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Studies on the potential use of entomopathogenic nematodes for biological control of animals in the stables

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Abstract: *Studies on the potential use of entomopathogenic nematodes for biological control of animals in the stables.* The study was aimed at selecting species and strains of entomopathogenic nematodes to be used in practical control of the housefly in stables, which should provide welfare of bred animals. Test insect, the housefly, and entomopathogenic nematodes of the family Steinernematidae and Heterorhabditidae were used in experiments. Laboratory strains of nematodes and those commercially available in Poland and in Europe were used in performed tests. Larvae, pupae and imagines of *M. domestica* were cultivated in the Institute of Organic Industry in Warsaw. Four groups were created for each nematode species. Not all nematode species and strains were equally pathogenic to houseflies.

Key words: biopreparations, entomopathogenic nematodes, housefly, animal welfare

INTRODUCTION

Excessive agricultural chemicalization leads to the degradation of the natural environment. Insecticides accumulate in plants, soil and ground waters and their remains are ingested by a consumer. They may pose a risk to human and animal life and health. Consequences after the contact with pesticides include the disturbances of nervous, respiratory and alimentary systems, skin wounds and even death.

The appearance of resistant pest races is an unfavourable effect of insecticide application. Insects often develop the resistance to more than one type of insecticides. Overuse of insecticides and application of substances from the same chemical group decreases their effectiveness from year to year. A disadvantage of chemical means for insect control is their broad spectrum of activity. They kill both harmful and beneficial organisms (Malinowski 2003).

Integrated method does not completely eliminate synthetic preparations. The term IPM was first introduced in 1959 by Stern. Chemical means should be applied only if the need arises (Stern et al. 1959).

The housefly is a synanthropic insect which makes problems in farm houses. The invasion of this insect stresses animals and causes economic losses. The housefly is a vector of viruses, bacteria, protozoans and fungi. Up to 6 million microorganisms may be found on its body. Its most intensive invasion takes place in the summer month (Ignatowicz 2000). Preventing invasions of this insect is troublesome, particularly when the interior of farm houses is permanently open to the external habitat. Therefore, breeders are advised to use several methods

of housefly control in order to obtain expected effects. Housefly control may be performed in several ways using physical, chemical and biological methods (Wojciechowska and Kamionek 2012). In this study entomopathogenic nematodes were used for this purpose.

Nematodes are used in the biological control of the populations of harmful insects. Control group consisted of housefly larvae with distilled water. Mortality and the extensity of infection of housefly larvae by various species and strains of Steinernematidae and Heterorhabdtidae nematodes were determined.

MATERIAL AND METHODS

Study material

Insects (*M. domestica*)
Studies were carried out on the housefly *M. domestica*, a species common in farm houses (stables). Experimental insects were cultivated in the Institute of Organic Industry in Warsaw. Patented substratum prepared in the Institute was used to breed houseflies.

Entomopathogenic nematodes (EPNs)
Steinernema affinis, *S. carpocapsae*, *S. feltiae*, *Heterorhabditis megidis*: Is, Ic WNZ /2009, biopreparations based on nematodes *S. feltiae*: Owinema (Oviplant), Entonem (Koppert), Nemaplus (E-nema) and biopreparations based on nematodes *Heterorhabditis bacteriophora*: Larvanem (Koppert) Nematop (E-nema) were used in this experiment.

Methods

Laboratory tests
Eleven species and strains of nematodes, including five commercial biopreparations were used in experiments on the housefly control in stables. Tests were made on Petri dishes 10 cm in diameter lined with a double layer of filter paper. Twenty housefly larvae were placed onto each dish and 5, 10, 20 or 50 invasive larvae of appropriate nematode species were instilled onto dish. Tests were made in three repetitions. Mortality was checked every 24 h for 5 days. The control consisted of larvae with 100 ml of water added instead of nematode suspension. After infection dead and live insects were counted and the former were dissected to check whether nematodes were the cause of their death (Figures 1 and 2).

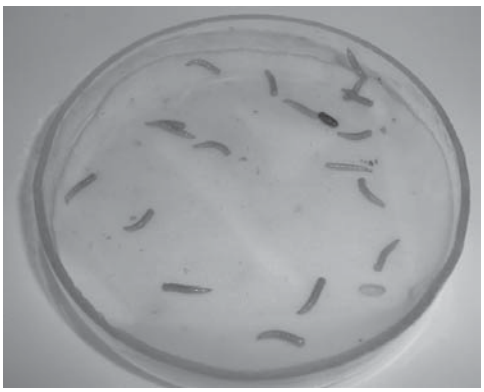

FIGURE 1. Infected larvae of the housefly (Wojciechowska 2013)

STATISTICAL ANALYSIS

In the development of the results of the experiments used a using multivariate analysis of variance (ANOVA). It allows the inference the significance of

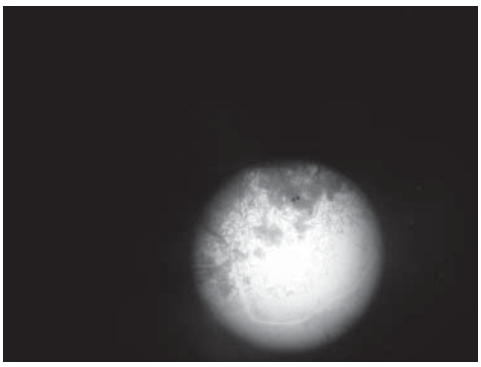

FIGURE 2. Nematodes visible in a dead larva (Wojciechowska 2013)

the impact factor of the test to the test parameter (extensiveness of infestation, the number of outlets). When we found a significant effect of a particular factor on a particular trait, with LSD test was evaluated, between which there are significant differences in average ($p < 0.05$). Calculations made using the computer program Statgraphic.

RESULTS AND DISCUSSION

The highest mortality in performed tests was noted with the use of *S. feltiae* (Owinema, Nemaplus) and *S. carpocapsae*. The lowest mortality was noted after application of *S. riobrave*, *H. bacteriophora* (Larvanem and Nematop) and *H. megidis* Ic and Is. Most extensive infection was recorded for *S. carpocapsae*, *S. feltiae* (Owinema) and *S. feltiae* (Nemaplus). The lowest extensity was characteristic for *S. affine*, *S. intermedia*, *S. riobrave* and *H. megoidis* Ic and Is (Tables 1 and 2, Fig. 3). The reasons

TABLE 1. Mortality and the extensity of infection of four-day larvae of *M. domestica* after application of various species and strains of Steinernematidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection	
		%	SD
S. affine	57^{b}	30^{bc}	3.67
S. intermedia	65^{c}	37^{c}	3.82
S. carpocapsae	80^{e}	70^{ef}	6.18
S. riobrave	55^{b}	42^{bd}	2.53
S. feltiae (Owinema)	90^{g}	75^{g}	6.58
S. feltiae (Nemaplus)	85^{f}	73^{fh}	6.26
S. feltiae (Entonem)	73^{d}	57^{de}	4.15

Different letters in columns denote significant differences at $p < 0.05$.

TABLE 2. Mortality and the extensity of infection of four-day larvae of *M. domestica* after application of various species and strains of Heterorhabditidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
H. megidis Ic	55^{b}	24^{b}	2.53
H. megidis Is	47^{a}	21^{ab}	1.17
H. bacteriophora (Nematop)	44^{a}	18^{a}	1.13
H. bacteriophora (Larvanem)	45^{a}	15^{a}	1.16

Different letters in columns denote significant differences at $p < 0.05$.

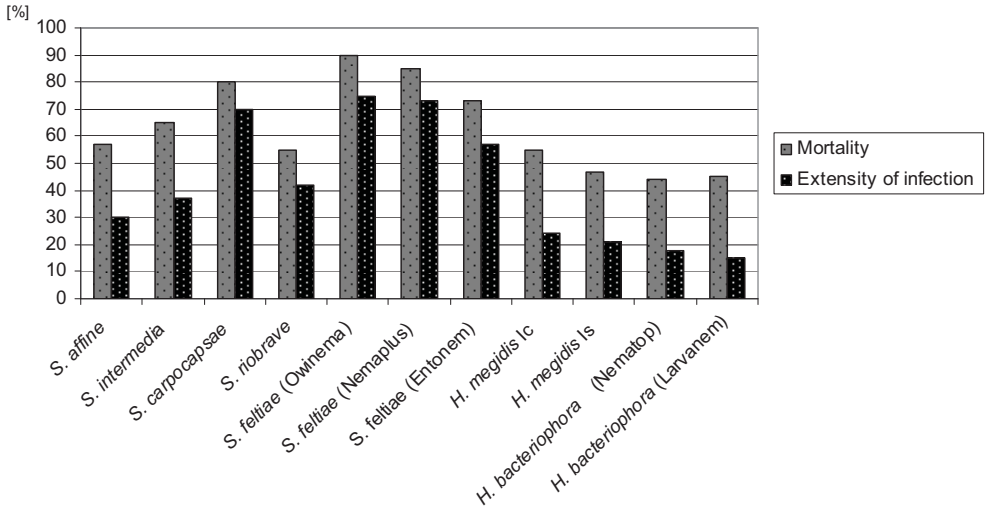

FIGURE 3. Mortality and the extensity of infection of four-day larvae of *Musca domestica*

of a higher extensity of infection in older larvae should be sought in the anatomy of natural openings, through which invasive larvae penetrate their body (Brzeski and Sandner 1974).

The highest mortality was noted after application of *S. feltiae* (Owinema, Nemaplus) and *S. carpocapsae*, the lowest – with *S. riobrave*, *H. bacteriophora* (Larvanem and Nematop) and *H. megidis* Ic and Is (Tables 3 and 4, Fig. 4).

The highest mortality was found in the case of *S. feltiae* (Owinema, Nemaplus) and *S. carpocapsae*, the lowest was obtained after application of *S. riobrave*, *H. bacteriophora* (Larvanem and Nematop) and *H. megidis*. Mortality and the extensity of infection were significantly lower in pupae than in housefly larvae. The former are immobile, which was probably the reason of their low mortality and extensity of infection (Tables 5 and 6, Fig. 5).

TABLE 3. Mortality and the extensity of infection of two-day larvae of *M. domestica* after application of various species and strains of Steinernematidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
S. affine	47^{b}	29^{b}	2.67
S. intermedia	39^{c}	25^{c}	2.82
S. carpocapsae	55^{e}	45^{e}	4.18
S. riobrave	45^{b}	24^{b}	2.53
S. feltiae (Owinema)	65^{f}	53^{f}	4.58
S. feltiae (Nemaplus)	60^{d}	49^{de}	4.26
S. feltiae (Entonem)	59^{c}	40^{cd}	3.15

Different letters in columns denote significant differences at $p < 0.05$.

TABLE 4. Mortality and the extensity of infection of two-day larvae of *M. domestica* after application of various species and strains of Heterorhabditidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
H. megidis Ic	20^{b}	15^{b}	2.53
H. megidis Is	16^{a}	12^{a}	1.17
H. bacteriophora (Nematop)	12^{a}	10^{ab}	1.13
H. bacteriophora (Larvanem)	10^{a}	7^{a}	1.16

Different letters in columns denote significant differences at $p < 0.05$.

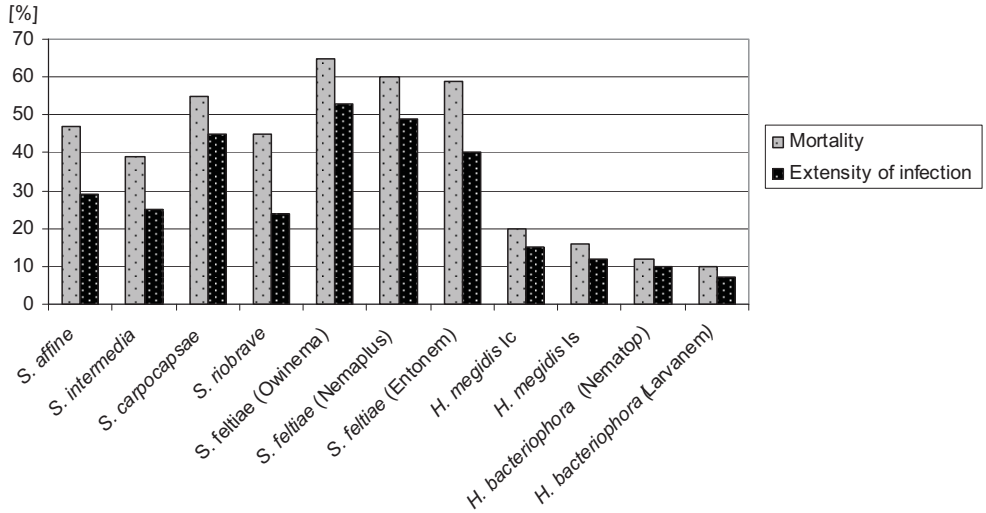

FIGURE 4. Mortality and the extensity of infection (%) of two-day larvae of *M. domestica*

TABLE 5. Mortality and the extensity of infection of *M. domestica* pupae after application of various species and strains of Steinernematidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
S. affine	25^{b}	3^{ab}	1.01
S. intermedia	37^{c}	4^{bc}	1.08
S. carpocapsae	45d	13^{de}	1.16
S. riobrave	30^{c}	9^{c}	1.14
S. feltiae (Owinema)	60^{e}	15^{e}	2.63
S. feltiae (Nemaplus)	55^{e}	12^{de}	2.53
S. feltiae (Entonem)	48^{d}	10^{d}	1.17

Different letters in columns denote significant differences at $p < 0.05$.

TABLE 6. Mortality and the extensity of infection of *M. domestica* pupae after application of various species and strains of Heterorhabditidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
H. megidis Ic	20[b]	4[b]	1.01
H. megidis Is	17[a]	3[a]	0.95
H. bacteriophora (Nematop)	14[a]	3[a]	0.95
H. bacteriophora (Larvanem)	11[a]	2[a]	0.95

Different letters in columns denote significant differences at $p < 0.05$.

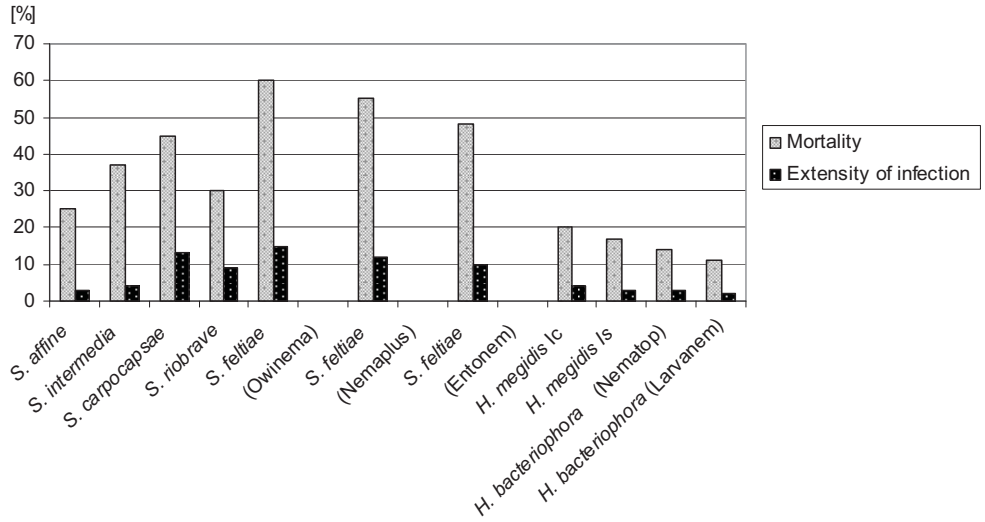

FIGURE 5. Mortality and the extensity of infection of *M. domestica* pupae

The highest mortality in performed tests was demonstrated after application of *S. feltiae* (Owinema and Nemaplus) and *S. carpocapsae* and the lowest with the use of *S. riobrave*, *H. bacteriophora* (Larvanem and Nematop) and *H. megidis* (Tables 7 and 8, Fig. 6).

Nematodes of the families Steinernematidae and Heterorhabditidae are effective in controlling many species of harmful insects. Studies have long been performed in many countries on nematodes as a means used in biological methods for pest control. They found practical application in controlling dipterans of the families Sciaridae and Phoridae in mushroom-growing cellars. The highest extensity of infection of *Lycoriella solani* Winnertz was found after application of *S. affinis*, *S. feltiae* (Nemaplus) and *S. feltiae* (Owinema). In the case of *Megaselia halterata* Wood the highest extensity was obtained with the use of *S. affinis*, *S. feltiae* (Nemaplus) and *S. feltiae* (Owinema) (Sznyk-Basałyga 2002).

Commercial preparations based on entomopathogenic nematodes are produced in many countries. In Poland the production of biopreparation Owinema

TABLE 7. Mortality and the extensity of infection of *M. domestica* imagines after application of various species and strains of Steinernematidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
S. affine	8^{b}	0^{ab}	0
S. intermedia	9^{c}	0^{ac}	0
S. carpocapsae	12^{d}	1^{bd}	0.55
S. riobrave	4^{a}	0^{a}	0
S. feltiae (Owinema)	13^{d}	2^{bd}	0.75
S. feltiae (Nemaplus)	11^{d}	2^{bd}	0.71
S. feltiae (Entonem)	10^{c}	1^{bc}	0.48

Different letters in columns denote significant differences at $p < 0.05$.

TABLE 8. Mortality and the extensity of infection of M. domestica imagines after application of various species and strains of Heterorhabditidae (%). Initial dose of nematodes for insect – 50

Nematode species	Mortality (%)	Extensity of infection (%)	SD
H. megidis Ic	7^{b}	0^{ab}	0
H. megidis Is	5^{a}	0^{a}	0
H. bacteriophora (Nematop)	8^{b}	0^{ab}	0
H. bacteriophora (Larvanem)	3^{a}	0^{a}	0

Different letters in columns denote significant differences at $p < 0.05$.

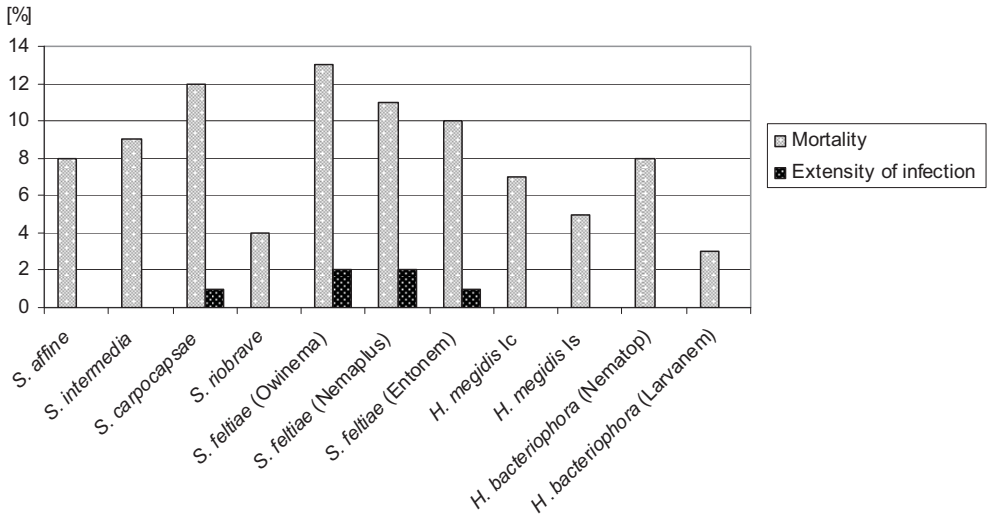

FIGURE 4. Mortality and the extensity of infection of *M. domestica* imagines

is based on *S. feltiae*. Biopreparations are considered safe in controlling pests in agriculture and animal breeding (Poinar 1979, Pezowicz 2005).

Unruh and Lacey (2001) used *S. carpocapsae* to control a fruit fly (*Carpocapsa pomonella* L.). Tomalak (2004) used *H. megidis* and *S. feltiae* against tree pests: the alder leaf beetle (*Agelastica alni* L.) and the oak flea beetle (*Haltica quercetorum* Foudras). This study showed that prepupae and pupae are sensitive to nematode infection. and that *H. megidis* was more effective than *S. feltiae*. The western flower thrips (*Frankliniella occidentalis*, Pergande) may also be controlled by nematodes (Borgemeister et al. 2002). In this case *Heterorhabditis* spp. was more pathogenic than *Steinernema* spp. Bednarek et al. (2002) controlled grubs of a cockchafer from the family Melolonthinae in forests. Also the pea leaf weevil (*Sitona lineatus* L.) is sensitive to nematodes (Jaworska and Ropek 1998). Mortality of these insects was 70–80%.

Insect species of different orders show differentiated susceptibility to nematodes. The easiest infected are the butterfly caterpillars. Beetles are resistant and the least mortality was found in dipteran populations (Dutky 1959).

M. domestica is an undesirable insect in stables since it harasses animals and carries diseases and parasites. Poland as a member of the EU should obey zoohygienic standards and norms. This includes also the control of houseflies.

In presented study, the tested nematode species and strains were more effective for four-day larvae of *M. domestica* (55–90% mortality) than for small, two-day larvae (39–65% mortality).

Pezowicz (2005) in her studies on the control of the lesser mealworm (*Alphitobius diaperinus* Panzer) showed that nematodes infected both young and older larvae. The extensity of infection of older larvae was, however, significantly higher. Higher extensity of infection of older larvae is an effect of natural openings, of the size of stigmas, through which the invasive larvae penetrate insect's body (Koppenhöfer et al. 2000). My studies revealed the same regularity – nematodes infected two-day larvae to a very small extent. According to Pezowicz (2005), nematodes penetrated all growth stages of the insect.

Dipteran larvae were controlled more effectively by nematodes of the family Steinernematidae than by those from Heterorhabditidae. The highest extensity of infection in laboratory tests was obtained after application of two nematode species *S. carpocapsae* and *S. feltiae* (Ovinema and Nemaplus). *S. feltiae* is also most effective in the control of other dipterans of the family Sciaridae in contrast with *S. carpocapsae* which showed much lower pathogenicity (Sznyk-Basałyga 2002). My studies do not confirm this observation since the extensity of infection was 70%.

S. affinis oraz *H. megidis* exerted the weakest effect on *M. domestica*.

Performed studies demonstrated that *S. feltiae* is the most effective nematode species in controlling the number of the housefly. Owinema and Nemaplus are the biopreparations adapted to be applied in practise. It is unfortunate that in Poland abandoned production Owinema.

CONCLUSIONS

1. Most sensitive to nematodes were the larvae of *M. domestica*. Four-day larvae were more sensitive than two-day ones.
2. Pupae and imagines were more resistant to nematode infection.
3. Larvae of the housefly were more effectively killed by nematodes of the family Steinernematidae than by those of the family Heterorhabditidae.
4. The housefly was most effectively killed by nematodes *S. feltiae* from biopreparations Owinema and Nemaplus and by *S. carpocapsae*.

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Streszczenie: *Biologiczne metody zwalczania muchy domowej w stajniach.* Doświadczenia miały na celu wybór gatunków i szczepów nicieni owadobójczych, które zostaną wykorzystane w praktycznym zwalczaniu muchy domowej w stajni, co powinno zapewnić dobrostan zwierzętom hodowlanym. Owad testowy – mucha i owadobójcze nicienie z rodziny Steinernematidae i Heterorhabditidae zostały wykorzystane w eksperymentach. Laboratoryjne szczepy nicieni i w Polsce i w Europie tych dostępnych na rynku zostały wykorzystane w przeprowadzonych testach. Larwy, poczwarki i owady dorosłe *M. domestica* były hodowane w Instytucie Przemysłu Organicznego w Warszawie. Cztery grupy zostały stworzone dla każdego gatunku nicieni. Nie wszystkie gatunki nicieni i szczepy były równie zjadliwe dla much.

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