Karaca *et al.*
Egyptian Journal of Biological Pest Control (2022) 32:84
https://doi.org/10.1186/s41938-022-00577-9

Egyptian Journal of
Biological Pest Control

RESEARCH

Open Access

Efficacy of some entomopathogenic fungi against tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Abstract

Background: Effects of entomopathogenic fungi (EPF); *Beauveria bassiana*, *Isaria fumosorosea* and *Pupureocillium lilacinum* against tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which is one of the most economic pests of tomato, were investigated in Petri dish, pot and greenhouse experiments. Commercial bioinsecticide Met 52 was also used in the experiments. In the Petri dish trials, *I. fumosorosea* applied to the third instar larvae by dipping method was recorded as the most effective entomopathogen, while the efficacy of *B. bassiana* was high in the spray and residue methods. In the pot experiments, entomopathogenic fungi were applied to tomato plants before and after infestation with *T. absoluta*.

Results: As a result of the applications, it was found that the EPF significantly reduced the formation of galleries on tomato leaves when applied before or at the beginning of tomato leafminer infestation. In the greenhouse experiments a registered insecticide Voliam Targo was also applied. All applications significantly reduced the numbers of galleries on tomato leaves than in the control plants, in both greenhouse trials, made in Antalya and Isparta Provinces. *I. fumosorosea* was the most effective one among the tested 3 EPF.

Conclusions: In this study, it was determined that the EPF *B. bassiana* and *P. lilacinum* can reduce tomato leafminer damage, while the efficacy of *I. fumosorosea* was the highest, and especially after two applications it was nearly effective as the registered insecticide.

Keywords: *Tuta absoluta*, Entomopathogenic fungi, Biological control, Efficacy

Background

Tomatoes are one of the most common and important vegetable crops in terms of production and consumption all over the world. The tomato leafminer, *Tuta absoluta* (Meyrick), has become the most destructive pest that causes significant losses, since tomato growers had no experience on this pest. The pest was recorded in Turkey in 2009 (Keçeci 2010). The pest can cause 80–100% yield loss in greenhouses or field grown tomatoes, if no control measure is applied (Durmuşoğlu et al. 2011). The pest can complete its life cycle in 28–29 days and has 10–12 generations per year (EPPO 2005). Different control methods have been used against *T. absoluta*. Pheromone traps have been used to catch the adults and decrease the population (Ünlü 2011). Chemical control has inevitably been used against the pest, because other control methods do not totally prevent its damage. Registered insecticides, mainly Azadirachtin, Metaflumizone and Spinosad have been used if leaves, twigs, stem or fruits are infested (Kılıç 2011). Although insecticides are considerably effective to reduce the pest population, they can also kill natural enemies and have negative effects on human health and environment (Topuz 2013). In addition, there are some reports on unsuccessful

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applications depending on the decreasing susceptibility of tomato leafminer against some synthetic insecticides (Desneux et al. 2010). Excessive use of insecticides causes an increase in production costs and harm on environment. Pesticide residue is another problem for tomato export (Durmuşoğlu et al. 2011). Because of the mentioned disadvantages of synthetic pesticides, biological control gained importance (Topuz 2013). Besides natural enemies, EPF are among the biological agents that were used against *T. absoluta. Isaria fumosorosea* (Wize) Brown and Smith, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin are the most common ones (Faria and Wraight 2007). *I. fumosorosea*, which has a wide host range, has been tried against different agricultural and forest insect pests as a mycoinsecticide (Konopicka et al. 2017). It was also tried against tomato leafminer. Blastospores of a strain of this fungus was applied onto tomato plants with *T. absoluta* eggs and it was found that the number of pest larvae significantly reduced (Zemek 2013). Similarly, *M. anisopliae* var. *anisopliae* and *B. bassiana* caused high mortality on the eggs of the pest (Pires et al. 2009). In another study applied in Turkey, efficiency of *B. bassiana* on eggs and first instar larvae of tomato leafminer, seven days after application by dipping method, were 42 and 4%, respectively, whereas *M. anisopliae* showed 92% efficiency against both stages of the pest (İnanlı et al. 2012). It was also tested against tomato leafminer and found to reduce the hatching ratio of the pest from 86 to 22% (Yüksel et al. 2017). The aim of this study was to investigate the possible use of three EPF against *T. absoluta,* and to compare their effects with Met 52, a mycoinsecticide containing spores (5.5×10^9 cfu/g) of *Metarhizium anisopliae* strain F52, and a registered insecticide, Voliam Targo 063 SC, under laboratory and greenhouse conditions.

Methods

Plant material

Three weeks old tomato seedlings of H2274 cultivar were regularly supplied from the commercial growers and transplanted to 20 cm diameter plastic pots containing sterilized soil-peat-manure mixture, one seedling per pot. Seedlings were then grown in a climatic room at the Department of Plant Protection, Faculty of Agriculture, Isparta University of Applied Sciences, at 25 ± 2 °C temperature, 60 ± 5% RH and 16:8 h. photoperiod. Tomato plants were transferred to separate growth chambers for tomato leafminer reproduction and in vivo experiments.

Tuta absoluta

Eggs, larvae and pupae of tomato leafminer, *T. absoluta*, were collected from the greenhouses in Antalya (situated in south-west Anatolia, between the longitudes 29°20'–32°35'East and latitudes 36°07'–37°29'North) and transferred to healthy tomato seedlings in the climatic rooms. Healthy plants were regularly transferred to the climatic room and changed with the severely damaged plants.

Entomopathogenic fungi (EPF)

Fungal material used in the study were *Beauveria bassiana*, *Isaria fumosorosea* and *Purpureocillium lilacinum* isolates obtained from the culture collection of the Mycology Laboratory of Plant Protection Department, Faculty of Agriculture, Isparta University of Applied Sciences. EPF were previously isolated from *Hyphantria cunea* Drury pupae and tested against this pest (Sullivan 2011). Fungal isolates were activated and reproduced on Potato Dextrose Agar (Merck). Spores of the fungi were transferred to a sterile glass bottle after scraping by a sterile scalpel and by washing with sterile distilled water containing 0.02% Tween 20, through two layers of cheesecloth, in order to separate mycelia. Concentration of the suspension was adjusted to 2×10^8 conidia/ml by adding some more spores or sterile water.

Petri trials

Third instar larvae of the moth were used in the Petri trials. Spore suspensions of the EPF were applied to the larvae, tenderly released from the leaf tissue, by three different methods. In the first method, larvae were transferred to cheesecloth and dipped into spore suspension for 5 s. Then, the larvae were transferred onto tomato leaflets on blotter paper in a sterile Petri dish. In the second method, larvae were first transferred onto tomato leaflets and then sprayed by the spore suspension. In the third method; tomato leaves were dipped into spore suspension for 5 s and placed onto blotter paper in a Petri dish. Then, larvae were transferred onto the leaves. For all methods, applications made with sterile distilled water, instead of spore suspension, were used as controls and the trials were performed by 20 replicate plates (5 larvae in each). Larval mortality rates were determined 3 and 7 days after the applications.

Pot trials

Tomato plants at 8–10 leaf stage grown in plastic pots in a clean growth room were used in the pot trials. Efficiency of the EPF was determined by three different methods. In the first method, spore suspension was prepared as in the Petri dish trials and sprayed onto tomato plants. These plants were then transferred to another climatic room with tomato moth infested plants and kept there for two days in order to ensure infestation. In the second method, tomato plants with 8–10 leaves were first kept for 2–3 days in the moth infested room and then sprayed with the spore suspensions and transferred to plexiglass cages in another room. In the third method, tomato plants were kept in the room with the infested plants for 6–7 days and waited for the pest larvae to form galleries on the leaves, then spore suspensions were applied and plants were transferred to plexiglass cages in a clean room. Plants sprayed with distilled water were used as control and the trials were performed with 12 replicate pots. In order to observe the development of the pest on the plants subjected to different applications, 2 leaflets from each plant in 6 replicate pots for each treatment were harvested every week for 4 weeks. Eggs, larvae, pupae and alive individuals of the pest and also the number of the galleries formed by the larvae were counted under a stereomicroscope. All leaves of the plants in other three pots were harvested after 15 days, and those of remaining three plants were harvested 30 days after the applications, and galleries formed by tomato moth and alive individuals were counted. Thus, damage caused by *T. absoluta* was evaluated both by weekly and two or four weeks after the applications.

Greenhouse trials

Trials were performed in two greenhouses, one of which was a grower's greenhouse about one decare in Kumluca District of Antalya Province, and the other with the same size, in the campus area of Isparta University of Applied Sciences, Isparta Province. Tomato seedlings were transferred to the greenhouses and regular agricultural practices were performed. Necessary measures were applied in order to prevent the possible pest and disease damage, but chemical control was not applied in the greenhouses. Before the applications, tomato plants in the greenhouses were protected in order to determine the natural infestation rates of the pest. In the greenhouse in Antalya Province, infestation rate of the pest was severe enough, while that in the greenhouse in Isparta was rather low and infested plants in the growth cages were transferred to the greenhouse and two pots per row were placed among the plants in order to increase the infestation rate. In the greenhouses, parcels with 60 plants for each treatment were formed for 6 treatments; 3 fungal isolates, bioinsecticide Met 52, a registered insecticide Voliam Targo 063 SC (Chlorantraniliprole 45 g/l + Abamectin 18 g/l, Syngenta Company) and control. Label rates of the insecticides and 2×10^8 spores/ml concentration of the fungal isolates were used in the trial. Applications in the greenhouses were made by spraying tomato plants with 8–10 leaves. One week after the first application, 30 plants in each parcel were sprayed again and then plants were controlled weekly for four weeks and larvae and galleries on the leaves were counted. Abbott formula was used to determine the efficiency of the applications (Abbott 1925). In addition, data on the numbers of death larvae and galleries were subjected to analysis of Variance and were statistically compared with Tukey test by using SPSS program (SPSS16_Mac_OSX_L).

Results

In vitro *efficiency of EPF*

When the effects of EPF in Petri dish trials were evaluated by Abbott formula, it was determined that the efficacy started 72 h after the application and all fungi caused death of almost all of the larvae 7 days after the applications. In the first application method (dipping), in which larvae were dipped into the spore suspensions of the EPF and transferred to clean tomato leaflets in a sterile petri dish, *B. bassiana* caused 87.65% mortality 72 h after the application, while *I. fumosorosea* showed the highest effect and killed all larvae after 7 days, as the bioinsecticide Met 52 (Table 1). In the second method (spraying), where spore suspensions were sprayed onto larvae transferred on tomato leaflets in the Petri dishes, the highest mortality was obtained by Met 52 and followed by *I. fumosorosea* on the third day, but all agents except *P. lilacinum* killed all larvae after 7 days. In the third application method (residue), where larvae were transferred onto and fed with tomato leaflets sprayed with spore suspensions, *B. bassiana* showed the highest mortality on the third day and on the seventh day its effect was similar with Met 52 causing 100% mortality.

When the results of Petri dish trials were statistically evaluated, there was significant difference among the mean numbers of alive larvae, 3 days after application with EPF, by dipping and spraying methods, while in the residue method they arranged in the same group (Table 2). There was no significant difference among the mean numbers of alive larvae 7 days after application

Table 1 Efficacy of entomopathogenic fungi against third instar *Tuta absoluta* larvae, 3 and 7 days after applications by three methods in petri dish trials

Time after applications (Days)	Applications	Mortality (%)		
		Dipping	Spraying	Residue
3	*Beauveria bassiana*	87.65	34.07	60.00
	Isaria fumosorosea	86.42	64.84	46.67
	Purpureocillium lilacinum	27.58	50.55	48.00
	Met 52	67.44	69.77	52.33
7	*B. bassiana*	98.68	100.00	100.00
	Isaria fumosorosea	100.00	100.00	98.57
	Purpureocillium lilacinum	97.37	98.90	94.29
	Met 52	100.00	100.00	100.00

Table 2 Mean numbers of alive third instar *Tuta absoluta* larvae, 3 and 7 days after the applications of entomopathogenic fungi by three methods in petri dish trials

Applications	Number of alive larvae					
	Dipping		Spraying		Residue	
	3 days	7 days	3 days	7 days	3 days	7 days
Beauveria bassiana	0.50^{d*}	0.05^{b}	3.00^{b}	0.00^{b}	1.50^{b}	0.00^{b}
Isaria fumosorosea	0.55^{d}	0.00^{b}	1.60^{d}	0.00^{b}	2.00^{b}	0.05^{b}
Purpureocillium lilacinum	2.85^{b}	0.10^{b}	2.25^{c}	0.05^{b}	1.95^{b}	0.20^{b}
Met 52	1.40^{c}	0.00^{b}	1.30^{d}	0.00^{b}	2.05^{b}	0.00^{b}
Control	4.05^{a}	3.80^{a}	4.55^{a}	4.55^{a}	3.75^{a}	3.50^{a}

[*] Means in the same column followed by the same letters were not significantly different from each other according to Tukey test ($P \leq 0.05$)

with EPF and all isolates were found to be effective as the bioinsecticide Met 52.

As a result of gallery counts in the pot trials, 15 and 30 days after the application of EPF, all fungi were found to be significantly decreased the numbers of galleries on tomato leaves, compared to controls, when applied before or after infestation. However, efficiency of the EPF decreased if the applications were made after infestation and in the third method, when applications were applied after gallery formation, where gallery numbers were higher than other methods (Table 3). Galleries could not be counted 30 days after applications on some plants, because of the severe damage on the leaves. Efficacy of the EPF as counted by Abbott formula was given in Fig. 1. As in the Petri dish experiments, there was no significant difference among the efficacy of the EPF against tomato leafminer damage. According to the pot experiments, it can be mentioned that the EPF should better be applied before or at the beginning of infestation and applications should be replicated not later than 15 days.

In the greenhouse trials performed in Antalya-Kumluca, according to the counts made one week after the applications, number of galleries on the leaves sprayed with *B. bassiana* and *P. lilacinum* were statistically arranged in the same group with the control plants, while *I. fumosorosea* significantly reduced the number of galleries, but not as Met 52 and insecticide Voliam Targo (Table 4). However, 7 days after the second application, all treatments significantly decreased the number of galleries on the leaves caused by *T. absoluta* compared to control, and *I. fumosorosea* arranged in the same group with the insecticide.

As a result of the greenhouse trial carried out in Isparta Province, the number of galleries on the tomato plants treated with EPF were significantly less than that of control plants. Especially after the second application, all treatments decreased the number of galleries and, *I. fumosorosea* and *P. lilacinum* were as effective as the insecticides. In the greenhouse trial in Antalya Province, the number of galleries on the tomato plants were rather high and very few fruits formed on the plants, while in Isparta Province, less humid than Antalya, the number of galleries were less and enough fruits were produced to evaluate the effects of treatments on fruit damage. Infested and healthy fruit numbers showed that all treatments significantly decreased

Table 3 Mean numbers of galleries caused by *Tuta absoluta* on tomato plants, 15 and 30 days after the application of entomopathogenic fungi at three methods in the pot dish trials

Applications	Mean numbers of galleries / leaf					
	Dipping		Spraying		Residue	
	15 days	30 days	15 days	30 days	15 days	30 days
Beauveria bassiana	12.00^{b*}	33.66^{b}	16.00^{b}	40.66^{b}	$36{,}33^{b}$	60.66
Isaria fumosorosea	10.33^{b}	30.00^{b}	13.33^{b}	46.33^{b}	$34{,}00^{b,c}$	55,33
Purpureocillium lilacinum	11.67^{b}	25.00^{b}	15.67^{b}	45.33^{b}	$25{,}33^{c}$	**
Met 52	9.00^{b}	33.33^{b}	14.00^{b}	39.33^{b}	$33.67^{b,c}$	59.67
Control	51.33^{a}	64.66^{a}	54.33^{a}	66.66^{a}	$56{,}33^{a}$	**

[*] Means in the same column followed by the same letters were not significantly different from each other according to Tukey test ($P \leq 0.05$)

[**] Galleries could not be counted because of the severe damage on the leaves

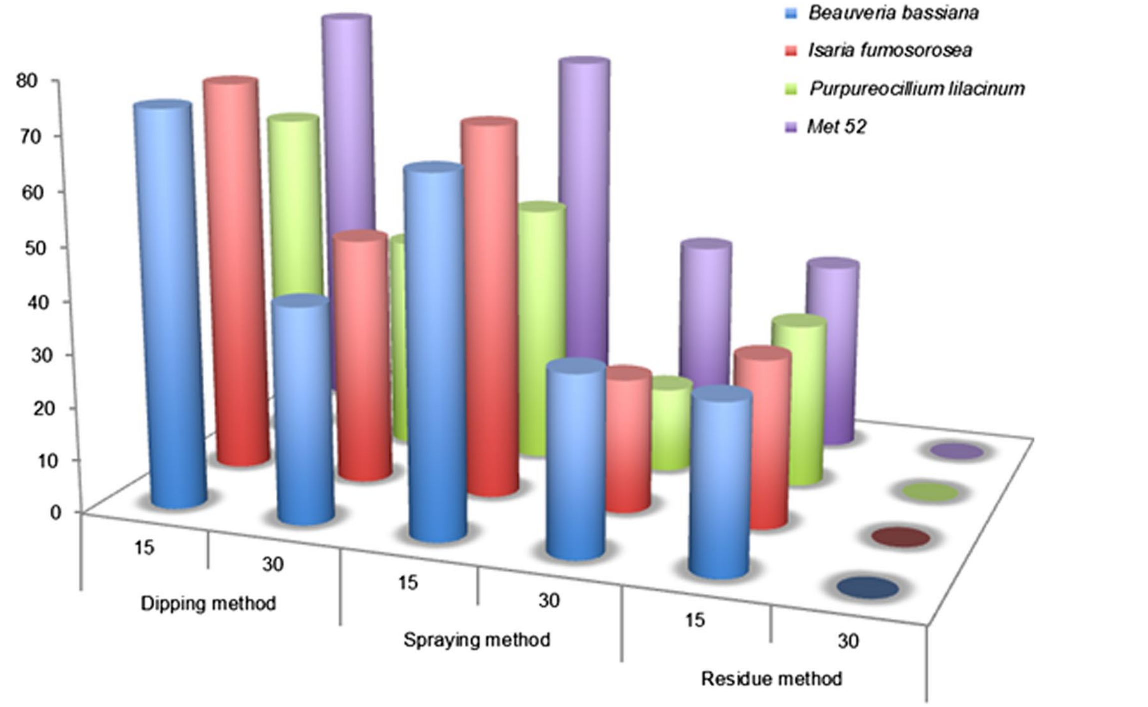

Fig. 1 Efficacy rates of the entomopathogenic fungi on the gallery formation on tomato leaves 15 and 30 days after the applications by three methods

Table 4 Mean numbers of galleries on the tomato plants in the greenhouses in Antalya and Isparta, 1,2 weeks after applications of entomopathogens (gallery/plant) and mean rates of infested fruits

Applications	Antalya		Isparta		
	gallery/plant		gallery/plant		Infested fruit (%)
	First week	Second week	First week	Second week	
Beauveria bassiana	39.10[a*]	38.25[b]	11.4[b]	14.1[b]	8.0[b]
Isaria fumosorosea	23.53[b]	24.37[c]	11.4[b]	9.9[b, c]	7.3[b]
Purpureocillium lilacinum	38.00[a]	37.73[b]	8.0[c]	12.5[b, c]	6.5[b]
Met 52	10.50[c]	9.70[d]	8.9[b, c]	9.3[c]	6.5[b]
Insecticide	13.00[c]	15.03[c, d]	6.1[c]	8.6[c]	7.0[b]
Control	37.73[a]	52.31[a]	15.0[a]	21.9[a]	12.7[a]

[*] Means in the same column followed by the same letters were not significantly different from each other according to Tukey test ($P \leq 0.05$)

the fruit damage and effects of EPF were similar with insecticides.

Discussion

Tuta absoluta is one of the most economic pest species causing significant damage on tomato plants. Chemical control is mainly used against tomato leafminer, however the commonly used insecticides have gradually lost effectiveness because the pest developed resistance. Thus, biological control gained importance (Gomez-Valderrama et al. 2014). As a result of this research performed by Petri dish, pot and greenhouse experiments, it was found that the EPF; *B. bassiana*, *I. fumosorosea* and *P. lilacinum* significantly reduced the damage caused by the tomato leafminer. The numbers of galleries on tomato leaves formed by the pest were reduced, especially with the applications of the EPF before or at the beginning of pest infestation. Pot and greenhouse experiments showed that all the EPF may reduce the damage caused by the tomato leafminer, however the highest effect similar to registered insecticides was obtained in the greenhouse trials by the two successive applications of *I. fumosorosea*. Various researchers have studied the effects of EPF against the tomato leafminer. Some of them compared the effects of EPF against different stages of the pest. *M. anisopliae* Qu-M558 and *B. bassiana* Qu-B928 isolates recorded 60–80% effective by spraying on the eggs of *T. absoluta* (Rodriguez et al. 2006a). In another study by the same researcher, 68% mortality rates were recorded as a result of spraying *M. anisopliae* var. *anisopliae* Qu-M558 and *Beauveria bassiana* Qu-B912 isolates on the third stage larvae of *T. absoluta* (Rodriguez et al. 2006b). In a research made in Turkey, efficiency rates of *B. bassiana* on the eggs and first instar larvae of tomato leafminer, 9 days after applications, were 67, and 12.5% and those of M. *anisopliae* were 100, and 92% (İnanlı et al. 2012). A strain of *I. fumosorosea,* applied to *T. absoluta* eggs significantly reduced the number of larvae (Zemek 2013). Abdel-Raheem et al. (2015) reported that the highest mortality rates against tomato leafminer were obtained by the applications of *B. bassiana* and *M. anisopliae* isolates on eggs, second and third instar larvae of *T. absoluta*, respectively. It was reported that *B. bassiana* preparation obtained by using liquid-semisolid fermentation technique was 95% effective on *T. absoluta* larvae (El Kichaoui et al. 2016). Yüksel et al. (2017) determined that the application of *B. bassiana* and *P. lilacinum* against the eggs of *T. absoluta* had the potential to control the pest. Similar to the obtained results, *B. bassiana* and *M. anisopliae* applied against *T. absoluta* larvae under laboratory and field conditions were found to be effective (Tadele and Emana 2017). In a study conducted under field conditions in Ethiopia, it was found that the application of *B. bassiana* was 74.14% effective against tomato leafminer (Shiberu and Getu 2017). It has been emphasized that *M. anisopliae* and *B. bassiana* isolates are important within the integrated control program against *T. absoluta* in tomato cultivation in greenhouse environment in Romania (Roxana 2018). Abebe (2019) reported mortality rates of *T. absoluta* larvae in pot and field experiments by *B. bassiana* application as 67.79 and 61.84%, and by *M. anisopliae* as 65.77 and 57.95%, respectively.

Conclusions

This study showed that EPF; *B. bassiana*, *I. fumosorosea* and *P. lilacinum* can be used against tomato leafminer. However, in vivo trials showed that the efficacy of EPF may change, depending on the timing of applications, and the severity of the damage caused by tomato leafminer related with the climatic conditions of the cultivation area. Thus, EPF should better be applied before or at the beginning of the infestation of the pest in order to decrease the damage effectively. Other cultural practices should also be applied to support the EPF.

Acknowledgements
Special thanks to the Ministry of Industry and Technology (Project Number: 00784.STZ.2011-1), Scientific Research Projects Coordination Unite of Süleyman Demirel University (Project Numbers: 2951-YL-11 and 2942-YL-11) and Bioglobal Company for their financial support and Gregory Sullivan who supplied the *Hyphantria cunea* pupae, from which entomopathogens were isolated.

Author contributions
GK, ABE, BAÇ, HA, and İK collaborated in the creation of the manuscript. GK, ABE, BAÇ and HA carried out the experiments, recorded data, interpreted the results, and wrote the manuscript. All authors read and approved the final manuscript during present study.

Funding
Not applicable.

Availability of data and materials
All data generated or analyzed during this study are included in this manuscript.

Declarations

Ethical approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

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

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Received: 28 February 2022 Accepted: 18 June 2022
Published online: 06 July 2022

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