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Research Article

Efficacy of integrated pest management tools evaluated against *Tuta absoluta* (Meyrick) on tomato in India

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ABSTRACT: South American tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an invasive pest on tomato and other solanaceous crops. In general, 20 to 30 % yield loss is caused by this pest and sometimes it may result in 100% damage, if timely management interventions are not followed. Though the pest was reported in India during 2014, presently it has spread to several tomato growing states. In the present study various IPM tools have been evaluated against this pest. As a long-term strategy of resistance breeding, genotype screening was carried out for identification of resistance sources from wild and cultivated tomato genotypes showing resistance/tolerance against *T. absoluta*. Among the evaluated wild and cultivated tomato genotypes, *Solanum pennellii* (Accession, LA 1940) was identified as a resistant source against *T. absoluta* both under choice and no-choice bioassays and is being used for resistance breeding. Various entomopathogens (*Bacillus thuringiensis, Metarhizium anisopliae, Beauveria bassiana* and *M. rileyi*), egg parasitoids (*Trichogramma chilonis, T. pretiosum* and *Trichogrammatoidea bactrae*), light traps, pheromone traps, synthetic insecticides, botanical origin insecticides were also evaluated for their relative efficacy. Among the egg parasitoids *T. pretiosum* and among synthetic chemicals, spinetoram 12 SC@ 1.25ml/l were found very effective for the management of *T. absoluta*. Yellow light traps were found as an effective component for integrated management of *T. absoluta*. Azadirachtin 5% EC at the tested concentrations showed highest mean radial growth (24.67 mm) with relatively less inhibition (16.51%) of *M. anisopliae* indicating these combinations can be effectively utilised in the eco-friendly management of *T. absoluta.* We reported natural incidence of *M. anisopliae* on *T. absoluta* larvae*,* causing up to 35 per cent mortality during 2016-17.

KEY WORDS: Entomopathogens, host plant resistance, IPM, light traps, pheromone traps, *Tuta absoluta*

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INTRODUCTION

South American tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a devastating pest of tomato that has undergone a rapid expansion since its first report from India and has the potential to occur throughout the year in the tomato ecosystem (Nitin *et al.,* 2017; Sridhar *et al.,* 2014). Presently, the pest has spread to other states like Maharashtra, Tamil Nadu, Andhra Pradesh, Telangana, Gujarat, Delhi, Chhattisgarh etc. Taram *et al.,* 2016). Application of chemical insecticides is the most commonly used practice for suppression of *T. absoluta* infestations. Though the pesticides used against this pest give satisfactory control, extensive use of insecticides may lead to the development of insecticide resistance (Siqueira *et al.,* 2000; Lietti *et al*., 2005). In general, 20 to 30 % yield loss is caused by this pest and may result in 100% damage, if timely management interventions are not followed. Entomopathogens like *Bacillus thuringiensis, Metarhizium anisopliae, M. rileyi* and *Beauveria bassiana* are eco-friendly and effective options both under polyhouse and open field conditions. At ICAR-Indian Institute of Horticultural Research, Bengaluru some of the components of IPM including biocontrol agents were evaluated for identifying effective treatments for including in integrated management of *T. absoluta.*

MATERIALS AND METHODS

Various experiments carried out at ICAR-IIHR for the management of *Tuta absoluta* are briefly described below.

Screening of tomato genotypes for resistance against *Tuta absoluta*

Twenty one genotypes (11 wild and 10 cultivated) were evaluated in the green houses by using the methodology of Maluf *et al.* (1997) and Rakha *et al.* (2017). Within twenty plants of each genotype, five plants were screened for the trichome density (both glandular and non glandular) on abaxial and adaxial surfaces of the tomato leaves by using scanning electron microscope (model: TM 3030 plus, Hitachi Co., Japan). Trichomes were grouped according to Luckwill (1943). *T. absoluta* damage parameter (larval numbers, per cent leaf damage and adult activity) were correlated with the trichome types to know whether any correlation exists between trichomes and level of resistance in the tomato genotypes.

Traps for monitoring of adults of *Tuta absoluta*

The solar light traps and pheromone traps were installed on 1st September 2016 @ one and eight per acre, respectively for monitoring of the pest. The insects trapped were recorded on daily basis starting from installation of traps in the tomato field and data was cumulated on weekly basis for the entire cropping period. Different colour light traps were installed in poly houses of tomato to observe the best light source for *T. absoluta* attraction.

Entomopathogens and egg parasitoids

Four entomopathogens, *viz*., *Bt* (1 ml/l), *Metarhizium anisopliae, M. rileyi* and *Beauveria bassiana* @ 1 x 10^8 cfu/ml were evaluated for their efficacy under field conditions. Five replications were used for each treatment including control. Two sprays were given after noticing 1-2 mines/leaf. Mortality of *T. absoluta* larva was recorded at weekly interval.

Egg parasitoids of *Trichogramma pretiosum, T. bactrae* and *T. chilonis* were released at weekly interval @ 50,000/ha for five weeks starting from first incidence of the *T. absoluta* observed in the light trap/pheromone trap. Each tomato plot for various treatments consisted of 8 m x 8 m measurement, each replicated five times. The observations on *T. absoluta* live mines/parsitism were recorded on 3, 7- and 10-days interval in each of the treatments.

Bioefficacy of insecticides

Eleven insecticides were evaluated against the pest during *rabi* (2016-17) under field conditions. The experiment was laid out in a randomised block design with 12 treatments including control, each replicated thrice. The seedlings (cv. Shivam) were transplanted during first week of October 2016. The tomato crop was raised as per the recommended package of practices, except plant protection protocols. A total of five sprays were given at fortnight interval. Per cent reduction in live mines of *T. absoluta* over control was assessed after each spray and healthy fruit yield was assessed at each harvest. Observations on live mines of *T. absoluta* were taken from five plants selected randomly from each plot (six leaves/plant). Observations were recorded on 3, 7, 10 and 14 days after the sprays. The per cent data on the incidence of *T. absoluta* was transformed to arcsine values before subjecting it to statistical analyses using ANOVA and DMRT. Being a newly invaded pest, baseline susceptibility of egg and larval stages of the pest were carried out with various groups of insecticides and data was assessed through $LC_{50.}$

Compatibility of pesticides with entomopathogenic fungi, *Metarhizium anisopliae*

Earlier, we reported natural incidence of entomopathogenic fungus, *Metarhizium anisopliae* on *T. absoluta* in tomato causing up to 35% mortality of the larvae. As different pesticides are being used in tomato ecosystem, for knowing the antagonising or synergizing effect of these pesticides *i.e.,* compatibility with the fungus, the present study was undertaken. The commonly used pesticides were tested against the entomopathogenic fungi, *M. anisopliae* by using poisoned food technique in Potato Dextrose Agar (PDA) medium (Moorhouse *et al*., 1992).

The experiment was carried out using a completely randomised design using seven pesticides at recommended (X) and double (2X) the recommended dose/concentration along with control (Table 1) each replicated thrice. Isolate of *M. anisopliae* obtained from cadavers of *T. absoluta* was used for the study. Hundred ml of PDA was sterilized and added with the target pesticides and 20 ml each was poured into 25 mm diameter sterile Petri dishes and were allowed to solidify under laminar flow cabinet. An agar disc of 5 mm mat of *M. anisopliae* was cut with cork-borer and was inoculated

Table 1. List of pesticides and doses used in the study

Treatments/ Pesticide formulations	Trade Names	Recom-mended dose (X)	Double the Recommended dose (2X)
T_{1-}Lamda cyhalo-thrin 5 EC	Reeva	0.5 ml/l	1 ml/l
T_{2-}Azadirachtin 5 EC	Neemazal	2 ml/l	4 ml/l
T_{3-}Indoxacarb 14.5 SC	Kingdoxa	0.75 ml/l	1.5ml/l
T_{4-}Thiomethoxam 25 WP	Actara	0.3 g/l	0.6g/l
T_{5-}Chlorant-raniliprole 18.5 SC	Coragen	0.3 ml/l	0.6ml/l
T_{6-}Carbendazim 50 WP	Bavistin	1 g/l	2 g/l
T_{7-}Mancozeb 75 WP	Tata M-45	2 g/l	4 g/l
T_{8-}Control		-	-

at the centre of the PDA plate. PDA with only mycelial disc, served as control. The Petri dishes were sealed with parafilm and incubated at room temperature for fungal growth. The diameter of growing culture, *i.e.,* the radial growth in each Petri dish was measured on 10th day after inoculation (DAI). The data were expressed as percentage growth inhibition of *M. anisopliae* in pesticide treated PDA (Hokkanen and Kotiluoto, 1992).

RESULTS AND DISCUSSION

Screening of tomato genotypes against *Tuta absoluta*

Among twenty-one genotypes screened against *Tuta absoluta*, six wild accessions, *viz. Solanum pennellii* (LA 1940); *S. chilense* (LA 1963); *S. arcanum* (LA 2157); *S. lycopersicum* (LA1257) and *S. corneliomulleri* (LA 1292, LA1274) were relatively resistant based on mean per cent damage and were further studied under *in vitro* conditions. Glandular trichomes (Type I, IV, VII) showed negative correlation in different genotypes of tomato with reference to larval number/plant, per cent damage and adult activity, while Type V (non-glandular) trichome showed negative correlation with larval number/plant Table 2, 3 and Fig. 2. Glandular (G) and non-glandular (NGTs) play important role in host plant resistance by affecting the performance of herbivores (Bitew, 2018). *S. pennellii* showed highest resistance both under choice and no choice conditions, hence selected for breeding for *T. absoluta* resistance and the trials are in progress. Host-plant resistance was explored by developing tomato accessions with high zingiberene and/or acylsugar contents resulting on low ovipostion rates and larval feeding of *T. absoluta* (Maluf *et al.*, 2010). Rakha *et al.* (2017) observed the role of glandular trichomes in host plant resistance against *T. absoluta*.

Traps

Highest number of *Tuta. absoluta* adults were trapped in December 2016 i.e., 79 and 104 per trap in solar light and pheromone traps, respectively followed by November 2016 (70 and 95) and least were trapped in the month of February 2017 (50 and 47). Weekly traps of *T. absoluta* in solar light traps and pheromone traps are presented in Fig. 1. Sex pheromone traps attracted only males. Few females were also trapped in light traps along with males, indicating their potential utilisation in the IPM programme

Table 2. Relative abundance of different types of trichomes in resistant lines of tomato against *Tuta absoluta* per 0.5 mm^2

Tomato wild genotypes	Accession no.	Abaxial surface								Adaxial surface							
		NG			G					NG			G				
		V	III	Total	I	IV	VI	VII	Total	V	III	Total	I	IV	VI	VII	Total
S. pennellii	LA-1940	0.00	0.00	0.00	18.67	10.67	4.33	0.00	33.67	0.00	0.00	0.00	7.67	15.00	5.00	0.00	27.67
S. chilense	LA-1963	79.33	147.67	227.00	0.33	0.00	0.00	0.00	0.00	40.00	20.33	60.33	0.00	0.00	0.67	2.00	2.67
S. corneliomulleri	LA-1274	1.33	7.00	8.33	22.67	15.67	0.33	0.00	38.67	6.67	1.33	8.00	11.67	14.00	2.67	0.00	28.33
	LA-1292	47.33	17.67	65.00	0.33	0.00	3.00	0.00	3.33	13.33	1.00	14.33	0.00	0.00	10.00	1.67	11.67
S. lycopersicum	LA-1257	37.67	193.33	231.00	0.00	1.33	9.00	0.00	10.33	112.00	1.33	113.33	0.00	0.00	5.67	13.33	19.00
S. arcanum	LA-2157	7.33	0.00	7.33	0.00	0.00	0.67	4.00	4.67	2.33	0.00	2.33	0.00	0.00	1.00	5.67	6.67

G-Glandular; NG-Non glandular

Table 3. Correlation matrix of different parameters of *Tuta absoluta* damage v/s trichomes*

Parameters of *T. absoluta*	Leaf surface	Non- Glandular Trichomes			Glandular Trichomes				
		V	III	Total	I	IV	VI	VII	Total
Larval nos.	Abaxial	-0.005	0.13	0.10	-0.46	-0.49	0.38	-0.15	-0.15
	Adaxial	-0.05	0.06	-0.01	-0.46	-0.46	0.51	-0.05	0.35
	Cumulative	-0.04	0.14	0.07	-0.47	-0.47	0.50	-0.09	0.22
% leaf Damage	Abaxial	0.11	0.22	0.22	-0.33	-0.31	0.03	-0.20	-0.31
	Adaxial	0.27	0.53	0.44	-0.29	-0.35	0.10	-0.19	-0.06
	Cumulative	0.23	0.33	0.32	-0.32	-0.34	0.09	-0.23	-0.17
Adult activity	Abaxial	0.12	0.05	0.09	-0.29	-0.27	0.54	-0.13	0.02
	Adaxial	0.02	0.05	0.03	-0.25	-0.30	0.70	-0.01	0.54
	Cumulative	0.08	0.06	0.08	-0.29	-0.31	0.52	-0.15	0.22

*Correlation coefficient values

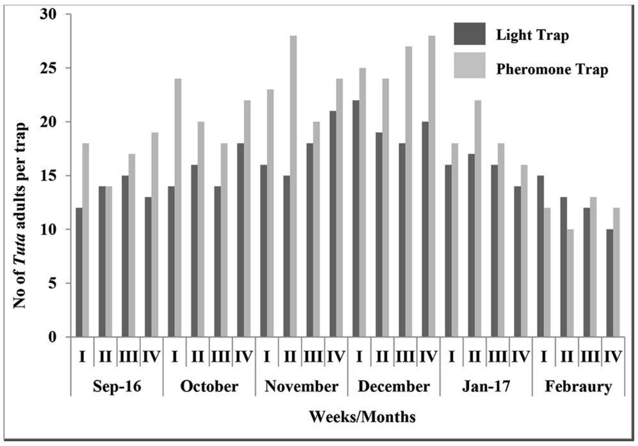

Fig. 1. Mean number of *Tuta* adults trapped/week in light trap and pheromone traps during 2016-17.

of this pest. Yellow incandescent bulb traps were found very effective in attracting *T. absoluta* followed by bluish white light traps. The pest has the potential to occur throughout the year in the tomato ecosystem (Nitin *et al.,* 2017).

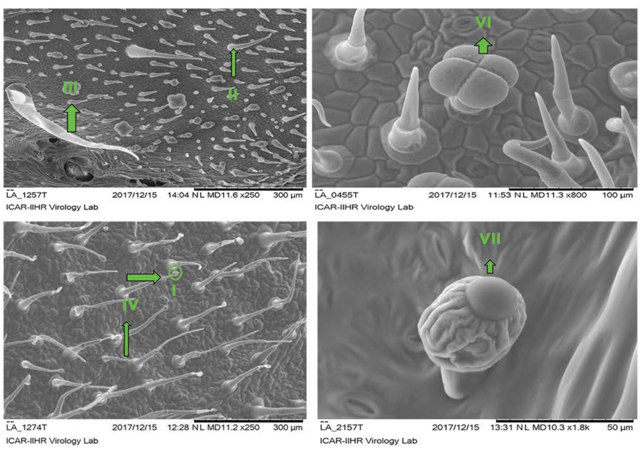

Fig.2. Different types (I - VII) of trichomes in tomato observed under SEM

Entomopathogens and egg parasitoids

Various entomopathogens have resulted in 70-81 % reduction in larvae of *Tuta absoluta* on tomato. Among them Bt was found most effective with 81.93% reduction in *T. absoluta* (Table 4). Among the egg parasitoids evaluated, *T. pretiosum* was found promising (45% parasitisation) followed by *T. chilonis* and *T. bactrae* (Table 5). Several biocontrol agents are used to control *T. absoluta* in open field and greenhouse tomato cultivation. *Bacillus thuringiensis* (Bt)-based insecticide formulations have been used to control *T. absoluta* in its native and invaded regions. Several studies have demonstrated the efficacy of Bt in controlling *T. absoluta* particularly first-instar larvae without any side effects on beneficial arthropods (Mollá *et al*., 2011). Several fungal species including *Metarhizium anisopliae* and *Beauveria bassiana* are reported to attack the eggs, larvae and adults of *T. absoluta.* Studies have revealed up to 54% mortality of *T. absoluta* adults by *M. anisopliae* (Pires *et al*., 2010). Faria *et al*., (2007) studied the efficacy of egg parasitoid, *T. pretiosum* against *T. absoluta* on tomato and observed upto 28% parasitisation.

Table 4. Efficacy of entomopathogens on *Tuta absoluta*

Treatment details	Trade name and Accession number	Dose	Mean reduction of *T. absouta**
Bacillus thuringiensis	Lipel (NCIM-2514)**	1g/l	81.93^{a} (64.82)
Beauveria bassiana (1 X 10^8 spores/ml)	Racer (NCIM-1216)**	3ml/l	75.11^{bc} (60.05)
Metarhizium anisopliae (1 X 10^8 spores/ml)	Pacer (NCIM-1311)**	3ml/l	70.59^{c} (57.14)
M. rileyi (1 X 10^8 spores/ml)	IIHR strain	3ml/l	77.36^{b} (61.77)
Control		-	0.00
	CV		6.62
	CD (p=0.05)		11.33

*Average of 2 sprays, three observations at 10 days interval

** Ajay Agro Tech Pvt. Ltd., Rajasthan

Efficacy of insecticides against *Tuta absoluta*

Among the different insecticides, spinetoram 12 SC @ 1.25g/L, (88.59 %), cyantraniliprole 10 OD @ 1. 8 ml/L (83.45 %), flubendiamide 480 SC @ 0.3ml/L (80.80 %), spinosad 45 SC @ 0.25 ml/L (78.99 %), indoxacarb 14.5 SC @ 0.75 ml/l (74.36 %) and chlorantraniliprole 18.5 SC @ 0.3 ml/L (74.26 %) were found effective against *T. absoluta* (Sridhar *et al.,* 2016). Baseline toxicity against *T. absoluta* egg and larval stages, spinetoram followed by spinosad, chlorantraniliprole, indoxacarb and flubendamide were toxic in the descending order. Botanical based Azadirachtin 5% EC at 2 ml/L was effective against *T. absoluta* resulting in 69.87 % reduction in live mines of *T. absoluta* and is relatively safe to the natural enemies also (Sridhar *et al*., 2016). Studies from others conducted elsewhere revealed that different insecticides were found effective against *T. absoluta* like spinosad (Bratu *et al*., 2015; Abdelgaleil *et al*., 2015), azadirachtin, emamectin benzoate, spinosad, chlorantraniliprole (Eleonora and Vili, 2014) chlorantraniliprole + abamectin (Ali *et al*., 2014), cyantraniliprole (Patricia *et al*., 2014), indoxacarb and chlorantraniliprole (Roditakis *et al*., 2013).

Compatibility of pesticides with *Metarhizium anisopliae* radial growth and growth inhibition

Among the insecticides (T_1 to T_5) tested, at recommended dose (x), azadirachtin 5% EC followed by chlorantraniliprole, showed maximum radial growth of 31.33 mm and 28.33 mm with least growth inhibition of *M. Anisopliae*, *i.e*., 10.28% and 18.71%, respectively. Similar results were obtained with these insecticides even at double the recommended dose (2x) with radial growth of 27 mm and 21 mm with growth inhibition of 22.75 % and 40.34%, respectively. Among the fungicides (T_6 and T_7) tested at X and 2X doses, carbendazim showed radial growth of 14 mm and 9.67 mm (*M. anisopliae*) with growth inhibition of 60.20% and 72.49%, respectively (Table 6). Thus, among the pesticides, Azadirachtin was relatively less toxic to *M. anisopliae* at the concentrations tested.

Spore count

Data on sporulation of *M. anisopliae* in relation to pesticides treated media are presented in Table 2. Among the various pesticides tested at two concentrations, the highest mean spore count was recorded in control (T_8)

Table 5. Per cent egg parasitisation of *Tuta absoluta* by *Trichogramma* species

Species of egg parasitoid	Percent parasitisation after release of egg parasitoids					Mean*
	I Week	II Week	III Week	IV Week	V Week	
Trichogramma chilonis	40.00	44.00	35.00	31.00	40.00	38.00[b]
Trichogrammatoidea bactrae	35.00	44.00	50.00	40.00	36.00	41.00[ab]
Trichogramma pretiosum	48.00	55.00	48.00	44.00	45.00	48.00[a]

Cumulative mean analysis results: SEM: 2.35; CD (p = 0.05) = 7.20 and cv = 12.35
*Means with the different letters are significant ($p > 0.05$) as analysed by Duncan Multiple Range Test (DMRT).

Table 6. Compatibility of various pesticides with *Metarhizium anisopliae*

Treatment/ Pesticide formulations	Performance of *M. anisopliae* in different pesticides								
	Radial growth (mm)			Growth inhibition (%) *			*Mean spore count* (1x10^8 spores/ml) #		
	X	2X	Mean	X	2X	Mean	X	2X	Mean
T_{1-} Lamda cyhalothrin 5 EC	16.67	14.67	15.67	52.11 (46.22)	58.16 (49.71)	55.14 (47.95)	2.70 (1.79)	2.20 (1.64)	2.45 (1.72)
T_{2-} Azadirachtin 5 EC	31.33	27.00	29.17	10.28 (17.93)	22.75 (28.44)	16.51 (23.86)	4.40 (2.21)	3.90 (2.10)	4.15 (2.16)
T_{3-} Indoxacarb14.5 SC	27.33	22.00	24.67	21.57 (27.33)	36.56 (36.79)	29.07 (32.21)	2.11 (1.61)	1.79 (1.51)	1.95 (1.56)
T_{4-}Thiomethoxam 25 WP	22.67	19.33	21.00	34.88 (36.08)	44.75 (41.99)	39.82 (39.10)	2.62 (1.76)	2.16 (1.63)	2.39 (1.70)
T_{5-} Chlorantraniliprole 18.5 SC	28.33	21.00	24.67	18.71 (25.21)	40.34 (39.12)	29.53 (32.83)	3.27 (1.94)	2.63 (1.77)	2.95 (1.86)
T_{6-} Carbendazim 50 WP	14.00	9.67	11.83	60.20 (50.96)	72.49 (58.38)	66.34 (54.59)	1.77 (1.50)	1.56 (1.43)	1.67 (1.47)
T_{7-} Mancozeb 75 WP	10.67	4.00	7.33	69.68 (56.65)	88.44 (70.57)	79.06 (62.84)	0.91 (1.15)	0.33 (0.88)	0.62 (1.03)
T_{8-} Control	35.00	35.00	35.00	-	-	-	5.00 (2.34)	5.00 (2.34)	5.00 (2.34)
SEM ±	0.73	1.14	0.65	1.90	2.12	1.43	0.06	0.04	0.04
CD at 5%	1.51	2.36	1.34	3.93	4.41	2.97	0.12	0.09	0.08

X: Recommended dose; 2X: Double the recommended dose; *Figures in parentheses are arcsine transformed values; # Figures in parentheses are square root transformed values

(5.00 x 10^8 spores ml^{-1}) followed by azadirachtin (T_2) (4.15 x 10^8 spores ml^{-1}) and chlorantraniliprole (T_5) (2.95 x 10^8 spores ml^{-1}) (Table 6). Possibility of combining botanicals with microbial for enhanced efficacy against insect pests was established earlier by (Antonio *et al*., 2001).

Thus from the present study, azadirachtin 5% EC at the tested concentrations showed highest mean radial growth (24.67 mm) with relatively less inhibition (16.51%) of *M. anisopliae*. These combinations can be effectively utilised in the eco-friendly management of *T. absoluta*.

Various IPM protocols for *T. absoluta* were worked out in other parts of the world (Goda *et al*., 2015) and needs to be standardised for indian conditions. The management options for *T. absoluta* should start from raising of healthy seedlings, as the pest causes the damage to the crop from seedling stage to final harvest of the crop. Hand picking and destruction of *T. absoluta* infested leaves and other plant parts, installation of light traps, pheromone traps minimises the population build-up of the pest. Though several management options are available for *T. absoluta*, there is a need for integrating them.

Being a newly invaded pest attacking major vegetable crops like tomato, these studies contributes for the development of effective IPM modules for *T. absoluta* by including effective treatments into the IPM modules which are environmentally friendly like light traps, pheromone traps, biocontrol agents like egg parasitoids, entomopathogens and eco-friendly insecticide molecules.

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