish: 15x90 mm) was used with Whatman qualitative #1 filter paper placed in it, *S. carpocapsae* 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 3<sup>rd</sup> 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

$$= \frac{\text{No. nematodes penetrated into insect larva}}{\text{No. added nematodes}} \times 100$$

## 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  $5.547 \times 10^5$  infective juveniles/gram of medium, respectively. Number of nematode in medium I, II and III were significantly different from that reared in its host (medium IV) (Figure-1). The nutrient source different from present in the culture medium. However number of nematode in medium I 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.



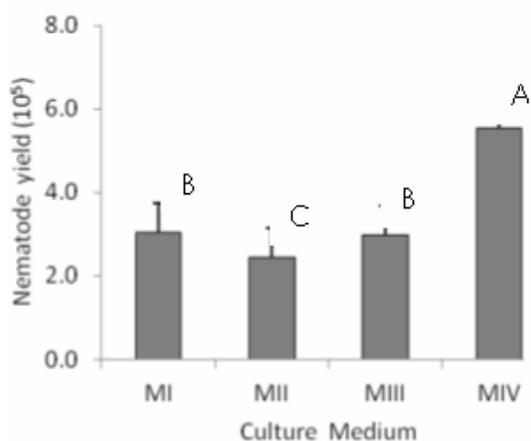
Nematodes reared in MII had lowest percent penetration while those reared in Medium I, III, and IV had no different percent penetration (Figure-2). This may concern with the nematode body width because nematodes from medium II had the biggest body size (Table-1) so they may faced some obstruction to 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 3<sup>rd</sup> 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..

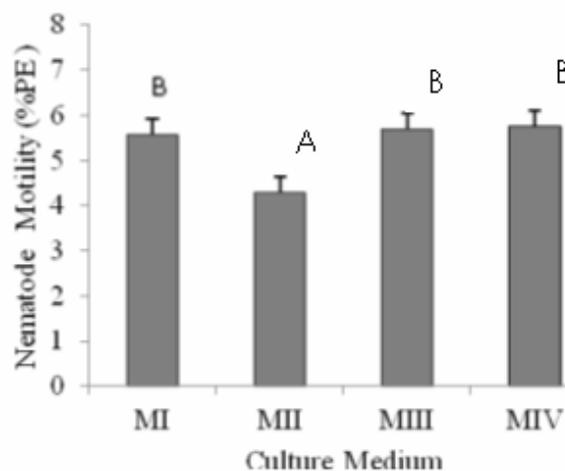
**Table-1.** Comparison of body length and width of the infective juveniles of *Steinernema carpocapsae* different culture media.

Culture medium	Body length ( $\mu\text{m}$ )	Body width ( $\mu\text{m}$ )
MI	536.4 $\pm$ 31.97a	25.3 $\pm$ 1.24b
II	579.8 $\pm$ 23.91d	26.2 $\pm$ 1.98c
III	572.4 $\pm$ 31.57c	25.4 $\pm$ 1.75b
IV	554.5 $\pm$ 37.06b	24.9 $\pm$ 1.73a

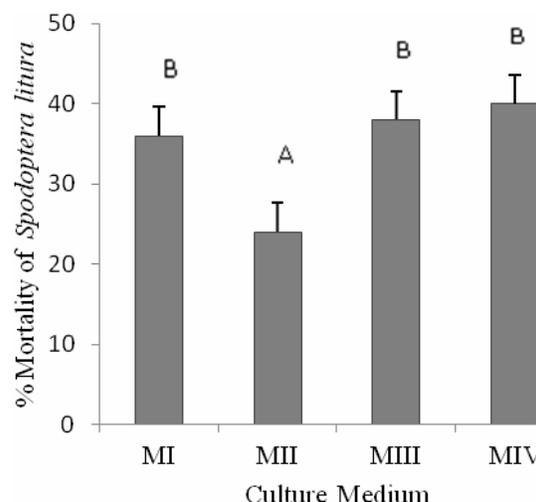
Means followed by the same letter in the same column are not significantly different at L.S.D at 5% significance level.



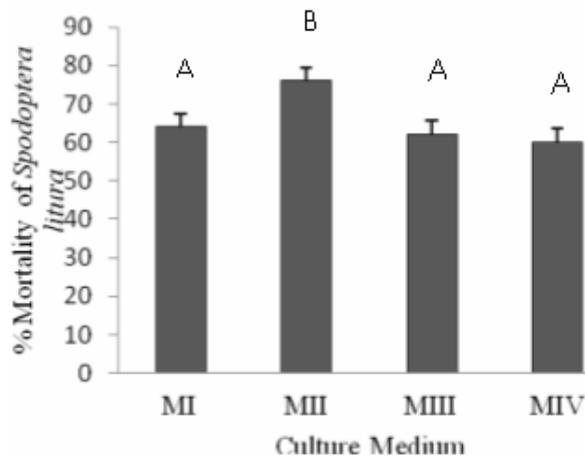
**Figure-1.** Comparison of nematode yields among four cultural media. Different letters above each bar indicate significant differences ( $P < 0.01$ ).



**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.



**Figure-4.** Comparison of percent mortality of last instar *Spodoptera litura* larvae exposed of *Steinernema carpocapsae* infective juveniles at 24 hours.

## CONCLUSIONS

MI 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.

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