Journal of Entomology

ansinet
Asian Network for Scientific Information

OPEN ACCESS Journal of Entomology

ISSN 1812-5670
DOI: 10.3923/je.2019.91.97

Research Article
Susceptibility and Biochemical Determination of House Fly (*Musca domestica* L.) to Lambda-cyhalothrin

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Abstract

Background and Objective: The house fly, *Musca domestica* L., has the ability to develop resistance against pyrethroid insecticides in a short exposure time. This study aimed to screen the susceptibility of house fly to lambda-cyhalothrin and determine the activities of metabolic enzymes in insect. **Materials and Methods:** House fly populations collected from different locations in Sharkia Governorate, Egypt were used. Bioassay experiments were carried out on 3rd larval instars of each population. Activities of metabolic enzymes were determined. **Results:** Data of bioassay experiments indicated that population collected from Kafr Sakr is 10.6-fold resistant to lambda-cyhalothrin. Other populations collected from San El-Hagar, Abo Kabir, Abo Hammad and Zagazig showed 8, 9.8, 6.9 and 7.5 fold of resistance as compared on LC_{50} levels. Biochemical assays revealed that San El-Hagar and Kafr Sakr populations have the highest significant activity of esterase, while Zagazig population showed the highest activity in carboxylesterase recording 2.58 fold higher than laboratory strain followed by populations from Kafr Sakr and San El-Hagar. Results of mixed function oxidase were significantly higher in both Zagazig and Abo Hammad populations with 1.3 and 1.3 fold than what recorded in the laboratory strain. Kafr Sakr and Abo Hammad populations showed the highest significant GST activity recording 174.7 and 170 mmol min^{-1} g^{-1} b.wt., respectively. **Conclusion:** The obtained results showed that populations collected from different locations in Sharkia Governorate have low to moderate resistance to lambda-cyhalothrin with the possible role of carboxylesterase. The existing resistance is a warning to carefully use lambda-cyhalothrin in control programs of this pest in the specified locations.

Key words: House fly, lambda-cyhalothrin, bioassay, carboxylesterase, GST, MFO

Citation: Ibrahim A. Hamed, Refat M. Sherif, El-Sayed A. El-Sheikh and Aly A. Shallaby, 2019. Susceptibility and biochemical determination of house fly (*Musca domestica* L.) to lambda-cyhalothrin. J. Entomol., 16: 91-97.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The house fly, *Musca domestica* L., is one of the major public health insect that adversely affects on both human and animal health by carrying more than 100 disease pathogens[1]. House flies are spreading not only in dairies and livestock facilities, but also in urban cities causing harmful effects for humans and domestic animals such as nuisance, aesthetic damages to different structures and also play a role in the mechanical transmission of pathogens[2].

Control methods for urban pests including house flies are depending mainly on insecticides for being fast acting, relatively cheap and convenient to use[3]. Synthetic insecticides have been used for decades as a principle control strategy for this pest. Pyrethroids are a group of insecticides that showed importance in pest control and used widely in the control of agricultural and health pests. They account for approximately 25% of the world insecticide market[4]. Lambda-cyhalothrin, a sodium channel modulator, has been used extensively for the management of various insect pests including *M. domestica*[5].

Due to the quick and easy of insecticides application, these methods become the choice of people to face house flies infestations. However, house flies have the ability to become resistant against insecticides used for their management usually in a short period of time[6]. Extensive applications have resulted in many incidents of resistance to these insecticides with different mechanisms of resistance i.e., metabolic resistance to pyrethroids in insects can be associated with increase in cytochrome P450 activity, increase in general esterase and elevated glutathione S-transferases due to high genes expression, enabling them to overproduce these types of enzymes[7,8]. The P450-mediated detoxification and sodium channel-mediated target site insensitivity are major mechanisms related to pyrethroid resistance development in house flies[9]. Increase resistance levels to insecticides in this pest has become a major issue due to the extensive and poorly optimized application of insecticides[10]. The purpose of this study was to investigate the current status of house fly susceptibility that collected from 5 localities in Sharkia Governorate, Egypt. In addition this study will provide baseline data that will help to define enzymes that might associate in house fly resistance to pyrethroid.

MATERIALS AND METHODS

Insects and rearing: A laboratory strain was established by collecting house fly larvae from fields in Zagazig, Egypt (during summer of 2016) and reared in the laboratory for 25 generations without any exposure to insecticides. A field strain was collected from dairy farms of 5 locations in Sharkia Governorate, Egypt (San El-Hagar, Abo Kabir, Abo Hammad, Kafr Sakr, Zagazig) during summer of 2017 and 2018. About 500 larvae of *Musca domestica* L., were collected from each location and brought to the laboratory for rearing. The larvae were reared on a medium containing of powdered milk, sugar, yeast and wheat bran at a ratio of 1.5:1.5:5:20 (w/w), respectively and made a paste with 65 mL water[11]. The previous ingredients were mixed to prepare the rearing media. By reaching pupation, around 100 pupae from each location were transferred into a cage (40×40×40 cm) provided with wire net from three sides to allow gas exchange as well as keeping adults inside the cage, while it provided with a hole covered with piece of cloth from the fourth side to allow dealing with insect. After adult emergence, they fed on powdered milk mixed with sugar (1:1) and cotton wick soaked with water was provided in a separate plastic cup. All the insects were maintained at 27±2°C, 60-65% RH and 14:10 h (light: dark) photoperiod.

Chemicals: Commercial grade formulation of lambda-cyhalothrin (Cycon 10% EC, Shoura Company, Egypt) was used for bioassays. α-Naphthyl acetate (α-NA), β-Naphthyl acetate (β-NA), diazoblue, sodium lauryl sulphate, α-naphthol, β-naphthol, methyl n butyrate (MeB), alkaline hydroxylamine, NaOH, HCl, $FeCl_3$, 1-chloro 2,4-dinitrobenzene (CDNB), ethanol, P-nitroanisole o-demethylation, NADPH, glucose-6-phosphate, glucose-6-phosphate dehydrogenase (G-6-P-D), acetone, CHCl3, ethanol, bovine serum albumin (BSA) were products of sigma chemical Co (St Lois, MO, USA). Bovine albumin standard was purchased from Stanbio laboratory (Texas, USA). Coomassie brilliant blue G-250 was from sigma (sigma chemical Co.). P-nitroanisole (purity 97%) was obtained from Ubichem Ltd. (Hampshire), while nicotinamide adenine dinucleotide phosphate (reduced form, NADPH) was from BDH chemicals Ltd. (Poole, England).

Bioassay: Bioassays were performed in the Bioassay laboratory at Plant Protection Department, Zagazig University during 2017 and 2018 according to Kristensen and Jespersen[12] with some modifications. Serial dilutions of 6 concentrations ranging from 50-350 μg mL^{-1} were prepared from lambda-cyhalothrin in distilled water (dH_2O).

The larval rearing medium (as mentioned previously) was mixed with each insecticide concentration in 3 replicates for each concentration. Fifty 3rd instar larvae were used per each replicate with total number of 150 larvae for each concentration. Mortality was recorded after 72 h from treatment at conditions of 27±2°C, 60-65% RH and 14:10 h (light:dark) photoperiod. All larvae that were unable to develop into pupae were considered died.

Biochemical determination

Larval homogenate for protein and enzyme determination: Larval homogenates were prepared as described by Zhang *et al.*[13]. Larvae were homogenized in distilled water (50 mg mL^{-1}). Larval homogenates were centrifuged at 8000 rpm for 15 min at 4°C. The supernatants, which were referred as enzymes extract, were stored at -20°C until used for enzyme activity determination within 2 weeks of extraction.

Total protein assay: Total proteins were determined by the method of Bradford[14]. Protein reagent was prepared by dissolving 100 mg of Coomassie Brilliant blue G-250 in 50 mL 95% ethanol. To this solution 100 mL 85 % (w/v) phosphoric acid was added. The resulting solution was diluted to a final volume of 1 L. Sample solution (50 µL) or 50 µL of serial concentrations containing 10-100 µg bovine serum albumin for preparation of standard curve were pipetted into test tubes. The volume in the test tube was adjusted to 1 mL with phosphate buffer (0.1 M, pH 6.6). Five milliliters of protein reagent were added to test tube and the contents were vortexed. The absorbance at 595 nm using Double beam ultraviolet/visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was measured after 2 min and before 1 h against blank. The blank was prepared by mixing 1 mL of phosphate buffer with 5 mL protein reagent.

General esterase assay: General esterase activity was determined according to Van Asparen[15] using α-NA or β-NA as substrates. The reaction mixture consisted of 5 mL substrate solution (3×10^{-4} M α-NA or β-NA, 1% acetone and 0.1 M phosphate buffer, PH 7) and 20 µL of larval homogenate. The mixture was incubated for 15 min at 27°C and then 1 mL of diazoblue color reagent (prepared by mixing 2 parts of 1% diazoblue B and 5 parts of 5% sodium lauryl sulphate) was added. The developed color was measured at 600 or 555 nm. Double beam ultraviolet/visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was used for α-and β-naphthol produced from hydrolysis of the substrate, respectively. The α-and β-naphthol standard curves were prepared by dissolving 20 mg α-or β-naphthol in 100 mL phosphate buffer, PH 7 stock solution. Ten milliliters of stock solution were diluted up to 100 mL using phosphate buffer. Aliquots of 0.1, 0.2, 0.4, 0.8 and 1.6 mL of diluted solution (equal to 2, 4, 8, 16 and 32 µg naphthol) were pipetted into test tubes and completed to 5 mL by phosphate buffer. One milliliter of diazoblue reagent was added and the developed color was measured as mentioned before.

Carboxylesterase assay: Carboxylesterase activity was measured according to the method described by Simpson *et al.*[16] using methyl n-butyrate (MeB) as a substrate. The reaction mixture contained 200 µL enzyme solution, 0.5 mL 0.067 M phosphate buffer, pH 7 and 0.5 mL MeB (4 mM). The test tubes were incubated at 37°C for 30 min. One milliliter of alkaline hydroxylamine (2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes and then 0.5 mL of HCl (1 part of concentration HCl and 2 parts of dH_2O) was added. The mixture was shaken vigorously and allowed to stand for 2 min. Half milliliter of ferric chloride solution (0.9 M $FeCl_3$ in 0.1 M HCl) was added and mixed well. The decrease in MeB resulting from hydrolysis by carboxylesterase was read at 515 nm, using Double beam ultraviolet/visible spectrophotometer (spectronic 1201, Milton Roy Co., USA).

Mixed function oxidase (MFO) assay: P-nitroanisole O-demethylation was assayed to determine the mixed function oxidase activity according to the method of Hansen and Hodgson[17] with slight modification. The standard incubation mixture contained 1 mL sodium phosphate buffer (0.1 M, pH 7.6), 1.5 mL enzyme solution, 0.2 mL NADPH, (Final concentration 1 mM), 0.2 mL glucose-6-phosphate (G-6-P, final concentration, 1 mM) and 50 µg glucose-6-phosphate dehydrogenase (G-6-P-D). Reaction was initiated by the addition of P-nitroanisole in 10 µL of acetone to give a final concentration of 0.8 mM and incubated for 30 min at 37°C. Incubation period was terminated by addition of 1 mL HCl (1N). P-nitrophenol was extracted with $CHCl_3$ and 0.5 N NaOH and absorbance of NaOH solution was measured at 405 nm using Double beam ultraviolet/visible spectrophotometer (spectronic 1201, Milton Roy Co., USA). An extinction coefficient of 14.28 mM cm^{-1} was used to calculate 4-nitrophenol concentration.

Glutathione S-transferase assay: Glutathione S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via the-SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-L-glutathione could be detected as described by the method of Habig *et al.*[18]. The reaction mixture consisted of 1 mL of the potassium salt of phosphate buffer (PH 6.5), 100 µL of GSH and 200 µL of larval homogenate. The reaction started by the addition of 25 µL of the substrate CDNB solution. The concentration of both GSH and CDNB was adjusted to be 5 mM and 1 mM, respectively. Enzyme and reagents were incubated at 30°C for 5 min. The increment in absorbance

at 340 nm using Double beam ultraviolet/visible spectrophotometer (spectronic 1201, Milton Roy Co., USA) was recorded against blank containing all reagents except the enzyme solution to determine the nanomole substrate conjugated/min/larva using a molar extinction coefficient of 9.6 mM cm^{-1}.

Statistical analysis: To estimate parameters of insect mortality in bioassay, the probit analysis program[19] was used which corrected against control mortality[20]. Data of biochemical parameters were analyzed using SPSS program 14 for windows. Least significant difference (LSD) was used through ANOVA at $p<0.05$.

RESULTS AND DISCUSSION

Bioassay experiments: Results of *M. domestica* susceptibility are presented in Table 1. In comparison with laboratory strain, populations collected from Kafr Sakr showed the highest resistance to lambda-cyhalothrin (10.6 folds) at LC_{50}. While at LC_{90}, there are no high differences among populations collected as they showed from 2.3 fold (San El-Hagar population) to 3.1 fold of resistance (Abo Kabir population) comparing to laboratory strain. Regression line slope showed high slope value in Kafr Sakr population (5.32) which indicating homogeneity among individuals and low slope value in Zagazig population (2.82) comparing with (1.47) for laboratory strain.

Biochemical assays: Results of total protein concentration (Fig. 1) showed that populations collected from Abo Kabir and Zagazig recorded the highest significant concentration followed by kafr sakr, while San El-Hagar and Abo Hammad populations had the lowest protein concentration which was not significantly different with laboratory strain.

Results of esterase activity (Table 2) showed that populations of San El-Hagar and Kafr Sakr are significantly higher in esterase activity with 1.4 fold in comparison with laboratory strain when α-NA was used as a substrate. While the lowest activity of esterase was recorded in the population collected from Zagazig when α-NA was used as a substrate. When β-NA was used as a substrate, the same trend in esterase activity was noted except in Zagazig population which showed non-significant activity with San El-Hagar and significant activity with the rest populations and laboratory strain.

The highest significant activity of carboxylesterase (Table 3) was recorded in Zagazig population which showed 2.58-times higher than what recorded in laboratory strain. Kafr Sakr population showed 1.18-time higher in carboxylesterase activity comparing with laboratory strain followed by San El-Hagar which was non-significantly different with Abo Hammad, Abo Kabir and laboratory strain.

Results of MFO activity (Fig. 2) showed markedly higher in the activity of enzyme in both Zagazig and Abo Hammad populations. The lowest activity of MFO was recorded in both San El-Hagar population and laboratory strain. Activity in Abo Kabir and Kafr Sakr followed the activity recorded in Zagazig and Abo Hammad populations.

Table 1: Susceptibility of *Musca domestica* larvae of different localities in Sharkia Governorate to lambda-cyhalothrin

				Resistance ratio (RR)*	
Localities	LC_{50} (μg mL^{-1}) (95% CL)	LC_{90} (μg mL^{-1}) (95% CL)	Slope±SE	LC_{50}	LC_{90}
San El-Hagar	155.34 (109.74-190.98)	337.09 (256.27-739.97)	3.81±0.65	8.0	2.3
Kafr Sakr	204.93 (194.34-214.70)	356.64 (332.35-390.76)	5.32±0.42	10.6	2.5
Abo Kabir	189.79 (147.05-241.13)	438.96 (313.05-1284.41)	3.52±0.61	9.8	3.1
Abo Hammad	132.89 (122.26-143.23)	366.05 (321.47-433.54)	2.91±0.22	6.9	2.5
Zagazig	143.87 (132.02-155.75)	410.14 (357.72-489.29)	2.82±0.21	7.5	2.9
Laboratory strain	19.29 (0.02-68.97)	143.36 (48.77-5612.0)	1.47±0.32	1.0	1.0

Number of larvae used for each experiment/location = 50, experiments were repeated 3 times, *Values of resistance ratio were calculated by dividing LC_{50} or LC_{90} of the respective population by LC_{50} or LC_{90} of the laboratory strain

Table 2: Esterase activity in *Musca domestica* larvae of different locations from Sharkia Governorate

	Esterases activity* (μg α-naphthol min^{-1} g^{-1} b.wt.)		Ratio**	
Locations	α-NA	β-NA	α- NA	β-NA
San El-Hagar	895.3±9.00[a]	259.7±8.5[a]	1.4	2.1
Kafr Sakr	899.7±19.6[a]	198.7±7.1[b]	1.4	1.6
Abo Kabir	857.3±15.7[b]	186.7±6.1[b]	1.3	1.5
Abo Hammad	866.7±7.7[b]	135.3±5.5[c]	1.3	1.1
Zagazig	683.0±6.1[c]	271.7±10.4[a]	1.04	2.2
Laboratory strain	659.0±7.8[d]	120.7±3.00[d]	1.0	1.0

*Values are shown as means±SD of 3 replicates, numbers followed by different letters within the same column indicate that data are statistically different at $p<0.05$, **Ratio is calculated as mean values of the collected populations/mean value of laboratory strain

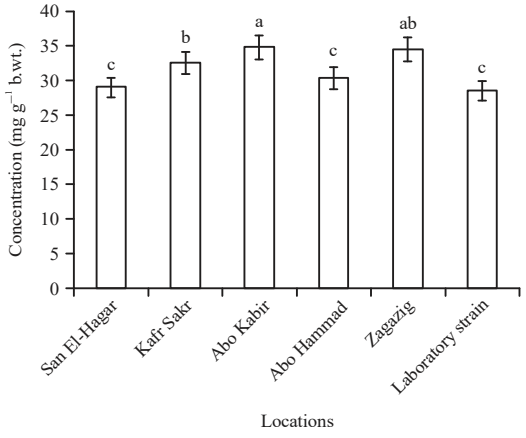

Fig. 1: Total protein concentration in *Musca domestica* larvae of different locations from Sharkia Governorate
Different letters on bars indicate that there are significant differences at p<0.05

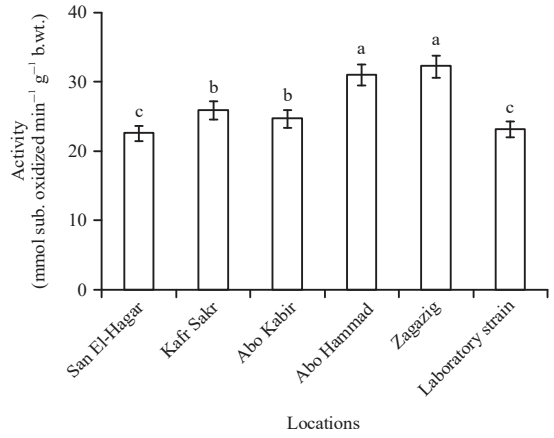

Fig. 2: Mixed function oxidase activity in *Musca domestica* larvae of different locations from Sharkia Governorate
Different letters on bars indicate that there are significant differences at p<0.05

Table 3: Carboxylesterase activity in *Musca domestica* larvae of different locations from Sharkia Governorate

Locations	Carboxylesterase activity* (μg product min^{-1} g^{-1} b.wt.)	Ratio**
San El-Hagar	39.7±2.4^{bc}	1.07
Kafr Sakr	44.0±2.9^{b}	1.18
Abo Kabir	32.3±2.2^{d}	0.87
Abo Hammad	31.6±2.1^{d}	0.85
Zagazig	96.03±6.0^{a}	2.58
Laboratory strain	37.2±2.1^{cd}	1.00

*Values are shown as Means±SD of 3 replicates, the activity of carboxylesterase was determined as μg product (enzyme substrate) min^{-1} g^{-1} b.wt., numbers followed by different letters within the same column indicate that data are statistically different at p<0.05, **Ratio is calculated as mean values of collected populations/mean value of laboratory strain

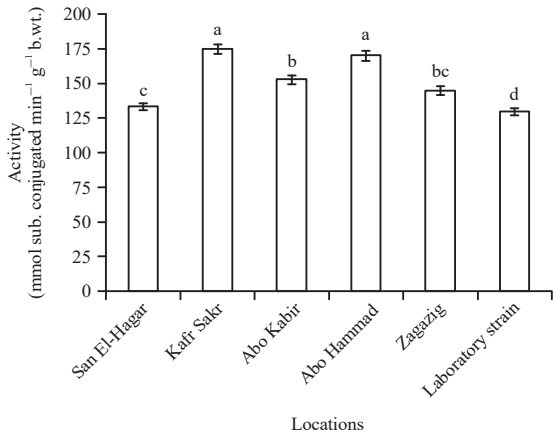

Fig. 3: Glutathione S-transferase activity in *Musca domestica* larvae of different locations from Sharkia Governorate
Different letters on bars indicate that there are significant differences at p<0.05

Results of glutathione S-transferase activity (Fig. 3) showed that the highest significant activity was recorded in both kafr sakr and Abo Hammad populations, while the lowest significant activity was recorded in the laboratory strain which showed non-significant differences with Zagazig and San El-Hagar populations. The activity of GST in Abo Kabir population followed the activity in Kafr sakr and Abo Hammad populations.

***Musca domistica* susceptibility:** Monitoring house fly susceptibility is important for the management control methods of this pest without harming the environment. Bioassay results showed 10.6 fold of tolerance in Kafr Sakr population to lambda-cyhalothrin. This result is similar to what Khan and Akram[21] found when they tested the susceptibility of a field strain collected from Pakistan against different insecticides. Their bioassay experiments resulted from 5.9-9.7 fold of resistance of newly collected field strain of house fly toward pyriproxyfen and diflubenzuron, respectively. Also in support of the current results, different levels of resistance were reported in the freshly collected field strain of house fly which showed from low resistance (2 fold (to very high levels of resistance)>1219 fold (depending on insecticides tested[13,22-27] ensuring carful use and regular monitoring of insect susceptibility in the environment toward different applied insecticides.

Activity of metabolic enzymes: Insects are naturally develop resistance against insecticides due to excessive use which promotes the development of high levels of resistance. Current results showed that different metabolic enzymes depending on the site of collection were higher in activity

comparing with the reference strain with emphasis on carboxylesterase and MFO. In agreement with these results, different studies have demonstrated that metabolic mechanisms (Esterases, MFOs and GSTs) are responsible for pyrethroid resistance due to their major role in detoxification of insecticides in a number of insect pests[28-30]. The increased in enzymes activity reported in the current study including esterase and/or cytochrome P450 monooxygenase reflect the major role of these enzymes as a metabolic mechanism to pyrethroid resistance in insect pests including house fly as previously implicated[31-33]. The high in activity of the determined enzymes according to the site of population might be due to exposure to other insecticides that metabolized by the same enzymes leading to cross resistance against different other insecticides as what previously reported with fenvalerate, cyhalothrin, cypermethrin, acephate, chlorpyrifos and methomyl[34-36].

On the other hand, a number of studies stated that cytochrome P450, GST and esterase enzymes not to be responsible for the main resistance mechanisms and other resistance mechanisms might be involved[22,24-25,37] which vary depending on different factors such as the insect species, the degree of pesticide resistance, spatial and temporal variations, or combination of these factors[37]. Taking into consideration insect species, repeated use of insecticides and the nature of habitats, enzymes determined in the current study might be good indicators for showing resistance development of house fly toward a specific insecticide.

CONCLUSION

It is important to screen the susceptibility of *M. domestica* to insecticides use from time to time in order to develop strategies for resistance management. In this manner the susceptibility of *M. domistica* from locations in Sharkia Governorate was determined against the most frequent insecticide use. It was noted from bioassay experiments that house fly was slight to moderate resistant to lambda-cyhalothrin (2.3-10.6 fold). The levels of resistance was associated with ~3 times higher in carboxylesterase activity comparing with <1.4-times in MFO and GST activity indicating the vital role of esterase enzymes in insect resistance.

SIGNIFICANCE STATEMENT

This study discovers the susceptibility status of house fly populations from Sharkia Governorate, Egypt to lambda-cyhalothrin and the possible role of carboxylesterase in resistance development that can be beneficial for applying the most suitable control strategies keeping in view the increasing problem of insecticide resistance and the effect on environmental pollution. This study will help the researcher to cover the critical areas of the possibility of repeating use of insecticides in house fly control. Thus a new theory on application of different control programs including those depend on the rotation of insecticides application may be arrived at.

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