

Mass production of the entomopathogenic nematode, *Steinernema carpocapsae* on *Tenebrio molitor* and *Spodoptera litura*

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Abstract. Authors. 2019. Mass production of the entomopathogenic nematode, *Steinernema carpocapsae* on *Tenebrio Molitor* and *Spodoptera litura*. *Biodiversitas* 20: 1344-1349. *Steinernema carpocapsae* is the most developed biological agents to control pests nowadays, to support this nematode as a biological agent mass production technique is required. *S. carpocapsae* can be produced via in vivo methods. In vivo production has some advantages, such as cheap, easy, and sustainable to be developed in small farm agriculture. The purpose of this research was to evaluate the mass production of *S. carpocapsae* using *Tenebrio molitor* and *Spodoptera litura* larvae as hosts. Twenty-five last instar larvae were inoculated with different concentrations of infective juvenile (IJ) (e.g., 100, 200, 300 and 400 IJ/larvae) of *S. carpocapsae* Asembagus. The production of *S. carpocapsae* with concentrations of 100, 200, 300 and 400 IJ/larvae on *T. molitor* and *S. litura* showed 3.62x10³; 6.29x10³; 1.13x10⁴; 1.52x10⁴; 1.13x10⁴; 1.46x10⁴; 1.54x10⁴; and 1.94x10⁴ IJ/larvae, respectively. The IJ harvest from *T. molitor* and *S. litura* shows the longest body length for IJ at a concentration of 400 IJ/larva of 437.48µm and 591.42µm, respectively. This study indicates that *T. molitor* and *S. litura* can be used to produce *S. carpocapsae*. The production of *S. carpocapsae* with a concentration of 400 IJ / larvae on *T. molitor* and *S. litura* showed the highest total yield and body length of IJ.

Keywords: In vivo, mass production, *Spodoptera litura*, *Steinernema carpocapsae*, *Tenebrio molitor*

INTRODUCTION

The utilization of chemical insecticides could trigger an explosive growth of the pest population. Such explosion of the pest population is possible when a pest becomes resistant to insecticides so that its population turns out of control. The higher a pest's level of tolerant of insecticides, the more the insecticide dosage is required (Nicolopoulou-Stamati et al. 2016; Parween et al. 2014). Moreover, chemical insecticides also bring other disadvantages, such as killing the predator and parasitoid, human poisoning, and environmental contamination (Kumar 2012; Horowitz and Ishaaya 2012). Those negative effects caused by chemical insecticides create an opportunity to develop an eco-friendly pest control as an alternative. One of the alternatives of eco-friendly pest controls is biological pest control, which focuses on the utilization of insect pathogen such as bacteria, fungi, viruses, and nematodes (Lazarovits et al. 2007; Kaya and Vega 2012; Lacey and Georgis 2012). One of the insect pathogens that are used as biological pest controller is entomopathogenic nematodes (EPNs) *Steinernema* sp. (Koppenhöfer et al. 2012; Dillman et al. 2012). Seven nematode families are utilized as biological pest agents including Mermithidae, Allantonematidae, Tetradomatidae, Phaenopsitylenchidae, Sphaerulariidae, Steinernematidae, and Heterorhabditidae (Lacey et al. 2001; Lewis and Clarke 2012; Van Zyl and Malan 2014; Půža 2015). However, nematode families of Steinernematidae and Heterorhabditidae are the most

developed biological agents to control pests nowadays (Glazer and Lewis 2000). *S. carpocapsae* that is categorized under the Steinernematidae family is proven to be potential to infect more than 250 species of insects from 75 families and 11 orders (Poinar 1990). Besides, there are more than 25 *Steinernema* strains isolated from various countries, and even it has been commercially produced in various formulations in some countries such as Austria, Germany, Japan, England, Columbia, Canada, and Australia (Grewal and Georgis 1999).

Steinernema is categorized under Steinernematidae family that can associate with bacteria *Xenorhabdus* sp. in eliminating the targeted pests (Boemare and Tailliez 2009; Stock and Hunt 2005). The *Steinernema* stadium that can cause high mortality of the targeted larvae is juvenile infective (IJ) stage. IJ enters the larvae through integument, spiracle, anus, and mouth. It is possible for IJ to penetrate the insect hosts when it is drawn by CO₂ produced by the targeted insect hosts (Batalla-Carrera et al. 2014). In case of penetrations through mouth or anus, IJ will go through the abdominal wall to reach hemocoel, while in case of penetration through spiracle, the juvenile will pass through the trachea wall to reach the hemocoel. The juvenile will release its symbiotic bacteria. The symbiotic bacteria shall form a supporting environment for nematode development by producing antibiotics that prevent the growth of other competitive microorganisms and turn the insect host cells as food sources for nematodes so that the insect host will be killed by septicemia (Constant et al. 2010; Poinar and Grewal 2012). The IJ is a phase where the larvae live

outside the pest hosts in which the IJ was created, the larvae are weather tolerant, and the larvae are able to infect new pest hosts (Lewis et al. 2006).

As a matter of fact, when compared with chemical insecticides, the use of nematode generally requires a higher price (Arthurs et al. 2004). Besides, nematodes have a shorter life span, low stability, and require specific knowledge to be applied, which include knowledge of the nematode maintenance until nematode storage (Umamaheswari et al. 2006). Such fact limits the utilization of nematodes as a biocontrol agent to farmers who are already equipped with the necessary skills and knowledge to use it (Shapiro-Ilan et al. 2002; Ehlers 2007). Nevertheless, in relation to a regulation and prohibition given through the Food Quality Protection Act of 1996 that stipulates the chemical insecticide usage limitation to prevent it from poisoning humans and contaminating the surrounding environment (Anonymous 1996) and in connection with the movement of eliminating chemical insecticide usage, it is considered as necessary to use a save biological pest controller especially through the utilization of pathogen *Steinernema* sp. (Koppenhöfer and Fuzy 2009). In addition to its eco-friendly characteristic, the use of nematode *Steinernema* sp. as a biological pest controlling agent offers more advantages, such as eco-friendly, high possibility of mass production (Georgis et al. 2006; Koppenhöfer and Kaya 2002; Bjorson and Schutte 2003), and highly tolerant of various kinds of chemical insecticides when applied together (Pino and Jove 2005; Elawad et al. 2007). In addition, nematodes actively seek for the targeted pest hosts Campbell and Lewis 2002), do not harm vertebrates (Ehlers 2003), and can be applied with a standard applicator, which is generally used for chemical pesticides (Wright et al. 2005).

A nematode mass production technique is required to support the usage of *S. carpocapsae* as a biological agent. The production of *S. carpocapsae* can be both *in vitro* and *in vivo*. The *in vitro* production is done by an artificial diet, while the *in vivo* production is done through insect hosts. *In vitro* production depends on specialized knowledge, modern technology availability, and complex materials (McMullen and Stock 2014).

In vivo production offers some benefits, which include the requirement of simple production tools and technologies, easy to be applied, and it produces higher quality nematodes (Grewal dan Georgis 1999). The *in vivo* production of *S. carpocapsae* solely depends on the availability of inexpensive pest hosts that could massively produce quality *S. carpocapsae* (Hazir et al. 2001).

Spodoptera litura (Lepidoptera: Noctuidae) and *Tenebrio molitor* L. (Coleoptera: Phylaridae) can be used as alternative pest hosts for some benefits, such as they can be used to produce various kinds of strain *Steinernema* spp., require easy maintenance and low maintenance cost, and able to massively produce IJ (Hazir et al. 2003; Gaugler and Han 2002). The *in vivo* production of *S. carpocapsae* relies on the fitness between the strain *S. carpocapsae* and the pest host. Only particular strains of *S. carpocapsae* can be produced in one kind of pest host for

several types of nematodes have typical characteristics. *S. carpocapsae* offers many benefits.

This study focused on mass production of *S. carpocapsae* Asembagus on *T. molitor* and *S. litura* larvae. It is paramount to obtain information on its production ability in *T. molitor* and *S. litura* in the necessary number and length of IJ and with an effective concentration for the production of *S. carpocapsae* isolates.

MATERIALS AND METHODS

Mass rearing of insect hosts

Larvae of *S. litura* were collected from Karangploso Experimental Station, Malang, East Java. The larvae were allowed to feed on leaves of Chinese cabbage (*Brassica rapa* var. *chinensis*) in a plastic jar (1.5 kg) that was covered with gauze for ventilation under laboratory condition. They were kept until developed into pupae. The pupae were collected and placed inside of another plastic jar up to adult emergence. Male and female moths were allowed to mating and produce egg masses. The egg masses were kept for hatching. The newly hatching larvae were collected and reared on cabbage leaves to produce 6th instar. The last instar of F2 generation was used for mass production of the nematodes (Prabowo et al. 2016).

Larvae of *T. molitor* were collected from Ngenep Market, Malang, East Java. The larvae were allowed to feed on carrot in a plastic jar (1.5 kg) that was covered with gauze for ventilation under laboratory condition. They were kept until developed into pupae. The pupae were collected and placed inside of another plastic jar up to adult emergence. Male and female weevils were allowed to mating and produce egg masses. The egg masses were kept for hatching. The newly hatching larvae were collected and reared on carrot to produce last instar of *T. molitor*. The last instar of F2 generation was used for mass production of the nematodes.

Nematodes

The isolates used in this study was *S. carpocapsae* isolated as Asembagus, East Java, Indonesia, and this isolates were the collection of Indonesian Sweetener and Fiber Crops Research Institute. IJ identification based on morphology and body length based nematode identification by Adam and Nguyen (2002). Stage III juvenile infective (IJ) was used in this research. Nematodes were reproduced *in vivo* in *T. molitor*. IJ was recovered using white traps method and after being harvested the IJ was then stored in a refrigerator (5 - 15 °C). All the IJs used in this study were harvested two weeks prior to the study to maintain its viability. The nematodes were acclimated in room temperature for one day prior to its use.

Mass production of nematodes

The IJ concentrations used in the study are categorized into 100 IJ/larva, 200 IJ/larva, 300 IJ/larva, and 400 IJ/larva with 25 larvae involved in each repetition. Stage three juvenile infective (IJ) was used for production. The inoculation of IJ to insect larvae by using a filter paper

method. First, the filter paper was cut by the surface area of a 10 cm diameter Petri dish. Next, the filter paper was then put under the Petri dish. Finally, the Petri dish was incubated at room temperature. The observed parameters were total IJ production since the first until last harvest, the total harvest of IJ, length of IJ harvested since the first until last harvest, and the average length of IJ.

Statistical analysis

The observed parameters were then analyzed via variance analysis with SPSS package. When a significant F-value ($p < 0.05$) was found, differences between observed parameter were compared using The Duncan's Multiple Range Test (DMRT) 5% mean separation test.

RESULTS AND DISCUSSION

Harvested *S. carpocapsae* nematodes on *T. molitor* and *S. litura*

The IJ production in *T. molitor* and *S. litura* hosts show harvested IJ between 1.93×10^3 - 3.55×10^4 IJ/larva. Both *T. molitor* and *S. litura* showed the highest production rate on the 4th and 6th day of 1.60×10^3 and 3.22×10^4 IJ/larva. After the highest production rate has been reached, the production continuously reduced up to the 20th day. The lowest production in both host larvae was found on the 20th harvesting day of 0.13×10^2 IJ/larva. Such 20th-production was not suitable to be harvested since it would cost a higher IJ production cost in both hosts. Low productions of IJ in its early harvesting times may be attributed to the early stage of the first generation IJ that was newly

penetrated into the host, which was not yet developed. As can be seen on the next harvests, there was increasing production of IJ because the IJs had multiplied inside the hosts. There was a decrease on the harvested IJ after the fifth harvest since the nematodes had consumed all the nutrition inside the hosts in 2-8 days since it was penetrated into the hosts. Therefore, the nutrition had been reduced drastically after the 5th day, correlating the reduced production rate of nematodes. The reproduction rate and harvesting ability of nematodes are greatly affected by the type of the host and nematodes. The results of this research proved that the nematodes harvest rate decreases after the fifth day of penetration and that it is a feasible trend. According to Zaki et al. (2000), the highest *S. carpocapsae* harvest from *Bombyx mori* larvae of 3.1×10^4 could be seen on the twentieth harvesting day. As reported by Mahar et al. (2004), the productions of IJ *S. carpocapsae*, *S. abbasi*, *S. karii*, and *S. riobravisi* on the 45th day were 1.6×10^4 , 5.9×10^2 , 4.2×10^2 , and 4.4×10^2 , respectively whereas there was 10^4 IJ *Heterorhabditis marelatus* production. Shapiro-Ilan et al. (2008) stated that nematodes heavily depend on the nutrition contained in the insect hosts for their fitness, reproduction capability, and success in infecting the hosts. Hence, the harvest on the 20th day since the IJ investment did not show any nematodes because there was not any nutrition left so that IJ was unable to reproduce. The harvest tests conducted in every two days up to the twentieth day showed that the IJ on *T. molitor* and *S. litura* reached a maximum number on the tenth day, afterward the tenth day and the IJ would decrease afterward (as shown in Table 1 and 2).

Table 1. Harvested *S. carpocapsae* IJ inoculated with concentration with different concentrations using *Tenebrio molitor* as host

Concentration of IJ (JI/larvae)	Harvested IJ (IJ/larvae)									
	Days of IJ harvested									
	2	4	6	8	10	12	14	16	18	20
100	1.93×10^3 a	7.83×10^2 a	4.73×10^2 a	0.97×10^2 a	1.07×10^2 a	1.07×10^2 a	0.73×10^2 a	0.33×10^2 a	0.23×10^2 a	0a
200	2.06×10^3 a	1.21×10^3 ab	7.33×10^2 a	1.26×10^2 a	1.87×10^2 a	1.36×10^2 a	1.17×10^2 a	1.20×10^2 a	0.27×10^2 a	0a
300	2.39×10^3 ab	1.60×10^3 b	7.47×10^2 a	1.27×10^2 a	2.23×10^2 a	6.10×10^2 a	3.37×10^2 a	1.93×10^2 a	0.53×10^2 a	0.13×10^2 a
400	3.35×10^3 b	1.60×10^3 b	9.83×10^2 b	3.17×10^2 b	2.27×10^2 a	7.73×10^2 b	6.20×10^2 b	5.56×10^2 b	0.63×10^2 a	0.15×10^2 a

Note: values followed by the same letter in the same column are not significantly different according to DMRT with a 95% confidence level

Table 2. Harvested *S. carpocapsae* IJ with a concentration of 100, 200, 300, and 400 IJ/larvae on *S. litura*.

Concentration of IJ (JI/larvae)	Harvested IJ (IJ/larvae)									
	Days of IJ harvested									
	2	4	6	8	10	12	14	16	18	20
100	1.28×10^4 a	1.64×10^4 a	1.85×10^4 a	1.89×10^4 a	2.28×10^4 a	1.16×10^4 a	4.80×10^3 a	4.40×10^3 a	2.61×10^3 a	0 a
200	1.60×10^4 a	3.65×10^4 ab	2.05×10^4 a	2.24×10^4 a	2.00×10^4 a	1.23×10^4 a	7.47×10^4 a	7.47×10^3 a	3.20×10^3 a	0 a
300	2.93×10^4 ab	2.51×10^4 b	3.09×10^4 a	1.87×10^4 a	1.76×10^4 a	1.17×10^4 a	9.07×10^4 a	6.40×10^3 a	3.47×10^3 a	0 a
400	2.99×10^4 b	2.67×10^4 b	3.22×10^4 b	1.99×10^4 b	1.88×10^4 b	1.22×10^4 b	1.01×10^4 b	9.50×10^3 a	3.55×10^3 a	0 a

Note: values followed by the same letter in the same column are not significantly different according to DMRT with a 95% confidence level

Table 3. Total of harvested *S. carpocapsae* IJ with a concentration of 100, 200, 300, and 400 IJ/larvae on *T. molitor* and *S. litura*

Concentration of IJ (IJ/larvae)	Total of IJ harvested (IJ/larvae)	
	<i>T.molitor</i>	<i>S.litura</i>
100	3,62x10 ³ a	1,13x10 ⁴ a
200	4,72 x10 ³ a	1,46x10 ⁴ b
300	6,29x10 ³ a	1,52x10 ⁴ c
400	8,51x10 ³ a	1,94x10 ⁴ c

Note: values followed by the same letter in the same column are not significantly different according to DMRT with a 95% confidence level

The highest production of IJ on *T. molitor* and *S. litura* was found in 400 IJ/larva concentrations producing 8.51 x10³ and 1.94x10⁴ IJ/larva respectively. The production of IJ shows a positive correlation with the initial IJ concentrations. The 400 IJ/larva concentrations produced the highest IJ followed by the 300 IJ/larva, 200 IJ/larva, and 100 IJ/larva concentrations (as demonstrated in Table 3). It is possible since the more IJ that is given, the higher the possibility it would penetrate into the insect hosts. The invested IJ will reproduce 2-3 generations and with a high reproduction rate of IJ leads to a higher possibility for its next generations to multiple. Thus, a high IJ reproduction is more likely to be found in an insect host with a high IJ concentration than the one with a low IJ concentration. A high IJ concentration goes together with a high harvest.

The use of alternative hosts has been frequently practiced in producing *S. carpocapsae*. Alternative hosts such as *Spodoptera litura*, *Plutella xylostella*, *Crociodomia binotalis*, *Galleria mellonella*, and *Heliothis virescens* (Miles et al. 2000), are proven to be reliable for production. Nonetheless, *G. mellonella* is the most frequently suggested host for its compatibility with various types of nematodes and its ability to produce a large harvest (Shapiro-Ilan et al. 2002). However, *G. mellonella* is quite rare in Asia especially Indonesia. Therefore, the development of alternative hosts besides *G. mellonella* that can make a high production rate is essential. *S. abbasi* and *S. riobrave* production on *G. mellonella* produces 2.2x10⁵ IJ/larvae (Elawad et al. 1999). The *S. carpocapsae* isolates inoculation number 30, 43, and 45 on *T. molitor* produces 8.3x10⁵-1.2x10⁶; 9.6 x10⁵-1.3x10⁶; and 1.2x10⁶ - 1.9x10⁶ IJ/ gram biomass, respectively. On the other hand, *S. carpocapsae* isolates number 30, 43, and 45 that are inoculated on *T. rufivena* respectively produce 9.3x10⁵-1.4x10⁶; 1.1x10⁶-1.3x10⁶; and 1.3x10⁶-2.1x10⁶ IJ/ gram biomass (Prabowo 2007). When measured up to IJ production on *G. mellonella*, *T. molitor*, and *Tirathaba rufivena*, the production of *S. carpocapsae* Asembagus isolates IJ on *T. molitor* and *S. litura* has a potency to be higher although the harvest is still lower than other hosts.

Length of *S. carpocapsae* nematodes harvested the different hosts

The quantity and quality of the harvested nematodes present the quality of the hosts in producing insect

pathogens. The quality of the harvested nematodes can be observed based on the quantity of the produced nematodes. Moreover, the harvest quality is demonstrated by the length of the produced nematodes. The length of IJ is determined by several factors such as the insect host, the concentration of nematodes, and the total nematodes production. The nematodes productions via *T. molitor* and *S. litura* showed IJ nematodes in the length of approximately 325-986µm (Tables 4 and 5). Both *T. molitor* and *S. litura* exhibited their highest production rates on the 6th and 10th day after the treatment with IJ length 647.67 and 986.67 µm respectively.

The Asembagus nematode isolates that are produced through *T. molitor*, and *S. litura* is relatively similar in size (325-986 µm) compared to other types of nematodes such as *S. carpocapsae* (325-488µm), *S. abbasi* (496-579µm), *S. kariii* (876-982µm), *S. riobrave* (561-701µm) and *H. marelatus* (588-700µm). Chaerani et al. (2018), stated that *Steinernema* and *Heterorhabditis* in Indonesia have body length of infective juveniles of 360-762 µm and 386-599 µm respectively. IJ which produced in *T.molitor* and *S.litura* showed a good result, this is possible because *S. carpocapsae* Asembagus isolates hold an adequate food synthesis level on *T. molitor* and *S. litura* hosts so that its production ability is sufficient to support its development like the four other nematodes. It shows that the length of the *S. carpocapsae* produced in *T.molitor* and *S.litura* has met the requirement to be utilized as a biological pest controlling agent.

The nematodes harvest from *T. molitor*, and *S. litura* shows the best length of IJ on the concentration of 400 IJ/larva of 437.48µm and 591.42µm, respectively. The early IJ concentration that was invested shows a reducing trend in IJ length. The investment of 400 IJ/larva could produce the longest IJ. It was then followed by 300 IJ/larva, 200 IJ/larva, and 100 IJ/larva concentrations (Table 6). The more the IJ that is invested on the host, the higher the possibility of IJ to penetrate into the hosts. Hence, it led to a more efficient production of IJ that allows IJ production in better length. According to Hazir et al. (2001), host nutrition shall affect the length of IJ that is produced. Nematodes that can efficiently synthesize nutrition in the insect hosts will produce IJ with a good length.

In addition, the type of insect host will also affect the IJ length. *S. litura* produces IJ in a better length than *T. molitor* with a respective length of 398.02 µm and 417.92 µm. As stated by bt Hazir et al. (2001), the nutrition in the host will affect the length of the produced IJ. The length of IJ harvested from *S. litura* is greater than that harvested from *T. molitor*. It is made possible because *S. litura* has 17-27 % larger biomass when compared with *T. molitor*, which only has 3.5-6 % biomass. High biomass allows IJ to consume more nutrition. Hence, it gets enough nutrition to develop. Sufficient nutrition is correlated with the production of IJ in a better length.

Table 4. Body length of infected juveniles of harvested *Steinernema carpocapsae* inoculated with different concentrations on *T. molitor*.

Concentration of IJ (JI/larvae)	Length of IJ (µm)									
	Days of IJ harvested									
	2	4	6	8	10	12	14	16	18	20
100	488,00 a	653,33 a	761 c	640,83 c	986,67 c	876,67 c	349,00 c	387,33 c	334,67 c	336,67 c
200	475,67 a	661,33 a	675,67 ab	483,33 ab	803,33 ab	453,67 ab	474,67 ab	370,33 ab	325,00 ab	285,33 ab
300	408,33 a	553,00 a	767,67 ab	362,00 ab	510,00 ab	623,33 ab	546,67 ab	358,33 ab	343,33 ab	186,67 ab
400	470,00 a	403,33 a	556,67 a	501,33 a	465,67 a	479,67 a	517,33 a	344,17 a	327,67 a	113,33 a

Note: values followed by the same letter in the same column are not significantly different according to DMRT with a 95% confidence level

Table 5. Length IJ of harvested *S. carpocapsae* nematodes with a concentration of 100, 200, 300, and 400 IJ/larvae on *S. litura*.

Concentration of IJ (JI/larvae)	Length of IJ (µm)									
	Days of IJ harvested									
	2	4	6	8	10	12	14	16	18	20
100	408,33 a	514,00 a	647,67 b	362,00 a	411,67 a	446,00 a	390,67b	358,33d	346,67 a	0 a
200	417,33 a	529,67 a	641,00 ab	387,33 a	415,33 a	434,33 a	362,33ab	369,50cd	343,33 a	113,33 b
300	432,33 a	416,00 a	545,67 a	393,33 a	456,67 a	479,33 a	361,67ab	358,50bc	333,33 a	113,33 b
400	467,33 a	403,33 a	556,67 a	501,33 a	472,00 a	461,33 a	333,00a	344,17a	327,67 a	113,33 b

Note: values followed by the same letter in the same column are not significantly different according to DMRT with a 95% confidence level

Table 6. Average of Length IJ of harvested *S. carpocapsae* nematodes with a concentration of 100, 200, 300, and 400 IJ/larvae on *T. molitor* and *S. litura*

Concentration of IJ (JI/larvae)	Length of IJ (µm)	
	<i>T.molitor</i>	<i>S.litura</i>
100	437,68 a	591,42 b
200	431,33 a	500,83 ab
300	401,17 a	465,93 a
400	398,02 a	417,92 a

Note: values followed by the same letter in the same column are not significantly different according to DMRT with a 95% confidence level

The production of *S. carpocapsae* with concentrations of 100, 200, 300 and 400 IJ/larvae on *T. molitor* and *S. litura* showed 3.62×10^3 ; 6.29×10^3 ; 1.13×10^4 ; 1.52×10^4 ; 1.13×10^4 ; 1.46×10^4 ; 1.54×10^4 ; and 1.94×10^4 IJ/larvae, respectively. The highest production of IJ on *T. molitor* and *S. litura* was found in 400 IJ/larva concentrations producing 8.51×10^3 and 1.94×10^4 IJ/larva respectively. The IJ harvest from *T. molitor* and *S. litura* shows the longest body length for IJ at a concentration of 400 IJ/larva of 437.48µm and 591.42µm, respectively. The production of *S. carpocapsae* with a concentration of 400 IJ / larvae on *T. molitor* and *S. litura* showed the highest total yield and body length of IJ. According to the harvest parameter and IJ length, the use of alternative hosts such as *T. molitor* and *S. litura* is relevant to boost the production of *S. carpocapsae* Asembagus isolates. Nevertheless, the production of *S. carpocapsae* via *T. molitor* is considered more efficient due to the availability of *T.molitor* in the market.

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