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Research Article
Biological Activity of Local Entomopathogenic Nematodes from Two Different Origins Based on Various Temperatures

Priyantini Widiyaningrum, Laily Fauziyah and Dyah Rini Indriyanti

Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, D6 Building 1st Floor, Sekaran Street, Gunungpati, 50229 Semarang, Indonesia

Abstract

Background and Objective: Biological activity such as reproduction rate, viability and pathogenicity of local entomopathogenic nematodes (EPNs) are influenced by ecological factors, one of them is temperature. In order to prepare the biopesticides product, the study aimed to compare their production rate, viability and pathogenicity of EPNs from Semarang isolate and commercial biopesticide under various temperatures. **Materials and Methods:** This study was designed by Completely Randomized Design one-way classification. Both EPNs were cultured *in vivo* condition with *Tenebrio molitor* larvae by white trap method. The reproduction rate of nematodes was calculated after 8 days of incubation and its viability test at six storage temperatures. The EPNs with the best viability was further tested for its pathogenicity on *Macrotermes* sp. The reproduction rate of nematodes were analyzed by using student's t-test, while one-way ANOVA was used to analyze the viability of EPNs, and pathogenicity of lethal dose value was calculated using Probit analysis. **Results:** The findings showed that the reproduction rate on EPNs from Semarang isolate was significantly higher ($p<0.05$) than EPNs from commercial biopesticide. Various temperatures significantly affected the viability on both nematodes, but under LSD test ($p<0.05$) revealed that viability at storage of 21, 24 and 27°C were not different of each other. **Conclusion:** The reproduction rate of EPNs from Semarang isolate was 35% higher than those originated from commercial biopesticide. The optimum viability of both EPNs was obtained at temperature ranged from 21-27°C. The LD50value of EPNs from Semarang isolate was better than nematodes originated from commercial biopesticide.

Key words: Biological activity, commercial biopesticide, local entomopathogenic nematode, reproduction rate, viability test

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Corresponding Author: Priyantini Widiyaningrum, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, D6 Building 1st Floor, Sekaran Street, Gunungpati, 50229 Semarang, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Entomopathogenic Nematodes (EPNs) from the genus of Steinernema (Rhabditida: Steinernematidae) and Heterorhabditis (Rhabditida: Heterorhabditidae) are known as potential biological control agents and have been widely used in various countries[1]. Many researchers in Indonesia have been succeeding in performing *in vivo* isolation of local EPNs with insect larvae, such as: isolates of Jember[1], Madurese[2], Pelabuhan Ratu[2], Malang[3] and Timorisland[4]. Many local isolates of EPNs has been explored and identified. Different methodologies were used to develop biopesticides with EPNs still limit the quality of the final product, reducing field efficacy and complicating application strategies[5]. Numbers of studies have revealed that Infective juveniles (IJs) of different EPNs differ in their ecological and behavioural traits with regard to their persistence and survival in the soil. Ecological character of each local isolate EPNs which tends to be different depends according to the geographical condition and the habitat[6-9] limits their distribution area. Thus, viability and pathogenicity of EPNs are highly influenced by many factors, include their abiotic environment, storage method and formulation[10]. Generally, mass cultivation of EPNs under *in vitro* condition tends to change their reproductive and morphometric character[11,12].

In order to develop the local EPNs of Semarang into the commercial biopesticide, there is a need to evaluate the ecological characteristics details of local EPNs, especially suitable temperature for their reproduction ability, viability and pathogenicity. This information is important to provide a significant assistance in studying local EPNs to become a mass-produced biopesticides by adjusting the environmental conditions. Genus Steinernema is a local EPN from Semarang that was recently explored and identified[13]. Indriyanti *et al.*[14] stated that the abundance of *Steinernema* sp. Infective juveniles (IJs) of Semarang isolates has been found in manure soil, i.e., 67.411×10^3 IJs/mL, with a clumped distribution pattern. These EPNs is potentially because it has been shown to proliferate in artificial media[15,16]. On the other hand, the commercial product of EPNs in the liquid media already can be found in local market. However, the quality control is questionable. Therefore, the same observation was done on commercial product of EPNs in a package from the local market. The study aimed to compare the reproduction rate, viability and pathogenicity of EPNs from different origins.

MATERIALS AND METHODS

This research was conducted in Biology Laboratory of Mathematics and Natural Sciences Faculty, Universitas Negeri Semarang, Indonesia. Local EPNs (*Steirnernema* sp.) was isolated from soil sample of the poultry farmin Gunungpati, Semarang and had been identified[17] to the level of genus by visual observation and morphological characterization as suggested by Nguyen[18] and Stock *et al.*[19]. The control sample consisted of the nematodes originated from the commercial pesticide 'Coleonema' based on the *Steinernema* spp. and used for control of insect pests of estate crop. Subterranean termites *Macrotermes* sp. employed in the pathogenicity test were obtained from the soil in the garden of Universitas Negeri Semarang.

Both of EPNs were cultured under *in vivo* condition with larvae of *Tenebriomolitor* (Coleoptera: Tenebrionidae) by filter paper in petridish inoculation method to reproduce IJs[20] at room temperature (28±1°C).As supporting data, soil temperature where the sample taken was measured by a digital thermometer and was repeated five times from different points.

Reproduction rate of EPNs: Petridish with diameter of 9 cm were put inversely on the bigger one (15 cm diameter). Filter paper then was put on the smaller petridish, while 20 mL of distilled water was poured into the bigger one till the filter paper touch the liquid surround. A gram of *Tenebriomolitor* (10-12 larvae) was put on the small, then it was inoculated with 5 mL suspension of 500 IJs. Larvae were intentionally infected (dead), then they were incubated in a dark room. Filter paper inoculation on both isolates was done in five replications. In this research, IJs were only harvested on day 8 after incubation. All suspension in the petridish then was filtered by Whatman number 1 and the volumes were measured. The reproduction rate of EPNs was observed with sampling method by counting dish and hand counter. Living IJs showed S pattern movement and J pattern when rest and mostly straight like a needle if it was dead[21,22]. The difference of IJs between two isolates was analyzed by Student's t-test.

Preparation of viability test: EPNs viability test at several temperatures was designed by Completely Randomized Design (CRD) one-way classification with an initial density of 2000 Ijs/mL was poured on petridish with 9 cm diameter with temperature treatments of 15, 18, 21, 24, 27 and 30°C. About 5 mL IJs suspension with aninitial density of 2000 IJs/mL was poured on petridish with 9 cm diameter then covered. Each treatment was done in five replications. All treatment was then incubated in dark room with the defined temperature. After 24 h of incubation, the viability of IJs was observed by the following formula.

$$\text{Viability}(\%) = \frac{\text{Number of life IJs after treatments}(24\text{h})}{\text{Number of life IJs before treatments}} \times 100$$

Pathogenicity test of EPNs on *Macrotermes* sp.: IJs of nematodes with the best viability were employed to examine the pathogenicity test on *Macrotermes* sp. The indicator of pathogenicity was observed based on the percentage of dead termites after 24 and 48 h of treatments. The test was done in five levels of dosage (0, 50, 250, 500, 750 and 1000 IJs/mL), each dosage was repeated five times. The media test used was *Polyethylene* plastic cups (7 cm diameter, 10 cm height) filled with 25 g of sterile sandy soil and 25 mL of distilled water to reach the field humidity. Each cup was filled with 50 worker termites and small pieces of board paper (2 g) as its hideout. All cups were incubated in dark condition at room temperature (28±1°C). Termite's mortality was observed after 24 and 48 h of incubation LC_{90} was observed by Probit analysis[23]. The LC_{90} is a concentration of EPNs which statistically can affect subterranean termite's mortality significantly by 90%. If the mortality in control group reach 5-20%, the data would be revised before it was analyzed with Abbot formula[24]:

$$\text{Corrected mortality}(\%) = \left[\frac{X-Y}{100-Y}\right] \times 100$$

Where:
X = Percentage of mortality in the treated samples
Y = Percentage of mortality in control sample

Statistical analysis: Data of viability from both local EPNs isolate was analyzed by one-way ANOVA and followed by LSD ($p<0.05$) if there was an effect of difference caused by the treatment. Difference test of optimum viability from both isolates was also employed.

RESULT AND DISCUSSION

The result of difference test on EPNs from both local isolate and commercial biopesticides were presented in Table 1.

The results indicated that EPNs from local isolate have a better ability to adapt to the environment that the one from the commercial product. It was assumed that each nematode has different ability to adapt in particular environment. When EPNs of commercial product was cultured in *in vivo* medium, they must adapt. Otherwise, EPNs isolated from soil in Semarang was still can reproduce well as in their natural environment. This is in line with those concluded that nematode isolated from nature has higher reproduction rate in the host than another isolate[12]. On the other hand, EPNs from commercial biopesticide were mostly cultivated in *in vitro* condition and tend to change their morphometric character[11,12]. Chaerani[11] found that length and width of IJs of *Steinernema* sp. which was cultured *in vitro* condition were shorter than *Steinernema* sp. grown *in vivo* condition with *Tenebriomolitor* larvae. Otherwise, Matuska-Lyzwa[12] proved that nematodes isolated from the soil and nematodes from the biopesticide were consistent with the standard sizes of species given in the references[25] and there were no significant differences by Nikdel *et al.*[26] they also showed that the morphological features of *S. kraussei* isolates collected from the natural environment were compatible with typical species.

Viability test of EPNs: Table 2 showed that viability of EPNs from Semarang isolate and commercial biopesticide isolate tend to increase along with the increase of storage temperature, then slightly fall when reaching 30°C. The highest viability in both EPNs was found at a temperature of 24°C. The result of the statistical analysis revealed that the temperature difference was significantly different ($p<0.05$) to viability.

At low temperatures (15 and 18°C) and the highest temperature (30°C), viability average was not significantly different (LSD, $p<0.05$), as well as at temperature of 21, 24 and 27°C. It happened in both EPNs isolate. The research of the local isolate of *Steinernema* sp. in some countries, such as India[27], *Steinernema* sp. appeared to be best adapted to temperatures between 15 and 30°C with an optimum temperature range of 25-30°C. This phenomenon strengthens the evidence that *Steinernema* sp. tolerates the varying

Table 1: Result of difference test on EPNs from local isolate and commercial biopesticides

	Resource of EPNs	
Parameters measured	*Steinernema* sp. from Semarang isolates	*Steinernema* sp. originated from the commercial biopesticide
-Reproduction rate (Ijs mL^{-1})	15.244×10^{3a}	11.252×10^{3b}
-Maximum viability of IJs (%)	77.99^{ns}	74.02^{ns}
-Optimum storage temperature (°C)	21-27	21-27

Notes: Indexes ^{a,b}indicate statistically significant differences between data in both columns, Indexes ^{ns}indicate statistically non significant differences(t-test, $p<0.05$)

Table 2: Viability (%) of EPN from Semarang isolate and commercial biopesticide after 24 h of treatment

	IJs viability average (%)	
Storage temperature (°C)	EPNs from Semarang isolate	EPNs from commercial biopesticide
15	61.77[a]	56.43[a]
18	61.82[a]	58.31[a]
21	73.14[b]	68.94[b]
24	77.99[b]	74.02[b]
27	77.77[b]	73.79[b]
30	49.85[a]	60.10[a]

Different letter in the same printed column shows a significant difference at 5% significance level, based on LSD posthoc test

Table 3: Mortality of *Macrotermes* sp. on various IJs dosage after 24 and 48 h of treatment

		Mortality (%)*	
EPNs	dosage (Ijs mL^{-1})	24 h	48 h
Semarang isolate	0	0	0
	50	20.25	35.84[a]
	250	28.27	81.86[b]
	500	38.82	82.30[b]
	750	40.51	83.63[b]
	1000	38.82	91.96[c]
Commercial biopesticide	0	0	0
	50	20.60	38.25[a]
	250	36.14	64.06[b]
	500	41.20	81.57[c]
	750	41.63	84.79[c]
	1000	40.77	94.33[d]

Different letter following the average of each isolate, shows a significant difference (LSD test, $\alpha<0.05$), *Mortality data was corrected by Abbot formula[24]

temperature in the different area among countries, depending on the ecological characteristics of each region.

The observation of soil temperature in where the sample was taken (depth of ±20 cm) showed an average temperature between 24-26°C. Viability test showed that EPNs tended to need the same temperature with original habitat to survive. Chen *et al.*[28] stated that one of the ecological characters affects the viability of EPNs is environmental temperature. In Indonesia, local isolates of EPNs, such as Madura, Jember and Tulungagung have adverse temperature range (25-30°C)[1,29]. The relationship between both the temperatures and wet and dry conditions was highly significant especially its effect of temperature on emergence of EPNs[30].

Ali *et al.*[31] stated that the viability and infectivity of local nematode decrease with the raise of temperature. The difference between environmental temperature and adaptation ability cause EPNs biopesticide from local isolate have a limited marketing place, only in areas that have similar ecological conditions and environmental temperature range. Shapiro-Ilan *et al.*[32] stated that EPNs could adapt to soil with proper moisture and temperature. The survival of EPNs depends on the adequacy of water in and around their bodies[9]. Research by Mejia-Torres and Saenz[7] showed that low environmental temperatures lowered the mobility and persistence of infective juveniles.

Pathogenicity test of EPNs on *Macrotermes* sp.: The EPNs pathogenic analysis was performed through a bioassay test to determine its ability to kill termites at least by 50% (LC_{50}) to 90% (LC_{90}). The mortality data were recorded after 24 and 48 h of treatment. At 24 h observation, no mortality of soil termites has reached 50% in all treatments. After 48 h of treatment, termite mortality over 50% was found in the concentrations of 250, 500, 750 and 1000 Ijs/mL (Table 3).The difference in IJs dose showed significant effect on the mortality of termites, but indicated that the higher levels of IJs doesn't lead to higher termites mortality.

At 24 h of observation, termites mortality was still below 50% in all treatments, which means that LD_{50} EPNs isolate could not be achieved within 24 h, but after 48 h. The Probit analysis showed that LD_{50} for EPNs from Semarang isolate reached 212 IJs/mL, while EPNs from commercial biopesticide reached 326 Ijs/mL. This data indicated that EPNs originated from the natural environments were more pathogenic than EPNs from commercial biopesticide. The ability of EPNs to kill insects is influenced by many factors. Storage methods, EPNs formulations and short shelf life greatly affect viability and infectivity[10]. From the genetic aspect, the genus of Steinernema is ambushing (silent, waiting) when it will attack the insects. It is in contrast to the more aggressive nature of the genus Heterorhabditis in search of prey[23]. The behavior in which termite workers have high mobility properties and the ambushing Steinernema are thought to be one of the reasons why LD_{50} is slower, i.e., 48 h after treatment. Once infected, the increased mortality become faster because of the nature of trophallaxis in termites leading to the spread and penetration of IJs more effectively. Sucipto[33] stated that the higher concentrations of IJs could result in increased competition among individual of IJs regarding the space and food. Brown *et al.*[34] mentioned the role of bacteria and toxins that study together in insect bodies causing EPNs to have the ability to kill very quickly. Insects infected by *Steirnernema* sp. will die within 24-48 h after the infection. Symbional bacteria that live in the digestive tract of nematodes under dormant conditions[35].

CONCLUSION

The reproduction rate of EPNs collected from Semarang local isolate is 35% higher than the reproduction rate of EPNs originated from commercial pesticide. The optimum viability

of both EPN is at the storage temperature of 21-27°C. The pathogenicity of EPNs from Semarang isolate is better than EPNs originated from commercial biopesticide.

SIGNIFICANCE STATEMENT

The study discovered that EPNs from local isolates is better than the commercial isolate in reproduction and pathogenicity, so, they could be used as a better alternate of pesticides than the commercial one. Thus, awareness of detailed characteristics of entomopathogenic nematodes may provide a significant assistance to researchers in studying the biopesticides and their mechanism that will be better adjusted to particular conditions of the environment.

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REFERENCES

1. Nugrohorini, 2010. Eksplorasi nematoda entomopatogen pada beberapa wilayah di jawa timur. J. Pertanian Mapeta, 12: 72-144.
2. Chaerani, Y.S., T.P. Priyatno, D. Koswanudin, U. Rahmat, Sujatmo, Yusuf and C.T. Griffin, 2007. Isolasi nematoda patogen serangga steinernema dan heterorhabditis. J. Hama Penyakit Tumbuhan Tropika, 7: 1-9.
3. Afifah, L., B.T. Rahardjo and H. Tarno, 2013. Eksplorasi nematoda entomopatogen pada lahan tanaman jagung, kedelai dan kubis di malang serta virulensinya terhadap *Spodoptera litura* Fabricius. J. HPT., 1: 1-9.
4. Santoso, T., J.A. Bunga and Supramana, 2009. Bioefficacy of entomopathogenic nematode, *Steinernema* sp. isolated from timor island as bioinsecticide on sweet potato weevil, *Cylas formicarius* (Fabr.) (Coleoptera: Brentidae). J. Biopesticides, 2: 87-91.
5. Cruz-Martinez H., J. Ruiz-Vega, P.T. Matadamas-Ortiz, C.I. Cortes-Martinez and J. Rosas-Diaz, 2017. Formulation of entomopathogenic nematodes for crop pest control-A review. Plant Protect. Sci., 53: 15-24.
6. Hussaini, S.S., 2017. Entomopathogenic Nematodes: Ecology, Diversity and Geographical Distribution. In: Biocontrol Agents: Entomopathogenic and Slug Parasitic Nematodes, Abd-Elgawad, M.M.M., T.H. Askary and J. Coupland (Eds.). CAB International, Boston, USA., pp: 88-142.
7. Mejia-Torres, M.C. and A. Saenz, 2013. Ecological characterisation of the Colombian entomopathogenic nematode *Heterorhabditis* sp. SL0708. Braz. J. Biol., 73: 239-243.
8. Mracek, Z., S. Becvar, P. Kindlmann and J. Jersakova, 2005. Habitat preference for entomopathogenic nematodes, their insect hosts and new faunistic records for the Czech Republic. Biol. Control, 34: 27-37.
9. Chen, S. and I. Glazer, 2005. A novel method for long-term storage of the entomopathogenic nematode *Steinernema feltiae* at room temperature. Biol. Control, 32: 104-110.
10. Hussein, M.A. and M.A. Abdel-Aty, 2012. Formulation of two native entomopathogenic nematodes at room temperature. J. Biopesticides, 5: 23-27.
11. Chaerani, 2011. Pembiakan nematoda patogen serangga (Rhabditida: *Heterorhabditis* dan *Steinernema*) pada media semi padat. J. HPT Tropika, 11: 69-77.
12. Matuska-Lyzwa, J., 2014. Ecological and morphological characteristics of *Steinernema kraussei* (Rhabditida: Steinernematidae): Comparison of nematodes isolated from the natural environments and originated from the commercial pesticide. Ecol. Questions, 19: 51-55.
13. Widyaningrum, P., N. Subekti and B. Priyono, 2016. Uji patogenitas nematoda entomopatogen isolat semarang *Steinernema* sp. pada rayap tanah *Macrotermes* sp. Prosiding Semnas Hasil Penelitian Inovasi IPTEKS Perguruan Tinggiuntuk Meningkatkan Kesejahteraan Masyarakat, Agustus 29-30, 2016, LP2M UNMAS Denpasar, pp: 178-182.
14. Indriyanti, D.R., A.D.H. Pribasari, D. Puspitarini and P. Widiyaningrum, 2014. Kelimpahan dan pola penyebaran nematoda entomopatogen sebagai agensia pengendali serangga hama pada berbagai lahan di semarang. J. Lahan Suboptimal, 3: 55-61.
15. Indriyanti, D.R. and N.L. Muharromah, 2016. Mass cultivation of entomopathogenic nematode in artificial media. Biosaintifika: J. Biol. Biol. Educ., 8: 111-118.
16. Widiyaningrum, P., N. Subekti and B. Priyono, 2017. Efektivitas tepung serangga sebagai media pembiakan nematoda entomopatogen isolat semarang (*Steinernema* sp.). Proceedings of the National Conference and Congress of Himpunan Pendidik dan Peneliti Biologi Indonesia, (HPPBI'17), Universitas Mataram, Lombok, Indonesia, pp: 112-117.
17. Widiyaningrum, P., A.B. Satria and S. Ngabekti, 2017. Viability of two local entomopathogenic nematodes on various pH media and their pathogenicity test against *Macrotermes* sp. Proceedings of the Asia International Multidisciplinary Conference, May 1-2, 2017, Universiti Teknologi Malaysia, Johor Bahru, Malaysia, pp: 24-25.

18. Nguyen, K.B., 2007. Methodology, Morphology and Identification. In: Entomopathogenic Nematodes: Systematics, Phylogeny and Bacterial Symbionts, Nguyen, K.B. and D.J. Hunt (Eds.). Nematology Monographs and Perspectives 5, Brill, Leiden, The Netherlands, ISBN-10: 90-04-15293-8, pp: 59-119.
19. Stock, S.P., C.T. Griffin and R. Chaerani, 2004. Morphological and molecular characterisation of *Steinernema hermaphroditum* n. sp. (Nematoda: Steinernematidae), an entomopathogenic nematode from Indonesia and its phylogenetic relationships with other members of the genus. Nematology, 6: 401-412.
20. Woodring, J.L. and H.K. Kaya, 1988. Steinernematid and heterorhabditid nematodes: A handbook of biology and techniques. Southern Cooperative Series Bulletin 331. Arkansas Agricultural Experiment Station, Fayetteville, AR.
21. Georgis, R. and R. Gaugler, 1991. Predictability in biological control using entomopathogenic nematodes. J. Econ. Entomol., 84: 713-720.
22. Laznik, Z. and S. Trdan, 2014. The influence of insecticides on the viability of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) under laboratory conditions. Pest Manage. Sci., 70: 784-789.
23. Finney, D.J., 1971. Probit Analysis. 3rd Edn., Cambridge University Press, Cambridge, London.
24. Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
25. Mracek, Z., 1994. *Steinernema kraussei* (Steiner, 1923) (Nematoda: Rhabditida: Steinernematidae): Redescription of its topotype from Westphalia. Folia Parasitol., 41: 59-64.
26. Nikdel, M., G. Niknam and N.E. Kary, 2011. First report of an entomopathogenic nematode, *Steinernema kraussei* (Rhabditida, Steinernematidae) from Iran. J. Entomol. Soc. Iran, 30: 39-51.
27. Lalramnghaki, H.C., Vanlalhlimpuia, Vanramliana and Lalramliana, 2016. Effect of temperature on the infectivity of entomopathogenic nematodes (Steinernematidae and Heterorhaditidae) isolated from Mizoram, Northeastern India. Sci. Vision, 16: 21-26.
28. Chen, S., J. Li, X. Han and M. Moens, 2003. Effect of temperature on the pathogenicity of entomopathogenic nematodes (*Steinernema* and *Heterorhabditis* spp.) to *Delia radicum*. BioControl, 48: 713-724.
29. Djunaedy, A., D.J.A. Fak and P. Unijoyo, 2009. Studi karakter ekologi nematoda entomopatogen heterorhabditis isolat lokal madura. J. Embryo, 6: 1-12.
30. Rahoo, A.M., T. Mukhtar, S.R. Gowen, B. Pembroke and M.A. Rahu, 2016. Emergence of *Steinernema feltiae* from infected *Galleria mellonella* cadavers in moist and dry conditions. Pak. J. Nematol., 34: 81-86.
31. Ali, S.S., R. Pervez, M.A. Hussain and R. Ahmad, 2007. Effect of temperature on survival of *Steinernema seemae*, *S. masoodi* and *S. carpocapsae* (Rhabditida: Steinernematidae) and their subsequent infectivity to prepupa of *Helicoverpa armigera* (Hubner). Arch. Phytopathol. Plant Prot., 40: 183-187.
32. Shapiro-Ilan, D.I., R. Han and C. Dolinksi, 2012. Entomopathogenic nematode production and application technology. J. Nematol., 44: 206-217.
33. Sucipto, 2008. Nematoda entomopatogen heterorhabditis isolat lokal madura sebagai pengendalian hayati hama penting tanaman hortikultura yang ramah pada lingkungan. Agrovigor, 2: 47-53.
34. Brown, S.E., A.T. Cao, P. Dobson, E.R. Hines, R.J. Akhurst and P.D. East, 2006. Txp40, a ubiquitous insecticidal toxin protein from *Xenorhabdus* and *Photorhabdus bacteria*. Applied Environ. Microbiol., 72: 1653-1662.
35. Wiratno and Rohimatun, 2012. Patogenisitas nematoda *Heterorhabditis* sp. terhadap kumbang daun kelapa *Brontispa longissima* gestro. J. Littri, 18: 137-142.