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Temperature and dose-size effects on infectivity and reproduction of entomopathogenic nematode, *Steinernema longicaudum* Gongju Strain

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Abstract : Effects of temperature and dose-size on infectivity and reproduction of Korean entomopathogenic nematode, *Steinernema longicaudum* Gongju strain were examined. The greater wax moth, *Galleria mellonella* larvae were exposed to 5, 10, 20, 40, 80, and 160 infective juveniles/larva in 60×15 ㎜ petri dishes and kept in 13℃, 18℃, 24℃, and 30℃ incubators. Each petri dish contained one larva weighed from 180 to 200 ㎎. Infectivity was observed everyday for 14 days and reproduction for 30 days. The infectivity of *S. longicaudum* was more influenced by temperature than by dose-size. Mortalities by *S. longicaudum* were lower at 13℃ at all concentrations but higher at 24℃ and 30℃ even at lower concentrations, 5 or 10 infective juveniles/larva. Lethal time was also shorter with increasing temperature and dosages. All host larvae died at 24℃ and 30℃ in 2 days at the rate of 160 infective juveniles per host while 83.3% of tested larvae died at 24℃ in 10 days and 90% at 30℃ in 6 days at the rate of 5 infective juveniles. Reproduction was also better with increasing temperature and dosages. The highest number of progenies was obtained at 30℃ in 6 days at the rate of 80 infective juveniles. However, progenies were not produced from cadavers at 13℃. Reproductive period was the shortest at 30℃ of all temperatures by 6 to 9 days. The results indicated that optimum temperatures for infectivity was 24℃ and 30℃ for reproduction.(Received March 13, 1999; accepted July 22, 1999)

Key words : entomopathogenic nematode, infective juveniles, infectivity, reproduction, *Steinernema longicaudum*, progeny.

Introduction

Entomopathogenic nematodes are lethal obligatory parasites of a broad range of insects (Stuart *et al.* 1998). These nematodes have worldwide distribution and comprise two genera, *Steinernema* in Steinernematidae and *Heterorhabditis* in Heterorhabditidae. *S. carpocapsae, S. glaseri, S. monticola,* and *H. bacteriophora* have been found in Korea by an extensive survey of entomopathogenic nematodes (Choo *et al.* 1995, Stock *et al.* 1997a, Stock *et al.* 1997b). These beneficial nematodes have been being surveyed to document effective Korean entomopathogenic nematodes against domestic insect pests. Meanwhile, *S. longicaudum* was isolated from soil samples of zoysiagrass at riparian in Gongju, Chungnam province (Stock *et al.* 1999). The sampling site contained white grubs of *Anomala cuprea* at the sampling time. Although the nematodes were effective against white grubs (Choo, unpublished data), study on virulence and reproduction needed to broaden the spectrum of this nematode in insect pest control. Although

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several factors have associated with nematode infectivity and reproduction, temperature and dosage of nematodes are important factors influencing on infection, development and reproduction (Milstead 1981, Molyneux 1985, 1986, Dunphy and Webster 1986, Mason and Hominick 1995, Henneberry *et al.* 1996). Thus, this study was conducted to clarify the effects of temperature and dose-size on infectivity and reproduction of Korean new isolate of entomopathogenic nematode, *S. longicaudum*.

Materials and Methods

Nematode source

S. longicaudum was isolated from soil samples collected in Gongju, Chungnam province, middle part of Korea. Soil samples were taken from 2~4 ㎡ with 5 subsamples at zoysiagrass in riparian sandy area. The white grubs of *A. cuprea* were distributed in the collecting site. The subsamples were combined resulting in ca. 800 mL of soil which was placed in a plastic bag. The soil was thoroughly mixed in the bag and a 250 mL subsample of soil was removed and placed into a 300 mL plastic container (Choo *et al.*, 1995). Six last instar *Galleria mellonella* larvae were added to each container and all containers were stored at room temperature (25±3℃) for 1 week. Dead larvae from each container were set up in a White trap (Woodring and Kaya, 1988) to collect emerging infective juveniles(Ijs). Nematodes isolated from traps were propagated in the last instar of *G. mellonella* larvae again. The adults and juveniles from cadavers were used for the identification of species. In addition, infective juveniles harvested from White traps were stored in sterilized distilled water at 10℃ for 5~21 days before use.

Temperature and dose-size effects on infectivity

Infective juveniles of *S. longicaudum* were inoculated into inverted 60×15 mm petri dishes containing moistened filter paper at the concentration of 5, 10, 20, 40, 80, and 160 Ijs/0.5 mL and one last instar of *G. mellonella* larva (180~200 mg) was placed in each dish. Control insects were treated with 0.5 mL of sterilized distilled water. The petri dishes were kept in 13℃, 18℃, 24℃, and 30℃ incubators and checked nematode infection everyday for 2 weeks. There were three replicates with 10 petri dishes per replicate for each concentration and each temperature.

Temperature and dose-size effects on reproduction

Reproduction of *S. longicaudum* in *G. mellonella* larvae was assessed. One cadaver infected by nematodes from each concentration or temperature was trapped in 100×15 mm petri dishes (Woodring and Kaya, 1988). The petri dishes were kept in 13℃, 18℃, 24℃, and 30℃ incubators again and daily produced progenies were checked everyday for 30 days.

Table 1. Effect of dose-size on mortality of *Galleria mellonella* larvae at various temperatures

Concentration (Ijs)	% Mortality[a] ±SD			
	13℃	18℃	24℃	30℃
5	10±10 Ba[b]	30±20 Bc	83.3±20.8 Aa	90±10 Aa
10	20±20 Ca	60±10 Bb	100±0 Aa	93.3±11.5 Aa
20	23.3±25.2 Ba	80±26.5 Aab	100±0 Aa	100±0 Aa
40	23.3±25.2 Ba	90±10 Aab	100±0 Aa	100±0 Aa
80	30±10 Ba	100±0 Aa	100±0 Aa	100±0 Aa
160	56.7±35.1 Aa	100±0 Aa	100±0 Aa	100±0 Aa

[a]Data obtained at everyday for 2 weeks. Data are averages of 3 replications ; in each, 10 larvae were assayed.

[b]Means within a column followed by different uppercase letters are significantly different; means within a row followed by different lowercase letters are significantly different (P<0.05; Student-Newman-Keuls test).

Statistical analysis

Relationship of temperature-time mortality data depending on concentration was analyzed using probit analysis. Infectivity and progeny production data were analyzed using Student-Newman-Keul test(ANOVA) (SAS Institute, 1996).

Results

Temperature and dose-size effects on infectivity

Infectivity of *S. longicaudum* was more influenced by temperature than by dose-size (Table 1). Mortalities by *S. longicaudum* were lower at 13℃ at all concentrations but higher at 24℃ and 30℃ even at low concentration, 5 or 10 Ijs/larva. High dosages of nematodes were needed to cause somewhat high mortality at lower temperatures. For example, larval mortalities at 13℃ were ranged from 10% to 30% at the concentrations of 5~80 Ijs/larva while 56.7% at the rate of 160 Ijs. At least 40 Ijs were needed to achieve 90% mortality at 1 8℃. However, 5 Ijs caused 83.3% mortality at 24℃ and 90% at 30℃. In addition, 10 Ijs were enough to obtain over 90% mortality at 24℃ or at 30℃.

Dosages and temperatures significantly influenced lethal time as well (Fig. 1A~F). The lethal time was shorter with increasing dosages. This was more distinctive at higher temperatures. One hundred mortality occurred at 24℃ and 30℃ in 2 days compared with 7 days at 18℃ when 160 Ijs were inoculated (Fig. 1F). On the contrary, 90% mortality was achieved at 30℃ in 6 days while 73.3% mortality was obtained at 24℃ in 10 days, 30% at 18℃ in 12 days, and 10% at 13℃ in 14 days at the concentration of 5 Ijs/larva (Fig. 1A).

Initial death also occurred later at lower temperatures than at higher temperatures at all concentrations. Differences in lethal time might be resulted from the pathogenicity of *S. longicaudum* depending on temperature. The LC_{50} of *G. mellonella* larvae for *S. longicaudum* was 189.9 Ijs/larva at 1 3℃, 8.4 Ijs at 18℃, 3.4 Ijs at 24℃, and 1.3 Ijs at 30 ℃ (Table 2).

Table 2. Lethal concentration at 50% for *Steinernema longicaudum* Gongju strain against last instar *Galleria mellonella* larvae in the laboratory

Temperature(℃)	LC_{50}	95% fiducial limits
13	189.9	89.1~1362.1
18	8.4	5.9~10.9
24	3.4	0.3~4.4
30	1.3	0.0~3.2

Temperature and dose-size effects on reproduction

The mean number of progenies produced was not significantly different depending on concentrations except low dosages, 5 and 10 Ijs/larva. The number of progenies was the highest at the rate of 80 Ijs at all temperatures (Table 3).

Table 3. Effect of dose-size on reproduction of *Steinernema longicaudum* Gongju strain in *Galleria mellonella* larvae at various temperatures

Concentration (Ijs)	Number of progenies[a] ± SD			
	13℃	18℃	24℃	30℃
5	0 A[b]	0±0 Aa	1,354±3,317 Aa	1,613±3,045 Ac
10	0 A	5,799±7,321 Aa	7,948±14,378 Aa	9,028±14,779 Abc
20	0 C	15,749±10,792 Ba	9,980±8,964 BCa	27,466±16,414 Aa
40	0 B	15,935±15,735 Aa	13,217±7,577 Aa	18,451±15,162 Aab
80	0 C	18,229±17,157 Ba	14,906±13,760 Ba	32,538±10,648 Aa
160	0 C	18,204±16,492 ABa	12,732±8,488 BCa	29,177±17,798 Aa

[a]Data obtained at everyday for 30 days. Data are averages of 10 replications.
[b]Means within a column followed by different uppercase letters are significantly different; means within a row followed by different lowercase letters are significantly different ($P<0.05$; Student-Newman-Keuls test).

Although *Galleria* larvae were infected at all concentrations at 13℃ or at the rate of 5 Ijs at 18 ℃, progenies were not produced from cadavers. At the concentration of 80 Ijs which produced the highest number of progenies, the mean number of infective juveniles emerged was 18,299, 14,906, and 32,528 at 18℃, 24℃, and 30℃, respectively.

The mean number of emerged progenies/day was 4,189 at 18℃, 5,919 at 24℃, and 17,784 at 30 ℃. Low concentration, 5 Ijs/larva obviously produced fewer progenies even at higher temperatures; 1,354 and 1,613 infective juveniles were emerged at 24℃ and 30℃, respectively. Temperature also influenced nematode production

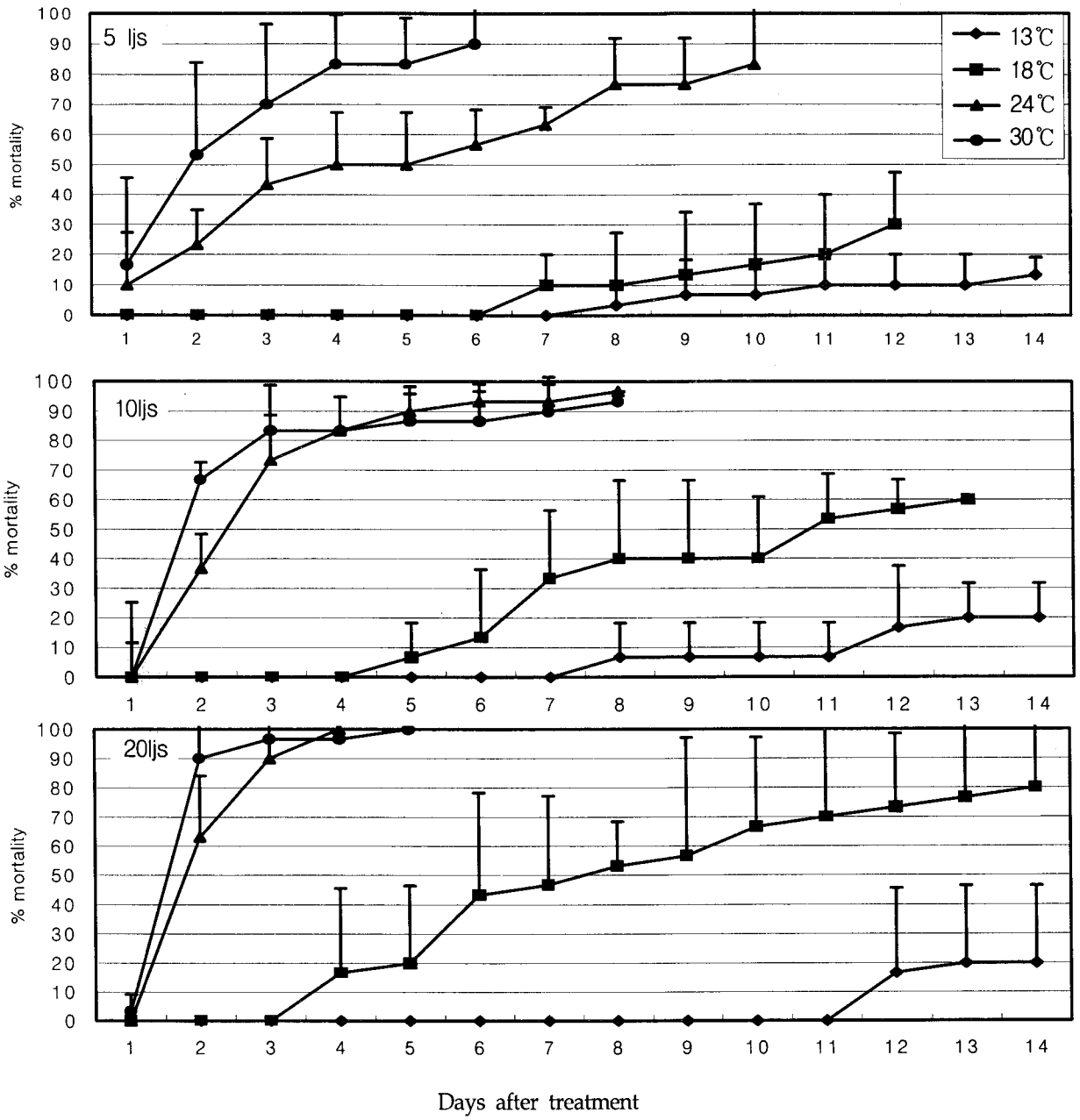

Fig. 1. Mortality of *Galleria mellonella* larvae exposed to *Steinernema longicaudum* Gongju strain.

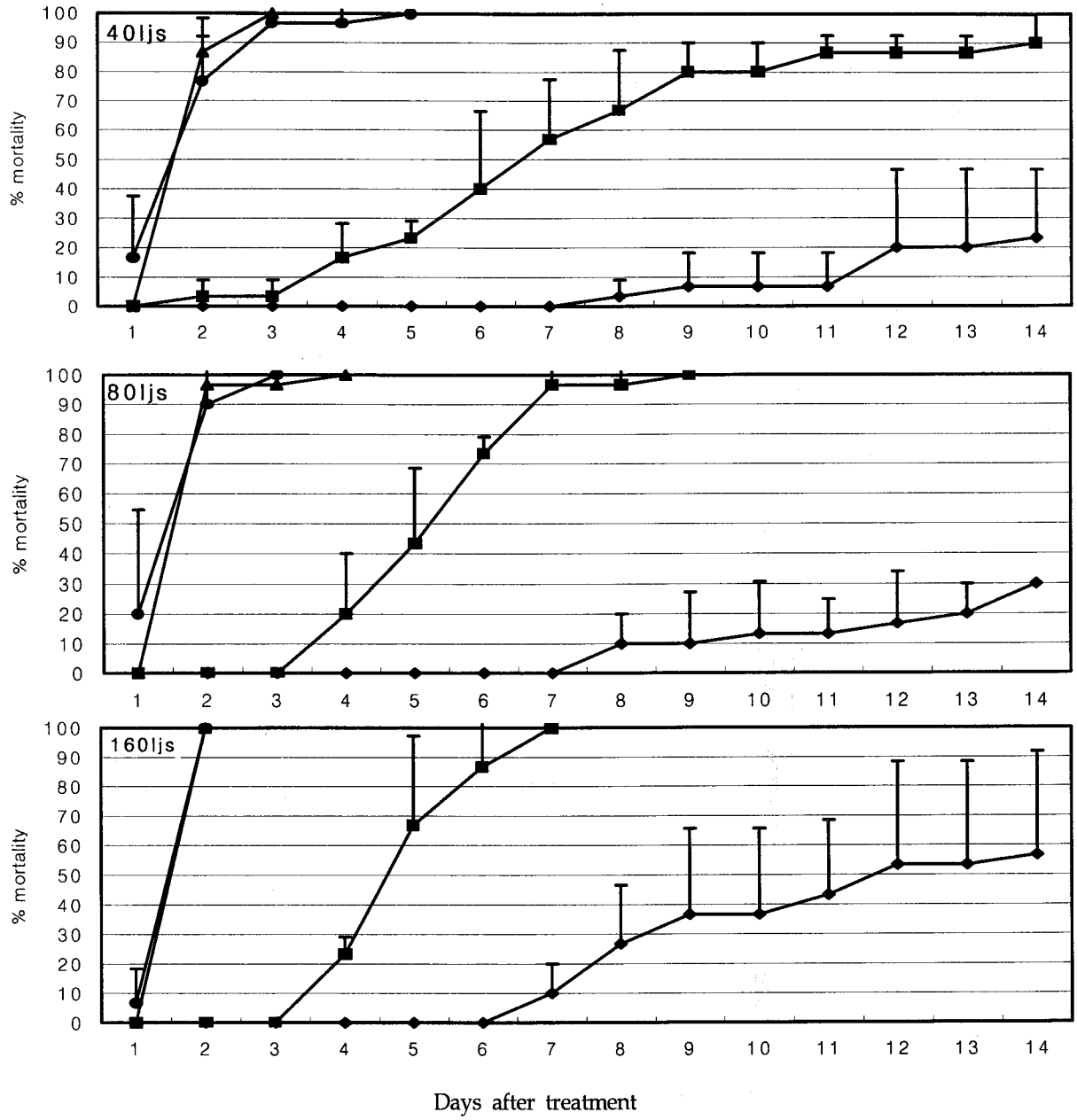

Fig. 1. Continued.

as shown in infectivity.

Although there were no significant differences among dose-sizes, progeny production was better with increasing dosages at the same temperatures (Fig. 2). Progenies were mainly emerged from the 8th day to 13th day at 18℃ (Fig. 2A), from the 10th day to 15th day at 24℃ (Fig. 2B), and from the 6th day to 9th day at 30℃ (Fig. 2C). Optimum temperature for reproduction was 30℃, that is, reproductive periods were generally shorter with the highest number of progenies of all temperatures (Fig. 2C).

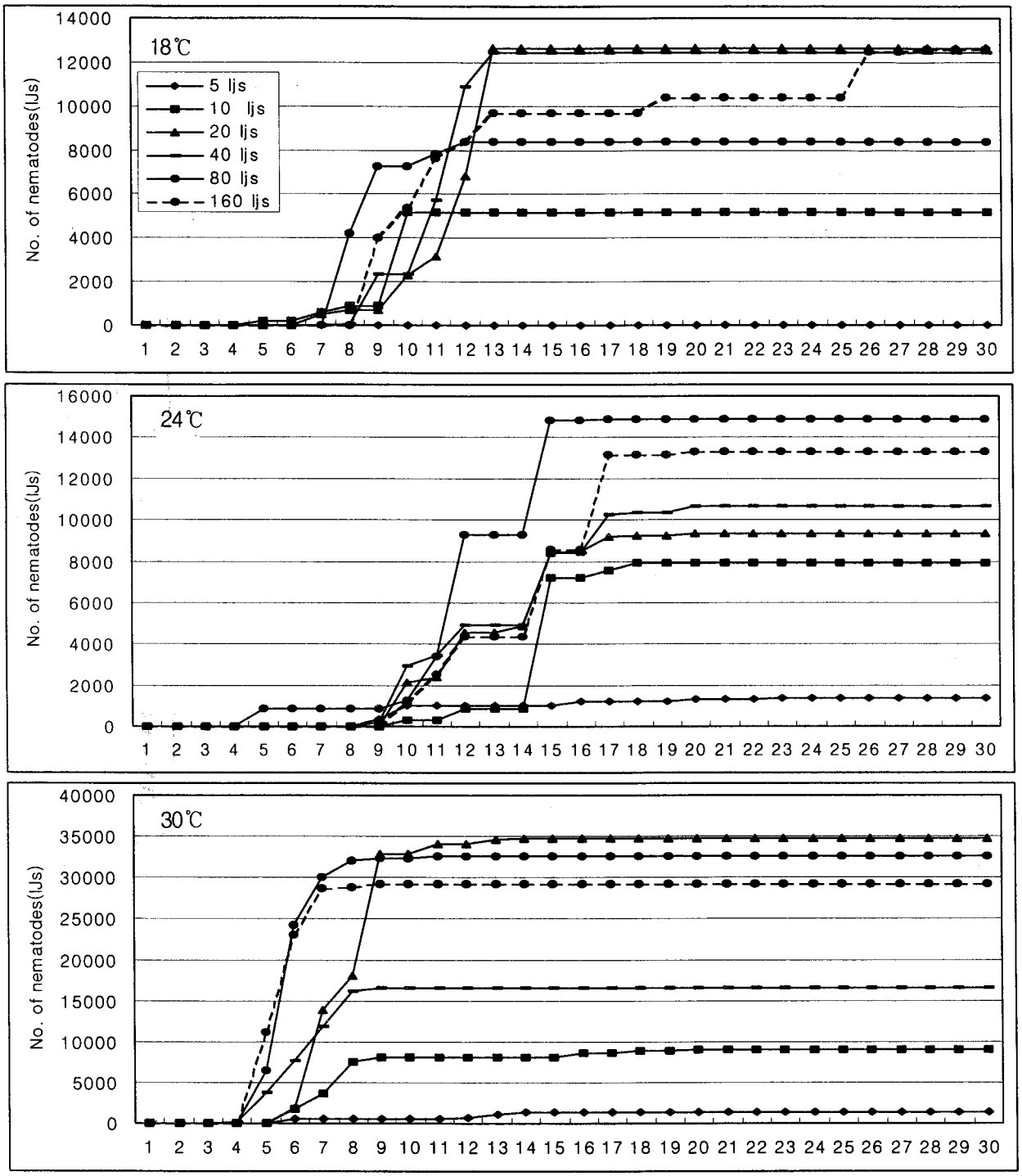

Fig. 2. Cumulative yield of *Steinernema longicaudum* Gongju strain in *Galleria mellonella* larvae in different temperature and concentration.

Discussion

Temperature and dose-size influenced infectivity and reproduction of Korean isolate of entomopathogenic nematode, *S. longicaudum* Gongju strain. *Galleria* larvae died earlier at higher temperatures and higher dosages. Temperature generally affects entomopathogenic nematode motility, survival, infectivity, development, and reproduction. The temperature range for infection and host-killing is both species and isolate dependent (Mason and Hominick, 1995). That is, LC_{50}s of *S. carpocapsae* DD-136 strain against *G. mellonella* larvae were 5.2 Ijs at 15℃, 2.1 Ijs at 20℃, and 1.5 Ijs at 25℃ and those of *S. carpocapsae* Mexican strain were 12.2 Ijs, 4.1 Ijs, and 3.5 Ijs at those temperatures, respectively. According to our observation, LC_{50} of *S. longicaudum* Gongju strain at 24℃ was similar to that of *S. carpocapsae* Mexican strain at 25℃ by 3.4 Ijs and 3.5 Ijs, respectively, but significantly different at low temperature. LC_{50} of *S. longicaudum* was 189.9 Ijs/larva at 13℃ while that of *S. carpocapsae* Mexican strain 12.2 Ijs/larva at 15℃. Infectivity of entomopathogenic nematodes may reflect their original climatic habitats. For example, 125 Ijs/grub of *S. riobravis* isolated from Texas caused no larval mortality at 25℃ but inflicted 45~71% mortality at 30℃ (Converse and Grewal, 1998). LT_{50} was longer at low temperature, 15℃ than high temperature, 25℃. LT_{50} of *S. carpocapsae* DD-136 strain were 142 hr at 15℃, 64 hr at 20℃ and 48 hr at 25℃ (Dunphy and Webster, 1986). This trend was the same in *S. carpocapsae* Mexican strain and *H. bacteriophora* NC-1 strain. Although temperature is favourable to nematodes, dosage rate sometimes affects infectivity. For instance, insect mortality by *S. riobravis* gradually increased with increasing numbers of infective juveniles to obtain high mortality in 24 hr but 15 Ijs/larva resulted in 100% mortality in 48 hr (Ricci *et al.*, 1996). However, 50 Ijs of *S. feltiae* were needed to achieve 100% mortality in 48 hr. Thus, dosage might be crucial factor to achieve sufficient mortality at a given temperature in a given time according to nematode species. *Galleria* larvae inoculated with higher doses of *H. bacteriophora* Oswego strain died earlier than those inoculated with lower doses (Flanders *et al.*, 1996). At low temperature, 12℃, *H. bacteriophora* required larger dosages to kill *Galleria* larva (Milstead, 1981). In addition, incubation period was longer at lower temperatures. *Galleria* larvae were killed by *H. bacteriophora* between 20.5 and 31.0℃ after 72 hr but it took 96 hr at 14.5℃. The minimum temperature was 11~12℃ and optimum temperature was 28℃ (Blackshaw and Newell, 1987). Although entomopathogenic nematodes killed host at low temperatures, reproduction was not occurred in our study like some other observations. However, nematodes were reproduced when the cadavers were transferred to high temperatures (Choo, unpublished data). This might be due to temperature-response of symbiotic bacteria, *Xenorhabdus* for steinernematids or *Photorhabdus* for heterorhabditids. The temperature range of infectivity, development, and reproduction of entomopathogenic nematodes in *Lucilia cuprina* was different depending on nematode species and strains (Molyneux, 1986). Interestingly, progeny production was not always influenced by inoculum rate although higher doses resulted in higher mortality of *Galleria* larvae. Temperature-reaction of entomopathogenic nematodes is variable depending on nematode species and strains. Development and reproduction of heterorhabditid nematodes occurred over slightly narrower temperature range than allowing infection to occur (Mason and Hominick, 1995) but steinernematids were more active at lower temperature and parasitized *L. cuprina* over a great temperature range (Molyneux, 1986). When 10 Ijs of *Heterorhabditis* were inoculated to *Galleria* larvae at various temperatures, *H. megidis* UK strain produced progenies at 10℃ but no reproduction was occurred in *M. megidis* Dutch strain, *H. zealandica*, and *H. bacteriophora* at the same temperature. Progeny production occurred at 15℃, 20℃, and 25℃ but the number of progenies was variable depending on species or strain and temperature (Mason and Hominick, 1995).

Nematode establishment, development, and

reproduction are also different depending on temperature and dose-size. Accordingly, temperature and dosage rate will need to be considered not only to enhance nematode efficacy in the field but also to propagate more progenies *in vivo* or *in vitro* production of *S. longicaudum*.

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온도와 농도가 곤충병원성 선충 *Steinernema longicaudum* 공주계통의 병원성과 증식에 미치는 영향

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요약 : 충남 공주지방의 강변 잔디밭에서 검출된 곤충병원성 선충 *Steinernema longicaudum* 공주계통의 병원성과 증식에 미치는 온도와 접종농도의 영향을 알아 보았다. 꿀벌부채명나방 유충 한 마리당 선충을 5, 10, 20, 40, 80, 160마리 농도로 처리하여 13, 18, 24, 30℃의 항온기에 보관하면서 14일간 치사율과 치사소요일수를 조사하였고, 동일 온도에서 30일 동안 감염태 유충의 증식수와 증식기간을 조사하였다. 그 결과 온도와 농도가 높을수록 병원성이 높게 나타났지만 농도보다는 온도에 의하여 더 많은 영향을 받았다. 즉, 13℃에서는 모든 농도에서 치사율이 낮았으나 24℃와 30℃에서는 5마리와 10마리의 낮은 농도에서도 높은 치사율을 나타내었다. 온도와 농도가 높아질수록 치사일수도 단축되어 24℃와 30℃에서 160마리 농도는 2일만에 기주를 100% 치사시켰으나, 5마리 농도는 24℃에서 10일만에 83.3%, 30℃에서 6일만에 90%의 기주 치사율을 나타내었다. 유충의 증식은 온도와 농도가 높아짐에 따라 양호한 경향이었지만 접종농도간에는 유의성이 인정되지 않았다. 저온인 13℃에서는 모든 농도에서 전혀 증식이 이루어 지지 않았다. 증식기간도 30℃에서 6~9일로 다른 온도와 비교하여 가장 짧았다. *S. longicaudum* 공주계통의 병원성 발현 최적 온도는 24℃였고 증식 최적 온도는 30℃였다.

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