RESEARCH

Evaluation of the entomopathogenic nematode, *Steinernema asiaticum* against the diamondback moth, *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) under screen house and field conditions

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Abstract

Background: The experiments were conducted at Research Farm, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. Infectivity and effectiveness of entomopathogenic nematode (EPN), *Steinernema asiaticum* infective juveniles (IJs) were evaluated against the diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) as foliar spray on cabbage plants in screen house with single (30,000 IJs per plant), two (15,000 + 15,000 IJs per plant) or three (10,000 + 10,000 + 10,000 IJs per plant) applications. In the field trial, *S. asiaticum* (25,000 or 50,000 IJs per plant) and Malathion 50 EC (0.05%) singly or in combination at half the concentrations were used.

Results: In screen house trial, all the treatments resulted in significantly high larval mortality than untreated plants. Split concentration application (15,000 + 15,000 IJs per plant) gave better DBM larval mortality (48.33%) compared to single application (36.00% at 30,000 IJs per plant). Comparing the two and three split applications, 15,000 IJs was significantly better (23.33% mortality) than 10,000 IJs (13.33%). In the field trial, *S. asiaticum* alone at 50,000 IJs resulted in 28.8% insect mortality compared to 18.0% in Malathion at 0.05%. Best results (37.5% mortality) were obtained by using nematode IJs at 25,000 per plant in combination with half the recommended concentration of Malathion (0.025%) leading to synergistic effect.

Conclusions: The study revealed that split application (15,000 + 15,000 IJs) of EPN *S. asiaticum* IJs proved better than single application of IJs at the same concentration (30,000 IJs) in the management of *P. xylostella* larvae in the screen house conditions. In the field experiment, the best effectiveness was obtained in combination of half the concentrations of EPN and insecticide (*S. asiaticum* IJs @ 25,000 per plant + Malathion @ 0.025%).

Keywords: Cabbage, Diamondback moth, *Plutella xylostella*, Entomopathogenic nematode, *Steinernema asiaticum*, Malathion, Management

Background

India is one of the main producers of cruciferous vegetables worldwide, ranking second in production of cabbage, *Brassica oleracea* var. *capitata* L. that belongs to family Brassicaceae. Of the total area of vegetable grown in India, 5% is occupied by cabbage (Anonymous 2016). The

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major constraint in the production of cruciferous vegetables is the ravages of lepidopteran insect pests of which *Plutella xylostella* (Linnaeus) (Fam.: Plutellidae), *Spodoptera litura* (Fabricius) (Fam.: Noctuidae), *Helicoverpa armigera* (Hubner) (Fam.: Noctuidae) and *Pieris brassicae* (Linnaeus) (Fam.: Pieridae) are economically important. Diamondback moth (DBM), *P. xylostella* is the most noxious pest of cabbage and cauliflower worldwide. Attack of DBM results into both yield loss and marketable value of the crop. If incidence of *P. xylostella* occurs at an early stage of crop, heavy yield loss may occur.

Management of DBM has become difficult since the pest has developed resistance to many organophosphates, carbamate and pyrethroid insecticides due to their overuse (Shelton et al. 1993). Alternatively, *Bacillus thuringiensis* (*Bt*) Berliner (Bacillales: Bacillaceae) has been used widely for the management of *P. xylostella* (Cherry et al. 2004), but extended use of *Bt* also has resulted in the emergence of resistant DBM populations in Asia and the Americas (Ferre and Van Rie 2002).

Entomopathogenic nematodes EPNs have become potential candidates for successful biological control agents of lepidopteran pests (Kaya et al. 2006). Their ability to attack a wide host range of insect hosts, active host search, killing the host within 48 h, ease of mass production and application, long-term efficacy, compatibility with agro-chemicals and environmental safety have prompted their commercial use in many countries. EPNs are commonly found in soils with high sand content. EPNs were isolated from the soil of Research Farm, Hisar, Haryana and three species of EPNs viz., *Steinernema asiaticum*, *Steinernema* sp. RB-5 and *Heterorhabditis bacteriophora* were found from the collection (Kumar et al. 2014). In our earlier studies, steinernematids species were recovered more compared to heterorhabditis species in Northern Indian (Hisar, Haryana). The Environment Protection Agency (EPA) in India, USA, Australia and many European countries has exempted EPNs from registration (Ehlers 2005). Current dilemma on DBM management firmly emphasizes the urgent need for integrated pest management approach, of which biological control is a major component. In this study, the susceptibility of *P. xylostella* to EPNs in screen house and field conditions was investigated and also their compatibility with recommended insecticide (Malathion) on cabbage crop.

Methods

Mass rearing of insects

The stock culture of Greater wax moth, *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) was maintained in laboratory on artificial diet (corn flour 200 g, wheat flour 100 g, wheat bran 100 g, milk powder 100 g, yeast 50 g, glycerine 100 ml and honey 100 ml). Late instar larvae were used for multiplication of EPNs. Stock culture of *P. xylostella* was maintained on cabbage leaves in the laboratory (Dwivedi and Mathur 2000). Third instar larvae were used for experimental purpose.

Mass rearing of EPNs

Three species of EPNs (*Steinernema asiaticum* (Weiser) (Rhabditida: Steinernematidae), *Steinernema* sp. RB-5 and *Heterorhabditis bacteriophora* (Poinar) (Rhabditida: Heterorhabditidae) were originally isolated from the soil of Research Farm, Hisar, Haryana. All the EPN isolates were found pathogenic to *P. xylostella* larvae in preliminary laboratory assays causing a significant mortality. *S. asiaticum* was adjudged the most pathogenic among the test nematodes on the basis of highest mean mortality (36.71%) and highest mortality (65.62%) after 96 h (Kumar et al. 2014). Indigenous strains of EPNs were reared in vivo on *G. mellonella* in laboratory. IJs were extracted from cadavers by 'White Trap' method (White 1927) and used for the experimental purpose.

Standardization of EPN formulation

To standardize the formulation, the IJs were applied in 50 ml of sterile water containing glycerine (1%) as antidessicant, Triton x-100 (1%) as spreader, PABA (Para Amino Benzoic Acid) (0.05%) and Congo red (0.05%) as UV protectants as adopted by Walia et al. (2008). While testing the efficacy of EPNs against larvae of diamondback moth, *P. xylostella*, it was noticed that PABA caused 60–70% EPN mortality, whereas Congo red proved safe to EPN. Therefore, PABA was replaced with Congo red in the final formulation. Congo red has been suspected to be considered as health hazardous, but at this low concentration it has recommended as UV Protectants (Hadapad et al. 2009). Moreover, Congo red is a sodium salt with negligible evaporation rate in the environment, stable at room temperature, oral LC_{50}—15,200 mg/kg, and teratogenicity occurs at 200 mg/kg/day and above. Hazardous class 4.1. No component in this product was listed as hazardous substance under clean water act and clean air act (Air Pollutants) according to US Federal Agency. Congo red is amenable to photo-degradable and not absorbed by plant and also not volatilized in the air. It is also used in tracing of certain human diseases pertaining to kidney, liver, brain, etc. (Yakupova et al. 2019). Even, if cabbage is processed and cooked, before processing upper two leaves were removed which are exposed to Congo red.

Green house evaluation

Earthen pots of (30 × 20 cm diameter) filled with field soil were transplanted (15–25 December) with cabbage

(cv. Golden acre) seedlings (@ 2 per pot). One month after transplanting, 10 individuals of 3rd instar larvae of *P. xylostella* were placed in the whorl of each plant. Different levels (30,000 IJs in single spray, 15,000 IJs in 2 sprays at 4-day interval and 10,000 IJs in 3 sprays at 4-day interval) per plant were sprayed in 50 ml of sterile water containing glycerine (1%), Triton x-100 (1%) and Congo red (0.05%). Hand atomizer was used to spray the mixture. After spraying, the plants were covered with paper cage so that larvae do not escape from the pots. Each treatment was replicated five times. In control, the plants were sprayed with 50 ml of water along with above-mentioned additives. Observations were recorded on dead larvae 24, 48 and 72 h after spraying. The dead larvae were taken to laboratory for estimating nematode population by the white trap method (White 1927). The data obtained were subjected to analysis of variance using the completely randomized design (CRD) (Snedecor and Cockran 1968).

Field evaluation

The experiment was conducted in a cabbage field at Research Farm, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India, in the month of April. Cabbage plant infested with *P. xylostella* larvae was randomly selected for evaluation, and to maintain equal number of larvae on each infested plant excess larvae were removed with camel hair brush. The uniformity of DBM larvae (i.e. 10 larvae/plant) was maintained by removing excess larvae from the plant. The movement of the larvae among the plants is very slow. There was no further scope of invasion of larvae during the experimental period (48 h). It was evident from the control plants where the larva was same after 48 h. Different treatments ($T1 = 50,000$ IJs with glycerine (1%) and Triton x-100 (1%), $T2 =$ Malathion 50 EC @ 0.05%, $T3 = 25,000$ IJs with glycerine (1%) and Triton x-100 (1%) + Malathion 50 EC @ 0.025%, $T4 = 25,000$ IJs with glycerine (1%) and Triton x-100 (1%), $T5 =$ control; water with glycerine (1%) and Triton x-100 (1%) per plant were applied in 50 ml of distilled water in the evening time. Hand sprayer was used to spray the mixture. Each treatment was replicated four times. Observations were recorded on dead larvae 24 and 48 h after spraying. To check the efficacy of EPNs against the larvae could have been correlated with the leaf damage. But, it was difficult to correlate the leaf damage with the population of this insect because larvae are very slow feeder and have slow growth. The data obtained were subjected to analysis of variance using completely randomized design (CRD) (Snedecor and Cockran 1968).

Results

Green house test

The bubble formation on the body of EPN-infected DBM larvae indicated early symptoms of infectivity. In the present study, dead larvae of *P. xylostella* turned to greyish/black due to infection of EPN, *S. asiaticum*. The fluid-filled bubbles harboured actively moving adults or infective juveniles (IJs) of EPNs. It is speculated that the gaseous emission resulting from the decomposing insect body by the bacterial symbionts was trapped in the thin membranous tissues. These bubbles finally burst and release the IJs (Fig. 1). However, Jang et al. (2012) reported the bubble formations due to catalase produced by the bacteria *Photorhabdus temperata* M1021 isolated from the EPNs.

The highest mortality (36%) of DBM larvae due to EPN after first spray was recorded at 30,000 IJs concentration, and it was significantly higher than the 15,000 and 10,000 IJs concentrations (Table 1). All the 3 concentrations of EPNs resulted in significantly higher mortality than adjuvants and water alone. The effect of time and its interaction with concentration was non-significant (Table 1). However, the data on the effect of second foliar spray in the case of split application of *S. asiaticum* once again revealed significantly higher larval mortality than the controls. Among the two concentrations, 15,000 IJs was significantly better mortality (23.33%) than 10,000 IJs (13.33%). The effect of time factor and its interaction with concentration was non-significant (Table 2). Pooled data of the first and second sprays revealed that split application (15,000 + 15,000 IJs) of *S. asiaticum* IJs proved better than single application of 30,000 IJs and resulted in 48.33% mortality (Table 3). The third treatment was originally intended for three sprays (10,000 + 10,000 + 10,000

Fig. 1 Bubble formation in the cadaver of *Plutella xylostella* infected by *Steinernema asiaticum*

Table 1 Larval mortality (%) of *Plutella xylostella* by *Steinernema asiaticum* in screen house after first foliar spray on cabbage

Time (h)	Concentrations of infective juveniles per plant					Mean (time)
	30,000	15,000	10,000	Water + adjuvants	Water alone	
24 h	29.00 (32.30)	19.00 (25.47)	19.00 (25.81)	03.00 (08.92)	01.00 (05.95)	14.20 (19.69)
48 h	36.00 (36.69)	27.00 (31.19)	29.00 (32.69)	03.00 (08.92)	01.00 (05.95)	19.20 (23.09)
72 h	43.00 (41.04)	29.00 (32.51)	32.00 (34.56)	03.00 (08.92)	01.00 (05.95)	21.60 (24.59)
Mean (concentration)	36.00 (36.68)	25.00 (29.73)	26.66 (31.02)	03.00 (08.92)	01.00 (05.95)	
Factors	**SE (m)**					**C.D. ($P = 0.01$)**
Time	(1.45)					(N. S.)
Concentration	(1.87)					(5.32)
Interaction (Time × concentration)	(3.25)					(N. S.)

Values in parentheses are angular-transformed ($x + 0.5$) values

IJs); however, all the left-over larvae turned into pupae by the time of application of third spray. So, the third spray could not be undertaken. The effect of time was also significant with a high larval mortality at each time interval. The interaction of time and concentration was, however, non-significant. Adults of the EPN (*S. asiaticum*) wriggling on the dead larvae of DBM (Fig. 2) were very prominent. The recovery of IJs from dead cadavers of *P. xylostella* after the first/second spray in different treatments ranged (95–439), and the differences were significant (Fig. 3).

Field evaluation

Maximum DBM larval mortality (37.5%) was recorded in treatment T3 (25,000 IJs + Malathion 50 EC 0.025%) which was significantly better than all other treatments (Table 4). As compared to control, minimum insect mortality (11.3%) was recorded in T4 (25,000 IJs), followed by 18.0% in T2 (Malathion 50 EC 0.05%) and 28.8% in T1 (50,000 IJs). All the treatments resulted in significantly higher larval mortality compared to control. The effect of time and its interaction with treatments was non-significant.

Discussion

Entomopathogenic nematodes (EPNs) have been widely utilized to manage wide range of insect pests both below and above ground (Bhairavi et al. 2021). They can be used solely as a biological control agents or combined with other management methods in integrated pest management (Kumar et al. 2013). Among the three indigenous EPNs, viz., *H. bacteriophora*, *S. asiaticum* and *Steinernema* sp. RB-5, *S. asiaticum* was the most virulent causing (65.6%) mortality of *P. xylostella* larvae after 96 h

and the number of nematode IJs recovered per insect larva was the highest (1226) in *S. asiaticum* (Kumar et al. 2014). In screen house conditions, split application (15,000 + 15,000 IJs) of *S. asiaticum* IJs provided (48.33%) mortality compared to single application (36% at 30,000 IJs per plant). In field conditions, maximum insect mortality (37.5%) was recorded in combination of half the concentration of insecticide with the split concentration of *S. asiaticum* (25,000 IJs + Malathion 50 EC @ 0.025%). Similarly, Koppenhöfer et al. (2020a) reported that splitting the concentration of *S. carpocapsae* (2.5×10^9 IJs ha^{-1}) into two applications provided significantly highest control of *Listronotus maculicollis* (Kirby) (Coleoptera: Curculionidae) larvae than in one application. The combination of imidacloprid with the split *S. carpocapsae* application also provided significantly higher control (88–95%) than the respective single-agent treatment. In another findings, *S. carpocapsae* efficacy was improved by 13% through splitting into two applications at half rate against larvae of the black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) (Koppenhöfer et al. 2022). The logic of using split application in screen house experiment was to enhance the chances of contact between IJs and *P. xylostella* larvae. But, the duration of 3rd instar larvae of DBM was very short (3–4 days) because of which the full potential of EPNs in the containment of *P. xylostella* was not fully realized. Therefore, splitting EPN into two applications at half rate could be a potential method for improving EPN performance as recorded in present studies. This is because after inundative applications of billions of IJs per ha^{-1} populations tend to decline pest population quickly as noticed in earlier studies (Ebssa and Koppenhöfer 2011). Other

Table 2 Larval mortality (%) of *Plutella xylostella* by *Steinernema asiaticum* in screen house after second spray in split application

Time (h)	Concentrations of infective juveniles (IJs) per plant				Mean (time)
	15,000	10,000	Water + adjuvants	Water alone	
24 h	18.00 (24.63)	09.00 (15.09)	00.00 (04.05)	00.00 (04.05)	06.75 (11.96)
48 h	25.00 (28.93)	13.00 (20.26)	00.00 (04.05)	00.00 (04.05)	09.50 (14.32)
72 h	27.00 (30.57)	18.00 (25.17)	00.00 (04.05)	00.00 (04.05)	11.25 (15.96)
Mean (Concentration)	23.33 (28.05)	13.33 (20.17)	00.00 (04.05)	00.00 (04.05)	

Factors	SE (m)	C.D. (*P* = 0.01)
Time	(1.59)	(N.S)
Concentration	(1.83)	(5.23)
Interaction (Time × Concentration)	(3.18)	(N. S.)

Values in parentheses are angular-transformed $(x + 0.5)$ values

Table 3 Larval mortality (%) of *Plutella xylostella* by *Steinernema asiaticum* in screen house after first and second spray,* on cabbage

Time (h)	Concentrations of infective juveniles per plant					Mean (time)
	30,000	15,000 + 15,000	10,000 + 10,000	Water + adjuvants	Water alone	
24 h	29.00 (32.30)	37.00 (37.34)	28.00 (31.43)	03.00 (08.92)	01.00 (05.95)	19.60 (23.19)
48 h	36.00 (36.69)	52.00 (46.28)	42.00 (40.49)	03.00 (08.92)	01.00 (05.95)	25.40 (27.67)
72 h	43.00 (41.04)	56.00 (48.88)	50.00 (45.27)	03.00 (08.92)	01.00 (05.95)	30.60 (30.01)
Mean (Concentration)	36.00 (36.68)	48.33 (44.17)	40.00 (39.07)	03.00 (08.92)	01.00 (05.95)	
Factors			**SE (m)**			**C.D. ($P = 0.01$)**
Time			(1.78)			(5.03)
Concentration			(2.29)			(6.50)
Interaction (Time × Concentration)			(3.98)			(N.S.)

Values in parentheses are angular-transformed ($x + 0.5$) values

*Planned third spray could not be applied as all the DBM larvae transformed into pupae

Fig. 2 Adults of *Steinernema asiaticum* wriggling on the dead larva of *Plutella xylostella*
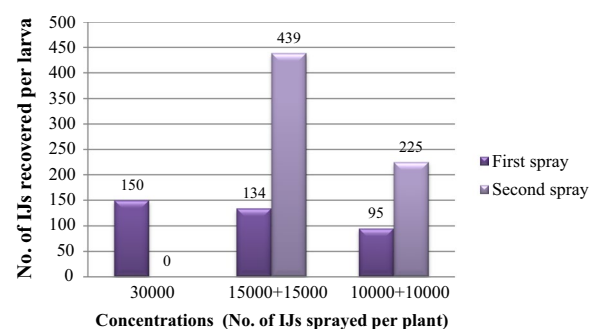
Fig. 3 Nematode multiplication (No. of IJs recovered per larva) in screen house experiment. (In third spray, 10,000 IJs could not be applied as all the larvae transformed in to pupae before the date of spray)

workers have also reported the efficacy of different *Steinernema* spp. against DBM (Belair et al. 2003).

Kaya and Gaugler (1993) documented that the field effectiveness of EPNs was very less than the laboratory tests. Soil moisture and temperature may affect the efficiency of those EPNs as they need some moisture for survival. DBM larvae cease feeding and may close their spiracles when sprayed by excessive amounts of water (Baur et al. 1995). This might explain the reduced percentage mortality in DBM larvae under field conditions when plants were drenched with the spray suspension, as compared to laboratory tests where the appropriate moisture percentage is maintained. UV light, warmer temperatures, dessication and the features of exposed foliage can promote lesser EPN activation in greenhouse circumstances than the laboratory conditions (Vashisth et al. 2013). Schroer et al. (2005a) reported that the same formulation decreased mobility of DBM larvae and at the same time provided conditions which enhanced nematode host seeking and invasion of the target insect. Waxy substance in the upper surface of leaves in cabbage perhaps leads to adherence of spray fluid containing nematodes. Spray fluid glued in form of droplet and move downward from leaf surface. Larvae feeding on the upper surface of leaves were attacked easily by the EPNs rather than larvae feeding on lower surface. DBM larvae preferably feed on lower surface of leaves (Saravanapriya and Subramanian 2006). Baur et al. (1995) also reported that *P. xylostella* mortality

Table 4 Evaluation of *Steinernema asiaticum* as foliar spray against *Plutella xylostella* on the cabbage plants under field conditions

	Treatment	Larval mortality (%)		Mean (time)
		24 h	48 h	
T1	50,000 IJs + adjuvants	26.3 (28.1)	31.3 (33.7)	28.8 (30.9)
T2	Malathion 50 EC @ 0.05%	18.0 (20.8)	18.0 (20.8)	18.0 (20.8)
T3	25,000 IJs + adjuvants + Malathion 50 EC @ 0.025%	36.3 (36.8)	38.8 (38.4)	37.5 (37.6)
T4	25,000 IJs + adjuvants	11.3 (16.2)	11.3 (16.2)	11.3 (16.2)
T5	Water + adjuvants	0 (4.05)	0 (4.05)	0 (4.05)
	Mean (time)	18.4 (21.2)	19.9 (22.6)	
Factors		**SE (m)**		**C.D. ($P = 0.01$)**
Treatment		(2.1)		(5.4)
Time		(2.6)		(N.S.)
Interaction (Treatment × Time)		(5.8)		(N.S.)

Values in parentheses are angular-transformed ($x + 0.5$) values

was higher in pubescent leaves than in glaborous leaves (cabbage plants). Susceptibility to nematodes was greater for early versus late 3rd instar DBM larvae (Baur et al. 1998). The factors outlined might have influenced present studies also, thereby leading to variation of results of efficacy in screen house and field studies.

Foliar application of EPNs exposes the IJs to the vagaries of dessication and UV radiation. The use of anti-desiccants and UV protectants along with surfactants into the nematode suspension was therefore necessitated (Schroer and Ehlers 2005). Schroer et al. (2005b) screened several adjuvants for toxicity on nematodes, plants or insects and tested different combinations of surfactants and polymers for their potential to improve nematode efficacy. Therefore, in present experiments, the concentrations of the adjuvants (glycerine, Triton X-100) were first standardized in the laboratory assays against *S. asiaticum* and only sub-lethal concentrations were used in this study. The nematodes were sprayed during the evening time between 5:30 to 6:30 PM to minimize UV and immediate desiccation and hence obtained good mortality in DBM larvae. Low humidity is a major limiting factor for nematode survival on foliage (Glazer 2002). Glazer (1992) recorded a drastic reduction in nematode efficacy 6 h after application to foliage. It was observed cessation in IJ movement after application to plant foliage, suggesting that further EPN uptake happened by insects consuming the IJs. There are other ways to optimize the efficacy of EPNs for greenhouse and field applications like preparing EPNs formulation, and EPNs can be combined with other control agents like chemical insecticides and other biological control agents like fungus and bacteria (Koppenhöfer et al. 2020b) as tested in the present studies. It

appeared that reduced concentrations of Malathion were less detrimental to EPNs and at the same time reduced the activity and feeding and growth activities of DBM larvae leading to enhanced mortality.

Conclusions

The efficacy of EPN, *S. asiaticum* against the DBM, *P. xylostella* larvae was tested under screen house and field conditions. Split applications (15,000 + 15,000 IJs per plant) of *S. asiaticum* were more effective than single application (30,000 IJs per plant) under screen house conditions. In field conditions, combinations of half the concentration of EPNs as well as insecticide (*S. asiaticum* @ 25,000 per plant + Malathion 50 EC @ 0.025%) proved more effectiveness than single treatment (*S. asiaticum* @ 50,000 per plant or Malathion 50 EC @ 0.05%). Splitting EPNs into two applications at half rate could be the potential method for improving efficacy of EPNs in the management of DBM, and also combination of half the concentration of Malathion with the split concentration of *S. asiaticum* provided significantly higher control than the respective single-agent treatment. The most effective, long-term way to manage pests is by using a combination of available pest control methods that work better together than separately, by the most economical means and with the least possible hazard to consumer as well as environment.

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Declarations

Ethics approval and consent to participate
The submitted article is the original work carried out by the authors and hence not been published elsewhere. The manuscript has not been submitted to any other journal for consideration. All authors have given their consent.

Consent for publication
The authors hereby give the consent for its publication in Egyptian Journal of Biological Pest control.

Competing interests
The authors declare that they have no competing interests.

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