International Journal of Agricultural Sciences
Volume 8 |Issue 1| January, 2012 | 232-237

RESEARCH PAPER

Evaluation of plant extracts against *Alternaria tagetica* under *in vitro* condition, and losses in the seed yield of African marigold due to the disease

DHIRENDRA SINGH BHADOURIA AND AVINASH SINGH TOMAR*
Department of Soil Science, College of Agriculture, R.V.S. Krishi vishvwa Vidhyalaya, GWALIOR (M.P.) INDIA

Abstract **:** All the tested botanicals significantly inhibited growth of *Alternaria tagetica* under all the three tested concentrations. All the botanicals were more effective under higher concentrations. Under lowest concentration (Leaf extracts @ 5%; oil, bulb and rhizome extract @ 1%). The neem oil and Eucalyptus oil where found best as these two were significantly superior over other plant extracts except Eucalyptus leaf extract. Under medium concentration (Leaf extracts @ 10%; oil, bulb and rhizome extracts @ 5%). Neem oil absolutely inhibited fungal growth of *Alternaria tagetica* and it was significantly superior over other plant extracts except Eucalyptus oil in which only 3.33 mm fungal growth of the pathogen was recorded. The maximum radial growth (81.67 mm) was recorded in control. Under higher concentration (Leaf extracts @ 20% ; oil, bulb and rhizome extracts @ 10%). Neem oil and Eucalyptus oil absolutely inhibited the fungal growth and these two were significantly superior over Iopmia leaf extract (50.33 mm), Lantana leaf extract (42.67mm), Parthenium leaf extract (36.00 mm), *Calotropis* leaf extract (27.67 mm), *Onion* bulb extract (17.67 mm) and *Datura* leaf extract (16.33 mm). Neem oil and Eucalyptus oil were statistically at par with *Ginger* bulb extract (4.00 mm), Neem leaf extract (5.00 mm), Eucalyptus leaf extract (6.33), Tulsi leaf extract (7.00 mm), Garlic bulb extract (7.00 mm) and Pudina leaf extract (9.00 mm). The maximum radial growth (76.33 mm) was recorded in control. The correlation coefficient "r = -0.9974** "shows a strong significantly negative correlation between the flower blight intensity and the seed yield. The regression equation "y = 14.61 – 0.1345 x" indicates 14.61 gm of seed yield of 10 flowers under disease free condition and thereafter the seed yield would decrease by 0.13 g with unit (1% each) increase of intensity of flower blight. The correlation coefficient r_1 = 0.9974** indicate that the seed yield losses were gradually and significantly increased with the corresponding increases in the intensity of flower blight. The regression equation "y_1 = -4.8892 + 0.965 x" indicates that with one unit of increase in the intensity of flower blight the seed yield loss would increase by 0.965 per cent. The correlation coefficient "r = -0.9870** " showed a highly significant and negative correlation between the flower blight intensity and the seed germination. The regression equation " y = 52.657 – 0.638 x " indicated 52.65 per cent seed germination under disease free condition and thereafter it decreased by 0.638 per cent with unit increase (1% each) in the intensity of flower blight.

Key Words **:** Flower blight, *Alternaria tagetica*, African marigold, Evaluation of botanicals

View Point Article **:** Bhadouria, Dhirendra Singh and Tomar, Avinash Singh (2012). Evaluation of plant extracts against *Alternaria tagetica* under *in vitro* condition, and losses in the seed yield of African marigold due to the disease. *Internat. J. agric. Sci.*, **8**(1): 232-237.

Article History **: Received :** *12.07.2011;* **Revised :** *22.10.2011;* **Accepted :** *06.12.2011*

INTRODUCTION

The leaf spot and flower blight of marigold incited by *Alternaria tagetica* (Shome and Mustafee, 1966) is a serious disease of marigold in the country and in northern Madhya Pradesh. The disease gets initiated as dark brown necrotic spots on leaves, stem and flowers. With the progress of the disease, the spots expand, coalesce which leads to drying of leaves. Now-a-days the disease has become a most important biotic constraint in the full exploitation of high yielding scented African marigold varieties.

Marigold (*Tagetes* sp. Linn.) is one of the most commonly grown ornamental and commercially exploited flower crop in India, *Tagetes erecta* (African marigold) and *Tagetes patula* (French marigold) are the dominant species of marigold in India. Out of these two *Tagetes erecta* is more popular in the

* **Author for correspondence.**

country.

The crop is known to be infected by a number of fungal and viral diseases. Out of these, leaf spot and flower blight caused by *Alternaria tagetica* is the most important disease of marigold. In northern Madhya Pradesh the leaf spot and flower blight disease has become a major biotic constraint in the full exploitation of high yielding scented African marigold varieties.

To study and to manage the problem of leaf spot and flower blight of marigold, the present studies were carried out during winter season 2006-2007 at the college of Agriculture, Gwalior.

MATERIALS AND METHODS

Experimental site :

The present studies were conducted at College of Agriculture, Gwalior during winter season of 2006-2007. Gwalior is situated in northern part of Madhya Pradesh at an elevation of 211.52 meters from mean sea level and lies between latitude and longitude 26°14' north and 78°15' east, respectively.

Climate:

The climate of Gwalior is subtropical. The rainy season normally starts from middle of June after commencement of southwest monsoon and last up to September. Maximum precipitation of rains occurred in the month July and August. Winter season runs from November to mid February, and hot summer season from April to mid June. October is the transitory month between rainy and winter season.

Soil:

The soil of experimental site was alluvial clay loam texture.

Experimental material:

Glassware:

During the experimentation, Corning make glassware was used. They were properly cleaned with cleaning powder followed by washing and were dried before use. The glassware's were sterilized in hot air-oven at 180^0C for two hours. The metallic appliances *viz*., forceps, inoculation needle etc, used during the study were sterilized by heating them on the flame directly.

Test organism:

Alternaria tagetica, the causal organism of leaf spot and flower blight of marigold was isolated from the infected plant parts.

Potato culture medium:

Potato dextrose agar medium containing following ingredients was used during the course of investigation

Peeled and sliced potato	:	200 g.
Dextrose	:	20 g.
Agar- agar	:	20 g.
Distilled water	:	1000 ml

For the preparation of PDA, Potato slices were boiled in 500 ml water until they became soft. The supernatant was filtered. In other 500 ml boiling water the powdered agar was poured. The supernatant of potato and melted agar were then mixed measured and the volume was restored to 1000 ml with hot water. It was again boiled for 5 minutes and then dextrose was added. The medium was poured into the flask and culture tubes. The mouth of flask and culture tubes were plugged with cotton plug and sterilized in an autoclave at the pressure of 15 pound / square inch (1.05kg/cm^2) for 15 minutes and the temperature 121.6 °C which is sufficient for proper sterilization finally streptomycin sulphate was incorporated into the melted medium. After this the medium was used under aseptic condition for bioassay of the test fungus.

Isolation :

The diseased plants were collected from the field. The infected leaf and flower tissues were washed and cut into small pieces and were surface sterilized by placing them in 0.1 per cent mercuric chloride solution for one minute and re-washed thrice with sterilized distilled water to remove the disinfectant. Thereafter, under aseptic condition pretreated pieces of material were inoculated into the culture tubes / pretrated containing sterilized potato dextrose agar medium. The inoculated tubes / plates were incubated at 24°C ± 1°C for 48 hours. Isolated pathogen was examined microscopically. Pure culture was obtained through sub-culturing and it was used for the study after the confirmation of pathogenicity test.

***In vitro* evaluation of botanicals:**

Botanicals are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. Many botanicals have been identified to be effective in the control of plant diseases. Among the 5280 species tested 1134, 346, 92 and 90 plant species possessed insecticidal, fungicidal, bactericidal, antiviral properties, respectively. Hence a laboratory experiment was conducted by adopting poisoned food technique to find out the optimum concentration of effective botanicals for the inhibition of fungal growth of *A. tagetica* under *in-vitro* condition. The botanicals *viz*., Neem (leaf and oil), Eucalyptus (leaf and oil), Datura (leaf), Lantana (leaf), Ginger (rhizome), Garlic (bulb), Onion (bulb), Tulsi (leaf), Pudina (leaf), Parthenium (leaf), Calotropis (leaf) and Ipomia (leaf) were used for the evaluation against the pathogen. The leaf extracts were used @ 5,10 and 20 per cent whereas rhizome, bulb and oil extracts were tested @ 1,5 and 10 per cent. A control treatment was also kept for

the comparison. All the treatments were replicated thrice.

Preparation of extracts:

For preparation of extracts, fresh leaves of respective botanicals were thoroughly washed in ordinary tap water and were dried in an oven at 60^0C for two consecutive days. After drying the leaves became brittle and easily crushed by mixer. These crushed leaves were sieved through fine sieve (52 mesh) and were stored in plastic bottles. These powdered leaves were used at the concentration of @ 5,10 and 20 per cent. The crude extracts of ginger (rhizome), garlic (bulb) and onion (bulb) were used at the concentration of @ 1,5 and 10 per cent. The oil extracts were also used at the concentration of @ 1, 5 and 10 per cent.

Assessment of seed yield losses:

A spray schedule trial with recommended fungicide (mancozeb @ 0.2%) was carried out to obtain the flowers of different degree of disease intensity. From each treatment ten flowers of uniform size were randomly selected and harvested separately along with their disease intensity.

Experiment details:

Treatment	:	7
T_1	:	One spray of mancozeb at 45 DAS
T_2	:	Two sprays of mancozeb at 45 and 55 DAS
T_3	:	Three sprays of mancozeb 45, 55 and 65 DAS
T_4	:	Four sprays of mancozeb at 45, 55, 65 and 75 DAS
T_5	:	Five sprays of mancozeb at 45, 55 65, 75 and 85 DAS
T_6	:	Six sprays of mancozeb at 45, 55, 65, 75, 85 and 95 DAS
T_7	:	Control (No spray)
Plot size	:	2 m x 3.2 m
Replication	:	3
Design	:	R.B.D.

Disease intensity and seed yield of ten tagged flowers were recorded separately then the per cent loss in seed yield was calculated as follows:

$$\text{Seed yield loss (\%)} = \frac{A - B}{A} \times 100$$

where,

A= Seed yield of 10 healthy flowers. In the absence of healthy flowers the least infected (infection in traces) flowers were treated as healthy flowers.

B = Seed yield of 10 diseased flowers

Further the correlation between per cent disease intensity and seed yield loss (%) was worked out

Thereafter the regression equation "Y=a + bx" developed to predict the per cent seed yield losses due to unit increase in flower blight intensity.

Role of seed borne inoculum on the germination of seed:

The seeds from infected (different category of infection) and healthy flowers were placed for germination in the plastic Petridishes containing moist blotter paper. For such study 100 seeds of each category of infection were separately placed and for comparison 100 seeds from healthy flowers were also placed for germination. The percentage germination under individual category of infection was recorded separately then correlation between the per cent disease intensity and per cent germination was worked out.

Statistical analaysis :

Correlation of regression studies:

Correlation and regression studies were carried out as follows:

$$r(xy) = \frac{Cov(xy)}{\sqrt{Var(x) X var(y)}}$$

Suggested by Chandel (1999)

$$b_{yx} = \frac{Cov(xy)}{Var(x)}$$

Regression line of y on x

$$y - \bar{y} = b_{yx}(x - \bar{x})$$

where,

r= Coefficient between x and y

Cov = Covariance between x and y

Var = Variance of x traits

Vari = Variance of y traits

$\bar{x}$ = Mean of x

$\bar{y}$ = Mean of y

The significance of treatments effect was tested with the help of 'F' (variance ratio), while for testing the significance between the treatment mean, critical difference was calculated.

Correlation and regression were also worked out by the method given by Johnson (1950). In a bivariate distribution (distribution involving two variables), it is important to find out the relationship (co-relation) between the two variables. Therefore, correlation co-efficients were worked out as per formula given below:

$$p(x,y) = \frac{Cov(x,y)}{\sigma x, \sigma y}$$

Cov (x,y) = Co- variance between two variables

x = Independent variable and

y = Dependent variable

$$Cov(x,y) = \frac{1}{n} \sum (x - \bar{x})(x - \bar{y})$$

□ x = Standard deviation of x

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

In-vitro* evaluation of botanicals against *A. tagetica:

The data presented in the Table 1 show that all the tested botanicals significantly inhibited growth of *Alternaria tagetica* under all the three tested concentrations. All the botanicals were more effective under higher concentrations. Under lowest concentration (leaf extracts @ 5%; oil, bulb and rhizome extract @ 1%), the Neem oil and Eucalyptus oil were found best as these two were significantly superior over other plant extracts except Eucalyptus leaf extract.

Table 1 : *In vitro* evaluation of botanicals against *Alternaria tagetica*

Treatment (Plant extracts)	Radial growth of fungal Mycelium in mm		
	*	**	***
T_1 : Neem leaf	51.00	29.00	5.00
T_2 : Eucalyptus leaf	40.33	23.67	6.33
T_3 : Datura leaf	54.67	35.67	16.33
T_4 : Calotropis leaf	60.67	51.33	27.67
T_5 : Parthenium leaf	61.00	54.00	36.00
T_6 : Lantana leaf	68.67	59.00	42.67
T_7 : Ipomia leaf	70.67	61.33	50.33
T_8 : Pudina leaf	55.00	33.67	9.00
T_9 : Tulsi leaf	54.00	25.00	7.00
T_{10} : Neem oil	36.33	0.00	0.00
T_{11} : Eucalyptus oil	38.00	3.33	0.00
T_{12} : Onion bulb	71.67	35.00	17.67
T_{13} : Garlic bulb	58.67	21.67	7.00
T_{14} : Ginger rhizome	59.33	15.00	4.00
T_{15} : Control	81.33	81.67	76.33
S.E.(m) ±	3.32	3.37	5.53
C. D. (P=0.05)	9.60	9.51	16.02

where,
* : Leaf extracts were used @ 5%, while oil, bulb and rhizome extracts were used @ 1%.
** : Leaf extracts were used @ 10%, while oil, bulb and rhizome extracts were used @ 5%.
*** : Leaf extracts were used @ 20%, while oil, bulb and rhizome extracts were used @ 10%.
Note : 1. The data are the mean of three replications.
2. The observations were recorded at six days after inoculation.

Under medium concentration (leaf extracts @ 10 %; oil, bulb and rhizome extracts @ 5%), the *Neem* oil absolutely inhibited fungal growth of *Alternaria tagetica* and it was significantly superior over other plant extracts except Eucalyptus oil in which only 3.33 mm fungal growth of the pathogen was recorded. The maximum radial growth (81.67 mm) was recorded in control.

Under higher concentration (leaf extracts @ 20% ; oil, bulb and rhizome extracts @ 10%), the *Neem* oil and Eucalyptus oil absolutely inhibited the fungal growth and these two were significantly superior over Ipomia leaf extract (50.33 mm), Lantana leaf extract (42.67mm), Parthenium leaf extract (36.00 mm), Calotropis leaf extract (27.67 mm), Onion bulb extract (17.67 mm) and Datura leaf extract (16.33 mm). Neem oil and Eucalyptus oil were statistically at par with Ginger rhizome extract (4.00 mm), Neem leaf extract (5.00 mm), Eucalyptus leaf extract (6.33), Tulsi leaf extract (7.00 mm), Garlic bulb extract (7.00 mm) and Pudina leaf extract (9.00 mm). The maximum radial growth (76.33 mm) was recorded in control .

Losses in seed yield of marigold due to *A. tagetica:*

A correlation study between the intensity of flower blight and corresponding seed yield was carried out and the data along with correlation coefficient and regression equation are presented in the Table. The correlation coefficient r = -0.9974** clearly showed a strong significantly negative correlation between the flower blight intensity and the seed yield. It means that the seed yield gradually and significantly decreased with the corresponding increase in the intensity of flower blight. The regression equation y = 14.61 – 0.1345 x indicated 14.61 g of seed yield of 10 flowers under disease free condition and thereafter the seed yield would decrease by 0.13 g with unit (1% each) increase of intensity of flower blight .

The correlation coefficient r_1 = 0.9974** (Table 2) indicates that the seed yield losses were gradually and significantly increased with the corresponding increase in the intensity of flower blight. The regression equation y = -4.8892 + 0.965 x indicated that with one unit of increase in the intensity of flower blight the seed yield loss would increase by 0.965 per cent

Role of seed borne inoculum on the germination of seed:

A correlation study between the intensity of flower blight and seed germination was carried out and the data alongwith correlation coefficient and regression equation are presented in the Table 3. The correlation coefficient r = -0.9870** clearly shows a highly significant and negative correlation between the flower blight intensity and the seed germination. It means that seed germination per cent gradually and significantly decreased, with the corresponding increase in the intensity of flower blight. The regression equation y = 52.657 – 0.638 x indicate 52.65 per cent seed germination under disease free condition and thereafter it decreased by 0.638 per cent with unit increase (1% each) in the intensity of flower blight .

It is obvious from the result of the present study that all the tested botanicals were more effective under higher concentrations. Under lowest concentration (Leaf extracts @ 5% ; oil, bulb and rhizome extract @ 1%), the Neem oil and Eucalyptus oil were found highly effective as these two were significantly superior over other plant extracts except Eucalyptus leaf extract.

Under medium concentration (Leaf extracts @ 10% ; oil, bulb and rhizome extracts @ 5%), Neem oil absolutely inhibited fungal growth of *Alternaria tagetica* and it was significantly superior over other plant extracts except Eucalyptus oil in which only 3.33 mm fungal growth of the pathogen was recorded. The maximum radial growth (81.67 mm) was recorded in control.

Under higher concentration (Leaf extracts @ 20% ; oil, bulb, and rhizome extracts @ 10%), the Neem oil and Eucalyptus oil absolutely inhibited the fungal growth and these two were significantly superior over Iopmia leaf extract (50.33 mm), Lantana leaf extract (42.67mm), Parthenium leaf extract (36.00 mm), Calotropis leaf extract (27.67 mm), Onion bulb extract (17.67 mm) and Datura leaf extract (16.33 mm). Neem oil and Eucalyptus oil were statistically at par with Ginger bulb extract (4 mm), Neem leaf extract (5.00 mm), Eucalyptus leaf extract (6.33), Tulsi leaf extract (7.00 mm), Garlic bulb extract (7.00 mm) and Pudina leaf extract (9.00 mm). The maximum radial growth (76.33 mm) was recorded in control.

Earlier Khanna and Chandra (1972), Meena and Mariappan (1993), Srinivas *et al.* (1997) and Dubey (2001) also reported that *Azadirachta indica* in different form is effective against *Alternaria* species. The volatile oils from Eucalyptus *citridora* was most effective against *Rhizoctonia solani* and *Helminthosporium oryzae* (Ramezani *et al.,* 2002).

A highly significant and negative correlation coefficient (r = -0.9974**) was found in between flower blight intensity and seed yield, this shows that the seed yield of marigold gradually and significantly decreased with the corresponding increase in the intensity of flower blight. The correlation study between the disease intensity and seed yield loss (r_1 = 0.997**) clearly indicates that seed yield losses gradually and significantly increased with the corresponding increase in the intensity of flower blight. Further, regression study indicates that with one unit increase in the intensity of flower blight the, seed yield loss would increase by 0.965 per cent.

The present finding is in agreement with Dhiman and Arora (1990) who also observed that leaf spot and flower blight infection plays a significant adverse effect on seed yield on infected flower of marigold. They reported that the

Table 2 : Correlation coefficient between intensity of flower blight (*A. tagetica*) and seed yield of African marigold

Treatments	Per cent disease intensity (x)	Seed yield of 10 flowers (g) (y)	Seed yield loss (%) (Y_1)
T_1 : One spray of manozeb (0.2%) at 45 DAS	45.83	8.20	41.13
T_2 : Two sprays of manozeb (0.2%) at 45 and 55 DAS	40.00	9.25	33.59
T_3 : Three sprays of manozeb (0.2%) at 45, 55 and 65 DAS	33.33	10.03	27.99
T_4 : Four sprays of manozeb (0.2%) at 45, 55, 65 and 75 DAS	22.50	11.73	15.79
T_5 : Five sprays of manozeb (0.2%) at 45, 55, 65,75 and 85 DAS	7.50	13.86	0.50
T_6 : Six sprays of manozeb (0.2%) at 45, 55, 65, 75, 85 and 95 DAS	3.33	13.93	0.00
T_7 : Control (No spray)	60.00	6.70	51.90

Correlation coefficient between x and y (r) : -0.997493004 **
Regression equation between disease intensity (x) and seed yield(y) : 14.61 - 0.1345x
Correlation coefficient between x and y_1 (r_1) : 0.997413 **
Regression equation between disease Intensity (x) and seed yield loss (y ') : - 4.8892 + 0.9653x
DAS = Days after sowing.
Note : The data are the mean of three replications.

Table 3 : Effect of *Alternaria tagetica* infection on the germination of seed

Treatments	Disease intensity on flowers (%) (x)	Seed germination (%) (y)
T_1 : One spray of manozeb (0.2%) at 45 DAS	45.83	21.0
T_2 : Two sprays of manozeb (0.2%) at 45 and 55 DAS	40.00	26.0
T_3 : Three sprays of manozeb (0.2%) at 45, 55 and 65 DAS	33.33	30.0
T_4 : Four sprays of manozeb (0.2%) at 45, 55, 65 and 75 DAS	22.50	37.0
T_5 : Five sprays of manozeb (0.2%) at 45, 55, 65,75 and 85 DAS	7.50	49.0
T_6 : Six sprays of manozeb (0.2%) at 45, 55, 65, 75, 85 and 95 DAS	3.33	52.0
T_7 : Control (No spray)	60.00	18.0

Correlation coefficient (r): -0.987020588 **
Regression equation (y): 52.657 - 0.6381x
The data are the mean of three replications
DAS = Days after sowing.

disease on an average cause a reduction of 28.21 per cent in seed yield.

The correlation coefficient r = -0.9870** showed a highly significant and negative correlation between the flower blight intensity and the seed germination. The regression equation y = 52.657 – 0.638 x indicate 52.65 per cent seed germination under disease free condition and thereafter it decreases by 0.638 per cent with unit increase (1% each) in the intensity of flower blight.

The present finding is supported by Dhiman and Arora (1990) who also observed that leaf spot and flower blight infection plays a significant adverse effect on seed viability of infected flower of marigold. They reported that the disease on an average causes a reduction of 53.53 per cent in seed germination. They observed that the seed obtained from diseased flowers produced 2 – 5 per cent sickly seedlings.

Conclusion:

– The oil extracts of neem and eucalyptus @ 5 per cent and leaf extracts of neem and eucalyptus @ 20 per cent were found very effective against *A. tagetica* under *in vitro* condition.

– The losses in seed yield and seed germination gradually increased with the corresponding increase in the intensity of flower blight.

– Regression study revealed that with one per cent increase in the intensity of flower blight, the corresponding losses in seed yield would increase by 0.965 per cent.

– Regression study also revealed 52.65 per cent seed germination under disease free condition and thereafter it decreased by 0.638 per cent with unit increase (1% each) in the intensity of flower blight.

REFERENCES

Chandel, S.R.S. (1999). *A hand book of agriculture statistics*. Achal Prakashan Mandir, Kanpur.

Dhiman, J.S. and Arora, J.S. (1990). Occurrence of leaf spot and flower blight of marigold (*Tagetes erecta* L.) in Punjab. *Punjab J. Res.,* **27** : 2, 231-236.

Dubey, A.K. (2001). Studies on leaf spot and flower blight of marigold. M.Sc. (Ag.) Thesis J.N.K.V.V. JABALPUR, M.P. (India).

Jhonson, O.P. (1950). *Statistical methods in research*. Prentice Holl Inc, New York.

Khanna, K.K. and Chandra, S. (1972). Antifungal activity in some plant extracts. *Proc. Nat. Acad. Sci.,* **42** : 300-302.

Meena, S.S. and Mariappan, V. (1993). Effect of plant products on seed borne mycoflora of sorghum. *Madras Agric. J.,* **80** : 383 – 387.

Ramezani, H., Singh, H.P., Batish, D.R., Kohli, R.K. and Dargan, J.S. (2002). Fungicidal effect of volatile oils from Eucalyptus *cirtriodora* and its major constituent citronellal. *New Zealand Plant Prot.,* **55** : 327-330.

Shome, S.K. and Mustafee, T.P. (1966). *Alternaria tagetica* sp. Nov. causing blight of marigold (*Tagetes* sp.). *Curr. Sci.*, **35** : 370-374.

Srinivas, T., Rao, K.C. and Chattopadhyaya, C. (1997). Effect of botanicals and chemical on the management of blight of sunflower. *Zeitschriftfur Planzenkrankheiton-Ptlanzensehutz,* **104** (5) : 523 – 527.

*_*_*_*_*_*