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# EVALUATION OF CARDINAL TEMPERATURES AND THERMAL TIME REQUIREMENT FOR GERMINATION OF *SCROPHULARIA STRIATA* AND *TANACETUM POLYCEPHALUM* (SCHULTZ BIP. SSP. *HETEROPHYLLUM*)

### ABSTRACT

*Scrophularia striata* and *Tanacetum polycephalum* are important medicinal plants in Iran which are rich in essential oils, bitter substances, and sesquiterpene lactones. The present study was conducted to compare four non-linear regression models (segmented, beta, beta modified and Dent-like) to describe the germination ratetemperature relationships of *Scrophularia striata* and *Tanacetum polycephalum* over eight and seven constant temperatures, respectively, to find cardinal temperatures and thermal time requirements to reach different germination percentiles. An iterative optimization method was used to calibrate the models and different statistical indices including RMSE, coefficient of determination  $(R^2)$ , and AICc were applied to compare their performance. The beta model was found to be the best model to predict germination rate of *Scrophularia striata* at D10, D50 and D90 (R2 = 0.96, R<sup>2</sup> = 0.97, R2 = 0.95; RMSE = 0.005, 0.001 and 0.001, respectively). According to this model outputs, the base, optimum, and the maximum temperatures for germination were estimated as  $1.21 \pm 0.39$ ,  $25.91 \pm 0.33$  and  $46.35 \pm 4.12$  °C, respectively. Also the segmented model was found to be the best model to predict germination rate of *Tanacetum polycephalum* at D10, D50 and D90 (R<sup>2</sup>)  $= 0.98$ ,  $R^2 = 0.98$ ,  $R^2 = 0.98$ ; RMSE = 0.067, 0.59 and 0.56, respectively). According to the model outputs, the base, optimum, and the maximum temperatures for germination were estimated as 0.44±1.15, 26.95±0.75 and 38.33±0.98 °C, respectively. It seems these two medicinal plants need moderate optimum temperature for seed germination.

Keywords: Cardinal temperatures, Seed germination rate, Thermal time, modeling

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## INTRODUCTION

*Scrophularia* genus belongs to Scrophulariaceae family and has five species in Iran (Mozafarian, 1999). *Scrophularia striata* grows in western regions of Iran. It has widely been used as a traditional medicine for treatment of diseases such as eczema, wounds, goiter, ulcers, cancer and fistulae. Both leaves and seeds of *S. striata* contain anti-cancer and cell growth enhancing agents (Ardeshiry lajimi *et al.*, 2010). Scrophulariaceae species have been known to be rich in iridous glycosides, mainly aucubin and catalpol (Park Su *et al.*, 2009). Amiri *et al.* (2011) identified 34 essence compounds in *S. Striata* that contains 90.3% of total essence in this plant. Essential oils of *S. striata* were linalool (18.3%), 6, 10, 14 trimethylpentadecane-2-one (8.4%), dibutyl phthalate (6.9%), and βdamascone (5.9%). *S. striata* extract may stimulate collagen synthesis, faster wound contraction, angiogenesis, vessel dilatation and decrease of inflammation, bleeding and edema (Shoohani *et al.*, 2010).

*Tanacetum polycephalum* belongs to the compositae family. This plant is an aromatic perennial plant which grows in Caucasia, Iraq, Iran and Turkey (Rechinger, 1986). These members of the daisy family are rich in volatile oils, bitters, and sesquiterpene lactones, which inhibit allergic, inflamatory responses, and are insecticidal.

They are extremely pungent, potent herbs and should be used with caution (Bown, 1995; Keskitalo *et al.*, 2001). Nori-Shargh *et al.* studied the oil of *T. polycephalum* Schultz Bip. ssp. *Heterophyllum* collected from different locations in Iran and found that the main constituents of the oil of the flowers were camphor (59.1%), camphene (14.9%) and 1,8-cineole (10.1%), whereas the leaf oil contained mainly camphor (53.5%), bornyl acetate (12.1%), camphene (10.9%), 1,8-cineole (7.8%) and borneol (6.1%) (Nori-Shargh, 1999).

*S. striata* and *T. polycephalum* are endangered medicinal plants because of excessive harvest from natural habitats for traditional use.

In order to determine the best planting date for crops, it is necessary to find the base  $(T_b)$ , optimum  $(T_o)$  and maximum temperatures  $(T_c)$  for seed germination which are known as cardinal temperatures (Bewley and Black, 1994). Cardinal temperatures are determined for many of agronomic crops while for most weed species and medicinal plants, they should be determined. Modeling of seed germination is known as a good approach in determination of cardinal temperatures, but it should be noted that due to unpredictable biological phenomena, they have some limitations. Usually a linear increase in germination rate is associated with increasing temperature from a base temperature  $(T_b)$  up to an optimum, then it shows linear reduction tread to a ceiling temperature (Garcia-Huidobro *et al.*, 1982; Steinmaus *et al.*, 2000; Bradford, 1990; 1995; 2002; Rowse and Finch-Savage, 2003). To perform seed germination modelling, two main concepts have widely been used by researchers: Empirical model and Mechanical models.

Empirical models can do a great job in various levels of the empiricism of matching individual data of germination overtime, while such models may need more empirical variables (Brown and Mayer, 1988). The empirical method may be useful for specific jobs, but it is difficult to elucidate the biological significance for appraisaling model parameters (Bradford, 1990).

Mechanical models are based on experimental quantifying of environmental effects on seed germination and seedling emergence. This approach has the highest chance of success in the long run (Bradford, 1990; Forcella *et al.*, 2000). It has been shown that mechanical threshold models for seed germination and seedling emergence have delivered some success (Forcella, 1993; Benech-Arnold and Sánchez, 1995; Allen *et al.*, 2000; Roman *et al.*, 2000; Bradford, 2002; Rowse and Finch-Savage, 2003). Kamkar *et al.* (2005, 2008) reported that segmented and logistic models could be used for determination of cardinal temperatures in three millet varieties and seedling emergence of wheat cultivar "Tajan". Other functions such as power (Stapper and Lilley, 2001), the beta (Yin *et al.*, 1997), the sigmoid, the exponential (Angus *et al.*, 1981) and intersected functions (Kamkar *et al.*, 2005, 2008) are widely used to describe crop responses to temperature.

These regression models estimate cardinal temperatures. In dent-like model at a lower temperature than optimum, linear relationships is exited between temperature and germination rate, while this relationship has also remained linear at higher temperatures than optimum but in reduction trend. In the segmented, with increasing the temperature, germination rate increases linearly till reach to optimum temperature, after this point a constant trend is produced. According to the literature, there is not any information about germination of these two medicinal plants and this study appears to be the first report about cardinal temperatures of these two species germination.

The objective of this study was to test various model responses and also to test whether beta and beta modified models can work better than segmented and dent-like in estimation of cardinal temperatures for seed germination in *S. striata* and *T. polycephalum*.

# MATERIALS AND METHODS

#### *Cardinal temperatures determination*

An experiment was performed to determine the cardinal temperatures of *S. striata* and *T. polycephalum*. The experiment was conducted using germinators with controlled environments in the Seed Laboratory, University of Tehran, Karaj, Iran. Four replications of 50 seeds were germinated in 9 cm diameter Petri dishes on two layers of Whatman No. 1 (9 cm diameter) filter paper containing 5 ml distilled water. The germination response was evaluated at eight constant temperatures of 5, 10, 15, 20, 25, 30, 35 and 40˚C for *S. striata* and seven constant temperatures of 5, 10, 15, 20, 25, 30 and 35 for *T. polycephalum*.

A seed was considered as germinated when its protruded radicle elongated at least 2 mm. The germinated seeds were counted every 24h under different temperatures. The time from the start of the imbibition to the last germination was considered the total time to maximum germination. The cumulative germination percentage was plotted against time (h). From this curve, the time to 50% germination (D50) was determined by fitting a logistic model to cumulative germination percentage (G) against time (t, h) as described by equation 1:

$$
G = \frac{G_x}{1 + exp[a \times (t - b)]}
$$

where:  $G_x$  is the maximum germination percentage, b is the time for 50% germination. The times for 10%, 50% and 90% germination were also determined by interpolation and are designated D10, D50 and D90, respectively (Marshall and Squire, 1996; Shafii and Price, 2001; Soltani, 2007). The reciprocal of the time taken for a given fraction of the seed population to germinate was considered to be the germination rate (GR).

To quantify the response of the germination rate of temperature and cardinal temperatures for germination, the following equation was used:

$$
GR = \frac{f(T)}{f_0}
$$

where:  $f(T)$  is a T function (reduction factor) that ranges between 0 at the base and maximum temperatures and 1 at the optimal temperature(s), and  $1/f<sub>o</sub>$  is the inherent maximum rate of germination at the optimal temperature estimated via an iterative optimization method. Therefore, the minimum number of hours for germination at the optimal temperature was calculted (Soltani *et al.* 2006). The *GR* also shows the germination rate of a given percentile. The Sigma Plot software was used to calibrate the models (beta, beta modified, segmented and dent-like) via an iterative optimization method (Table 1). To determine the best estimates of the parameters (lower biases of the intercept from 0 and the slope from 1 are criteria for increased reliability), (*RMSE*; Equation 3), the coefficient of determination  $(R<sup>2</sup>)$ ; Equation 4), and the intercept and slope of the regression equation of predicted vs. observed germination rate were used. MAE was used because it avoids compensation between probable under- and over-prediction as follows:

$$
RAMSE = \sqrt{\left(\frac{1}{n}\right) \times \sum \left(Y_{obs} - Y_{pred}\right)^2}
$$

where: *Yobs*: observed value, *Ypred*: predicted value, *n*: number of samples (Timmermans, 2007).

and

$$
R^2 = \frac{SSR}{SST}
$$

where: *D<sub>i</sub>* is the difference between measured and calculated values, *SSR* where:  $D_i$  is the difference between measured and calculated values, both is the total *SS* is the total *SS*  $(\sum_{i=1}^{n} (L_i - L))$ .  $Y_i$  is the observed value and  $\hat{L}$  is the correspondent estimated value. The parameters estimated by non-linear models were exposed to descriptive statistical analysis for the pooled datasets, after which the best estimated values were used to calculate the thermal time needed for each germination percentile. Lower *RMSE* and  $R^2$  near to 1 show better model estimation.  $i=1$ 

To determine the best model in the estimation of cardinal temperature, Akaike Information Criterion (AIC) is used. This index explain the amount of reduction RSS, value of reduction from a degree of freedom of error and model complexity (Burnham and Anderson, 2002).

$$
AIC = n \times Ln\left(\frac{RSS}{n}\right) + 2 \times k
$$

where: *RSS* is Residual Sum of Square, *n* – number of observation and *k* is a number of model parameters.

It is possible to use corrected *AIC* (*AICc*) index instead of using *AIC*. This index is used to determine most accurate model (Butler and King, 2004; O'Meara *et al.*, 2006).

$$
AICc = n \times Ln\left(\frac{RSS}{n}\right) + 2 \times k + \left(\frac{2 \times k \times (k+1)}{n-k-1}\right)
$$

The model that produces a more accurate estimation is the one with the lower AICc value. Although the best model is the one that produces lower *AICc*, but there is a method that by using is, we are able to explain, rank and fit different models. This method is perform with calculation of *∆i*.

$$
\Delta_i = AICc - minAICc
$$

where: *minAICc* is the minimum value of calculating *AICc* among all models, and it belongs to the model that best fitted. If  $\Delta I < 10$ , then it means that there is no significant difference between models and model with higher *AICc* could also be fitted well. While *∆I*>10, then model with higher *AICc*, is not suitable and could not be fitted well.

## *Thermal time determination*

The daily thermal time (DTT) was calculated as:

$$
DTT = (T_{o1} - T_b) \times f(T)
$$

where  $f(T)$  is the *T* function,  $T_{ol}$  is the lower optimum *T*, and  $T_b$  is the base *T*. The first components of daily thermal time are the constant and non-optimal temperatures that affect the daily thermal time through *f(T)*.

Table 1



Models that were fitted to germination rate vs. different constant temperatures

Beta, segmented and dent-like, where  $T$  is the temperature,  $T_b$  the base temperature,  $T_o$  the optimum temperature,  $T_{ol}$  the lower optimum temperature (for 3-piece segmented function),  $T_{ol}$  the upper optimum temperature (for 3-piece segmented function), Tc the maximum temperature, c is the shape parameter for the beta function which determines the curvature of the function and d is the parameter of the beta modified function which indicates the sensitivity of the germination rate to temperature.

# RESULTS AND DISCUSSION

In this study,  $AICc$  and  $R^2$  were the main indices for selection of the best model for evaluation of cardinal temperatures of the two species. Estimated parameters for the dent-like, segmented and beta (4 and 5 parameter) models for different seed germination percentiles of *S. striata* and *T. polycephalum* seed is shown in Table 2 and Table 3 respectively. Also predicted and observed seed germination rate of *S. striata* and *T. polycephalum* for different germination percentiles (D10, D50 and D90) using following models:

- $\bullet$  beta (a),
- beta modified (b),
- segmented (c)
- $\bullet$  dent-like  $(d)$

The models are shown in Fig. 1 and Fig. 4, respectively. Beta five-parameter model was shown to be more successful to evaluate cardinal temperatures of *S. striata* than other models.

According to this model, calculated *AICc* indexes were equal:

- -308.02, for D10
- -361.53 for D50
- -371.88 , for D90,



Fig. 1. Predicted (lines) versus observed (symbols) seed germination rate of *S. striata* at different constant temperatures for different germination percentiles (D10, D50 and D90) using beta (a), beta modified (b), segmented (c) and dent-like (d) models

Moreover, this model was most reliable for D10, D50 and D90, due to the higher determination coefficient between observed and predicted values ( $R^2$  = 0.96, 0.97 and 0.95 for pooled data). According to beta five- parameters model cardinal temperatures were  $T_b$  (4.30  $\pm$  1.29, 3.25  $\pm$  0.79, 1.21  $\pm$  0.39), To (25.28  $\pm$  0.54, 26.23  $\pm$ 0.36, 25.91  $\pm$  0.33) and  $T_c$  (40.21  $\pm$  0.33, 42.32  $\pm$  0.16, 46.35  $\pm$  4.12) for D10, D50 and D90 respectively (Table 2). Furthermore beta modified model had the lowest accuracy in predicting the cardinal temperatures of *S. striata* because of the lowest  $R^2$  and the highest AICc than other models. In beta modified model,

AICc was -234.24, -286.29 and -345.15 for D10, D50 and D90, respectively (Table 2). For *T. polycephalum* segmented model had the highest accuracy in predicting cardinal temperatures. According to this model, calculated *AICc* index was -60.52, -80.56 and -87.04 for D10, D50 and D90, respectively. In addition, this model was most reliable for D10, D50 and D90, because of the higher determination coefficient between observed and predicted values ( $R2 = 0.98$ , 0.98 and 0.98 for pooled data) (Table 3). According to segmented models cardinal temperatures for *T. polycephalum* were  $T_b$  (2.55  $\pm$  0.95, 1.70  $\pm$  0.83, 0.44  $\pm$ 1.15), To  $(27.36 \pm 0.81, 23.56 \pm 0.61, 26.95 \pm 0.75)$  and  $T_c$   $(38.74 \pm 1.24, 39.42)$  $\pm$  1.02, 38.33  $\pm$  0.98) for D10, D50 and D90, respectively (Table 3).

Table 2

Estimated parameters for the SEGMENTED, BETA, BETA MODIFIED and DENT-LIKE models for different germination percentiles of *S. striata* seed

Parameter <sup>1</sup>	Segmented			Beta		
	D10	D50	D <sub>90</sub>	D10	D50	D <sub>90</sub>
T <sub>b</sub>	$5.60 \pm 0.67$	$3.70 \pm 0.54$	$1.78 \pm 0.94$	$4.30 \pm 1.29$	$3.25 \pm 0.79$	$1.21 \pm 0.39$
$T_{o}$	$26.14 \pm 0.69$	$26.64 \pm 0.41$	$25.89 \pm 0.63$	$25.28 \pm 0.54$	$26.23 \pm 0.36$	$25.91 \pm 0.33$
$\rm T_c$	$44.29 \pm 1.16$	$42.42 \pm 0.53$	$41.33 \pm 0.68$	$40.21 \pm 0.33$	$42.32 \pm 0.16$	$46.35 \pm 4.12$
$f_o$	$7.42 \pm 0.26$	$22.70 \pm 0.47$	$39.62 \pm 1.22$	$7.83 \pm 0.27$	$24.59 \pm 0.54$	$40.87 \pm 1.65$
$\boldsymbol{c}$				$4.83 \pm 0.21$	$2.12 \pm 0.17$	$1.07 \pm 0.49$
$R^2$	0.94	0.97	0.95	0.96	0.97	0.95
<b>RMSE</b>	0.009	0.001	0.001	0.005	0.001	0.001
<b>AIC</b>	$-291.70$	$-367.31$	$-375.79$	$-312.02$	$-366.53$	$-376.88$
AICc	$-283.70$	$-359.31$	$-367.79$	$-308.02$	$-361.53$	$-371.88$
$\Delta_i$	24.32	2.21	4.08	$\overline{0}$	$\mathbf{0}$	$\mathbf{0}$
Parameter <sup>1</sup>	Beta modified			Dent-like		
	D10	D50	D <sub>90</sub>	D10	D50	D <sub>90</sub>
T <sub>b</sub>	$8.17 \pm 1.31$	$5.00 \pm 2.50$	$1.51 \pm 0.86$	$5.60 \pm 0.70$	$3.70 \pm 0.56$	$1.82 \pm 0.94$
$T_{o}$	$26.43 \pm 0.71$	$26.37 \pm 0.69$	$25.99 \pm 0.93$			
$T_c$	$41.72 \pm 0.60$	$40.76 \pm 0.41$	$40.20 \pm 0.48$	$44.29 \pm 1.21$	$42.42 \pm 0.54$	$41.00 \pm 0.59$
$T_{01}$				$25.05 \pm 0.83$	$25.39 \pm 0.92$	$25.83 \pm 0.54$
$T_{o2}$				$27.11 \pm 0.35$	$27.49 \pm 0.09$	$27.13 \pm 0.02$
$f_{o}$	$8.60 \pm 0.31$	$26.34 \pm 0.83$	$45.69 \pm 2.04$	$7.84 \pm 0.78$	$24.07 \pm 0.30$	$39.28 \pm 0.78$
$R^2$	0.91	0.92	0.88	0.94	0.97	0.95
<b>RMSE</b>	0.011	0.003	0.002	0.009	0.001	0.001
AIC	$-242.24$	$-294.29$	$-353.15$	$-289.70$	$-365.31$	$-374.21$
AICc	$-234.24$	$-286.29$	$-345.15$	$-285.70$	$-361.31$	$-370.28$
$\Delta_i$	73.78	75.23	76.72	22.32	0.21	1.59

 $T_b$ ,  $T_a$ ,  $T_c$ ,  $T_a$ ,  $T_a$ ,  $T_a$ ,  $f_a$  and care base temperature, optimum temperature, maximum temperature, lower limit of optimum temperature, upper limit of optimum temperature, minimum time to reach a given percentile, parameter of beta function, coefficient of regression, respectively

The base and the maximum temperatures for different percentiles did not show any significant difference for all tested models for *T. polycephalum*. The beta-modified and dent-like models, were also reliable for D10 and D50 (Table 3), because  $R^2$  was high for both models. According to the segmented model for percentiles of D10, D50 and D90, the basic temperature varied be-





Estimated parameters for the SEGMENTED, BETA, BETA MODIFIED and DENT-LIEF models for different germination percentiles of *T. polycephalum* seeds

*Tb* , *To* ,*Tc* ,*To1* , *To2* , *fo* and care base temperature, optimum temperature, maximum temperature, lower limit of optimum temperature, upper limit of optimum temperature, minimum time to reach a given percentile, parameter of beta function, coefficient of regression, respectively

Dent-like model produced the lowest RMSE for D10, D50 and D90 (0.01, 0.004 and 0.003, respectively) compared to other models for *T. polycephalum* (Table 3). In this study, the germination rate was very sensitive to temperature, in order that it was slow at low temperatures. The germination rate is maximum at optimum temperature and by increasing and decreasing in temperature, germination rate decreases. Decreasing of germination rate at low temperatures is related to decrease imbibition rate of seed (Bewley and Black, 1994). Khan *et al* (2001) investigated the effects of different temperature regimes on germination of *Kochia scoparia* and reported that the temperature had a significant effect on germination, and germination rate was higher at higher temperatures. Several reports indicate an increasing effect of temperature on the speed of germination up to a certain point (Hardegree and Winstral, 2006; Bannayan *et al.*, 2006).

Other studies suggest that the typical germination rate increases linearly with increasing temperature in a suitable range of temperature, but at higher temperatures it decreases (Mwale *et al.*, 1999). Adam *et al.* (2007) stated that the germination response to temperature could be different depending on the species or populations within a species. Decrease of germination rate at low temperatures is related to decreased seed imbibition rate (Begley and Black, 1994). Tabrizi *et al*. (2007) by Evaluation of various models on germination of two agricultural and natural populations of Thyme (*Thymus Transcaspicus*) reported that beta five-parameter model has the most reliable of cardinal temperatures for germination on natural population of this species. In addition, among different nonlinear regression models (Dent-like, segmented and beta), segmented model was found to be the best model to predict germination rate of opium poppy (Kamkar *et al.*, 2012). In segmented model, relative changes in development rate is plotted separately for temperatures lower and more than optimum temperature. The optimum temperature calculated from the intersection of two regression lines and base temperature and maximum are intercept of the regression line at lower and more temperatures than the optimum temperature, respectively (Phartyal *et al.*, 2003).



Fig. 2. *f(t)* Values for different constant temperatures based on beta model

Calculated *f(t)* for constant temperatures used in this research based on the beta model for *S. striata* and segmented model for *T. polycephalum* are illustrated in Fig 2 and Fig 5 respectively. They show an increase trend to 25°C then starts to decrease for the two species. This suggests that the optimum temperature for two species is around 25°C .Using the estimated parameters of the segmented model, each germination percentile will be achieved when *DTT* = *TT*, or  $f(T) = f_0$ , or  $f(T)/f_0 = 1$ . It is clear that temperatures closer to optimum temperature have a small reducing effect on germination rate.



Fig. 4. Predicted (lines) vs. observed (symbols) germination rate of *T. polycephalum* seeds at different constant temperatures for different germination percentiles (D10, D50 and D90) using beta (a), beta modified (b), segmented (c) and dent-like (d) models



Fig 5. *f(t)* Values for different constant temperatures based on the beta model



Fig 6. Thermal time (degree-hours) required for different germination percentiles in based on pooled data, when *T*=*To*

The calculated thermal times for each germination percentile based on pooled data are represented in Fig. 3 and Fig 6 for *S. striata* and *T. polycephalum* respectively. Thermal time required for 10, 50 and 95% germination in *S. striata* is 7, 23 and 43 degree-days respectively. Also thermal time required for 10, 50 and 95% germination in *T. polycephalum* is 170, 460 and 780 degree-hours respectively. Kamkar et al (2012) reported that the thermal time required to reach 50 and 95% germination in opium poppy was 57.27 and 87.55 degree-days, respectively. The thermal time requirement for a developmental process (like germination), offers a measure of physiological time required to complete the process. In addition, thermal time is the number of degree days required for a developmental process based on a set of physiological temperatures during the process and expression of time in thermal units. It eliminates the time dependence of biological process because of temperature change (Trudgill *et al.*, 2005). Thermal time required for each developmental stage is calculated by inversion the slope of the regression function of development rate versus temperatures below the optimum temperature (Thornley, 1987).

Our results confirmed the certainty of the estimated parameters and the reliability of the beta for *S. striata* and segmented model for *T. polycephalum*. In other words, the regression between the degree day sums and the mean temperatures for this experiment confirmed independently between the degree day sums and the temperatures of traits. This independency has fully explained by Bonhomme, (2000) for using degree day's unit in such experiments. This study suggests that the bilinear-shape response model of germination rate of temperature can be used to estimate the cardinal temperatures of *T. polycephalum.* In this model, the germination rate is regressed separately against temperature for two extreme of temperatures (below and above optimum temperature). Base temperature and maximum temperature are the intercepts of each regression line (Covell *et al.*, 1986; Phartyal *et al.*, 2003). The results of the present study confirmed that in the absence of other limiting factors (e.g., light, water), seed germination of *S. striata* and *T. polycephalum* is highly influenced by temperature. In addition, our results indicate that the germination rate of *S. striata* based on the beta model and *T. polycephalum* based on the segmented model exhibite sharply defined cardinal temperatures.

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