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Variation of Alkaloids Content of Vinca Rosea *(Catharanthus roseus* L.) Under Different Meteorological Conditions of Five Governorates of Egypt.

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ABSTRACT

The aim of the present study was the investigation of the alkaloids content of the dry herb of *Catharanthus roseus* under different meteorological conditions of five governorates of Egypt; Alexandria, Gharbia, Giza, Minia and Wady Gadead. There was a strong correlation between average temperature and the percentage of alkaloids in the plant. The amount of accumulated alkaloids increased at medium temperatures to a level of (27-29°C), then sudden reduced at higher average temperature (30°C). This was evident in Wady Gadead governorate in the summer. When the average temperature was raised to (33.60°C) in Wady Gadead, it recorded the lowest alkaloids compared to other governorates.

Key words: *Catharanthus roseus* L, Vinca rosea, Meteorology, Temperature, Alkaloids.

Introduction

Vinca rosea *(Catharanthus roseus*) is a species of *Catharanthus* native and endemic to Madagascar. Other English names occasionally used include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle, and "Old-maid". In the wild, it is an endangered plant. It is however widely cultivated and is naturalized in subtropical and tropical areas of the world. It is an evergreen subshrub or herbaceous plant growing to 1 m tall. The leaves are oval to oblong. The flowers are white to dark pink with a darker red centre and basal tube. The fruit is a pair of follicles (Brickell (1996).

The most important genus in Apocynaceae family is Catharanthus. Periwinkle is cultivated as an ornamental plant almost throughout the tropical and subtropical world (Hey wood, 1971). It grows in abundance as a naturalized plant in many regions, particularly in arid coastal locations. As an ornamental plant, it is appreciated for its hardiness in dry and nutritionally deficient conditions, popular in subtropical gardens where temperatures never fall below 5 to 7°C. It is noted for its long flowering period, throughout the year in tropical conditions, and from spring to late autumn in warm temperate climates. Full sun and well-drained soil are preferred for growth. It thrives in the heat of summer. The species has long been cultivated for herbal medicine and as an ornamental plant. In traditional Chinese medicine, its extracts have been used to treat numerous diseases including diabetes, malaria and Hodgkin's disease. Its alkaloids help in relieving muscle pain, depression of central nervous system and wasps stings. It is used in case of nosebleed, bleeding gums, mouth ulcers and sore throats. It is also used internally for loss of memory, hypertension, cystitis, gastritis and enteritis, diarrhea and raised blood sugar levels (Taylor and Fransworth, 1973).

Vinca *(C. roseus*) is well known in folk medicine for the treatment of diabetes (Harridy, 1986). It is used in plant pathology as it is a rich source of alkaloids. Alkaloids is a class of organic compounds composed of carbon, hydrogen, nitrogen, and usually oxygen that are often derived from plants such as *C. roseus*. Alkaloids have physiological effects render vinca plant medicinal values. Some vinca alkaloids are powerful muscle relaxant, others are dilator of the pupils of the eyes.

Vincristine, Vinblastine, Ibogaine, Yohimbine, Reserpine, Ajmalicine, Lochnerine, and Vincaleukoblastine proved to be active alkaloids in *Catharanthus roseus.* Perivine is a representative alkaloid of average molecular weight present in *C. roseus* plant (Masoud *et al.*, 1968). It is also the major alkaloids formed in *C. roseus* leaves. Balbaa *et al*., (1976) pointed out that, more than 100 alkaloids have been isolated from the different parts of the plant and all parts contain alkaloids. The roots are regarded as purgative, vermifugal, depurative and haemostatic and are used to relieve toothache. The roots are used to control dysentery. Vietnamese choose the root of the white flowered variety of vinca to treat malaria. Alkaloids, vincaleukoblastine or vinblastine and leurocristine or vincristine have been employed for the past sixteen years in cancer chemotherapy. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia. Six alkaloids proved to be active in *C. roseus*, two are now commercially available. Vinblastine is used mainly for the treatment of Hodgkin disease and chorion epithelioma. Vincristine is also a cytotoxic agent and is used principally in leukaemia treatment in children and short remissions (Trease and Evans, 1978). They added that at least 70 alkaloids have

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now been isolated from Vinca.; for example, ajmalicine, Lochnerine, serpentine and tetrahydroalstonine. Vincristine and vinblastine are of a particular interest. Reda (1978) reported that, the most active stage of alkaloid biosynthesis in all organs of *Catharanthus roseus* was at the start of flowering, when the highest total alkaloid content was found.

Dahatonde and joshi (1982) reported that, *C. roseus* is considered as a commercial crop due to the active substances content, since the foliage base stem and root contain over sixty five alkaloids of which vincristine and vinblastine are now employed in cancer treatment, also reserpine and ajmalicine are used in controlling high blood pressure. The foliage was reported to be rich in vincaleukoblastine (VLB) alkaloids, while, root and base stem contain ajmalicine alkaloids. Sukmar *et al.*, (1981) reported that, Vincaleukoblastine is administered mainly in the treatment of Hodgkin's disease and choriocarcinoma. Leurocristine is employed mainly in childhood leukemia and breast cancer. Combined vincaleukoblastine and leurocristine when injected weekly on patients with malignant lymphoma produced beneficial results in 43 percent of the patients. A combination of leurocristine and the synthetic drugs prednisone and methotrexate has been more successful in achieving regression of adult leukemia than leurocristine alone. Dramatic remission of leukemia in children has been attained with leurocristine, prenisolone, 6-mercaptopurine and methotrexate administered together over thirteen months. More recently, leurocristine, prednisone and the antibiotic daunomycin have given encouraging results. Many other combinations with periwinkle alkaloids have been tried with varying degrees of success in cancer therapy. Vinca rosea plant can be used as diuretic antidysenteric and wound healing (Raghuvanshi and Chauhan,1982). Frequent or prolonged administration of small doses of *Catharanthus* alkaloids in cancer therapy has caused platelet damage. Patients may exhibit transient side effects such as hair loss, nausea, vomiting, abdominal cramps, phlebitis, dermatitis, loss of deep-tendon reflexes, temporary mental depression, headache, nosebleed, loss of appetite, stomatitis and, bleeding of old peptic ulcers. In animals and humans, excessive dosage over long periods has resulted in convulsions, damage to the central nervous system and sometimes fatality.

Relative humidity may be defined as the ratio of the water vapor density (mass per unit volume) to the saturation water vapor density, usually expressed in percent. As temperature goes up, saturated vapor pressure goes up as well, and relative humidity will go down.

Transport of alkaloids across the tonoplast into the vacuolar space was characterized as an active, energy requiring mechanism, which was sensitive to the temperature and pH of the surrounding medium (Deus and Zenk., 1984). Sen and Datta (1986) reported that, in *Catharanthus roseus* leaf, individual alkaloids differed with season but the total was less affected. (Shukla,1997) reported that, as the temperature at which the isolation process of alkaloids from the dried leaves of *C. roseus* was increased from 26° to 42° C, the alkaloid yield increased by 0.30 to 0.38%. PH had little effect on alkaloid yield which increased by 0.07 to 0.13%, as the PH increased from 8.5 to 9.0. Paul and Jean (2008) pointed out that, vinca plant is adapted to hot, dry or windy slopes, bright sunshine, well-drained soil and prolonged summer heat.

Materials and Methods

The present study was carried out during seasons of two successive years 2009 and 2010 (winter, spring, summer and autumn) in five governorates of Egypt; Alexandria (Montaza Park), Gharbia (Gemmaiza research station), Giza (agricultural museum garden-Dokky), Minia (Malawy research station) and Wady Gadead (Garden of South Valley University). Laboratory experiments were conducted in the agriculture research center. Seeds of *Catharanthus roseus* were obtained from medicinal and aromatic plant department of horticulture institute of agriculture research center in Dukky. In the two years, the seeds were sown in pots for two months before transplanted to the experimental filed on January, April, July and October respectively. Each season consists of four sowing dates, winter (January, February and March), spring (April, May and June), summer (July, Augustus and September), autumn (October, November and December). In each season, four cuts were taken at the end of March, June, September and December respectively.

Vinca seeds were planted in pots of 15 cm in diameter at November, February, May and August respectively, and then transferred to pots of size 30 cm in diameter at January, April, July and October respectively, during the agricultural seasons. The plant age when taking readings is five months from cultivation to study the effect of the variance in the climatic factors on alkaloids contents.

A description of the main climatic factors air temperatures °C and relative humidity %s (max, min and mean) during the period of the study was obtained from the ministry of agriculture where monthly meteorological report was taken. The herb was oven dried at 60 °C until a constant weight (g) was obtained. Total alkaloids content % of dry herb was done according to the method described by Masoud *et al.,* (1968).

Extraction of alkaloids:

a) Put one gram of dry milled material in a flask with a narrow slot. *b)* Add 3-5 ml of 25% ammonia solution to the sample and leave to dry completely. *c)* Add 15 ml of chloroform, then close well for 24 hours. *d)* Filter the sample with a filter paper and wash three times with chloroform 5 ml each time. *e)* Add 0.4-0.5 ml of

2% sulfuric acid and then add ammonia solution to the residue to reach a PH 10. *f)* For extraction of alkaloids add 10 ml of chloroform (3 times) and likely mix for 10 minutes using the separating funnel to separate the chloroform layer from the acid layer. *g)* Take the lower layer of chloroform and place it in a flask with the addition of anhydrous sodium sulfate to get rid of the moisture. *h)* After filtration the flask and the filter paper were washed with 5 ml chloroform, and then concentrate chloroform layer in a water path at 80 °C until the volume became approximately 2 ml. *i)* Leave the solution to cool and then add 5 ml of chloroform with the addition of a drop of violet gentian indicator and 5 ml of glacial acetic acid with good shaking *j)* The solution then titrated by using 0.01 N perchloric acid until the green color appear.

$$\% \text{ of alkaloids} = \frac{V/1000 \times N \times MW}{\text{Sample wt.}} \times 100$$

Statistical analytical comparison of alkaloids content was performed using the "Least Significant Difference (L.S.D)" test at the 0.05 level, as described by Snedecore and Cochran (1982).

Results

1- Alkaloids content of the dry herb of *C. roseus* L under different meteorological conditions in Alexandria governorate.

Tables (1&2) shows the alkaloids contents (%) in the dry herb of *C. roseus* L during seasons of two successive years (2009 and 2010), air temperatures (max, min and mean °C) and relative humidity %s (max, min and mean) in Alexandria. It is clear that, in the first year (2009), winter alkaloids % was (0.22) when air temperatures °C (max, min and mean) were (20.2), (9.6) and (14.9) respectively while relative humidity %s (max, min and mean) were (99), (64) and (82) respectively. In spring, it was (0.33) when air temperatures °C (max, min and mean) were (28.4), (16.4) and (22.0) respectively and relative humidity %s (max, min and mean) were (99), (60) and (79) respectively. In summer, alkaloids % was (0.41) when air temperatures °C (max min and mean) were (32.6), (21.7) and (27.2) respectively while relative humidity %s (max, min and mean) were (99), (24) and (61) respectively. In autumn, alkaloids % was (0.28) when air temperatures °C (max, min and mean) were (24.1), (13.2) and (18.6) respectively and relative humidity %s (max, min and mean) were (98), (62) and (80) respectively. The mean of alkaloids % was (0.31) when the means of air temperatures °C (max, min and mean) were (26.33), (15.23) and (20.68) respectively and relative humidity %s (max, min and mean) were (99), (52) and (75) respectively.

In the second year (2010), alkaloids % recorded (0.23) in winter when air temperatures °C (max, min and mean) were (20.8), (9.7) and (15.2) respectively and relative humidity %s (max, min and mean) were (99), (59) and (79) respectively. Spring alkaloids % was (0.31) when air temperatures °C (max, min and mean) were (27.3), (17.0) and (22.1) respectively and relative humidity %s (max, min and mean) were (99), (61) and (80) respectively. In summer, it recorded (0.38) when air temperatures °C (max, min and mean) were (32.4), (24.1) and (28.3) respectively and relative humidity %s (max, min and mean) were (92), (50) and (71) respectively. Autumn alkaloids % was (0.26) when air temperatures °C (max, min and mean) were (25.2), (15.7) and (20.5) respectively and relative humidity %s (max, min and mean) were (93), (55) and (74) respectively. The mean of alkaloids % was (0.30) when the means of air temperatures °C (max, min and mean) were (26.43), (16.63) and (21.50) respectively and relative humidity %s (max, min and mean) were (96), (56) and (76) respectively.

Table 1: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Alexandria governorate during seasons of 2009.

Seasons	Alkaloids %	Air temperature °C			Relative humidity %		
		Max	Min	Mean	Max	Min	Mean
Winter	0.22	20.20	9.60	14.90	99	64	82
Spring	0.33	28.40	16.40	22.00	99	60	79
Summer	0.41	32.60	21.70	27.20	99	24	61
Autumn	0.28	24.10	13.20	18.60	98	62	80
Mean	0.31	26.33	15.23	20.68	99	52	75

Table 2: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Alexandria governorate during seasons of 2010.

Seasons	Alkaloids %	Air temperature °C			Relative humidity %		
		Max	Min	Mean	Max	Min	Mean
Winter	0.23	20.80	9.70	15.20	99	59	79
Spring	0.32	27.30	17.00	22.10	99	61	80
Summer	0.38	32.40	24.10	28.30	92	50	71
Autumn	0.26	25.20	15.70	20.50	93	55	74
Mean	0.30	26.43	16.63	21.50	96	56	76

2- Alkaloids content of the dry herb of *C. roseus* L under different meteorological conditions in Gharbia governorate.

Tables (3&4) shows alkaloids contents (%) in the dry herb of *C. roseus* L during seasons of two successive years (2009 and 2010), air temperatures (max, min, and mean °C) and relative humidity %s (max, min, and mean) in Gharbia governorate. It is clear that, in the first year (2009), alkaloids % was (0.18) when air temperatures °C (max, min and mean) were (19.8), (9.8) and (14.8) respectively while relative humidity %s (max, min and mean) were (82), (39) and (60) respectively. In spring, it was (0.31) when air temperatures °C (max, min and mean) were (28.1), (16.8) and (22.5) respectively and relative humidity %s (max, min and mean) were (84), (27), and (56) respectively. In summer, alkaloids % was (0.40) when air temperatures °C (max, min and mean) were (33.5), (21.6) and (27.6) respectively while relative humidity %s (max, min and mean) were (81), (36) and (58) respectively. In autumn, alkaloids % was (0.24) when air temperatures °C (max, min and mean) were (23.8), (13.7) and (18.7) respectively and relative humidity %s (max, min and mean) were (79), (32) and (55) respectively. The mean of alkaloids % was (0.283) when the means of air temperatures °C (max, min, and mean) were (26.30), (15.48) and (20.90) respectively and relative humidity %s (max, min and mean) were (81), (33) and (57) respectively.

In the second year (2010), alkaloids % recorded (0.22) in winter when air temperatures °C (max, min and mean) were (20.1), (9.7) and (15.8) respectively and relative humidity %s (max, min and mean) were (79), (36) and (57) respectively. Spring alkaloids % was (0.32) when air temperatures °C (max, min and mean) were (28.3), (17.1) and (22.7) respectively and relative humidity %s (max, min and mean) were (82), (29) and (56), respectively. In summer, it was (0.37) when air temperatures °C (max, min and mean) were (32.20), (20.70) and (26.45) respectively and relative humidity %s (max, min and mean) were (82), (39) and (60), respectively. Autumn alkaloids % was (0.24) when air temperatures °C (max, min and mean) were (22.7), (12.5) and (17.6) respectively and relative humidity %s (max, min and mean) were (80), (35) and (57) respectively. The mean of alkaloids % was (0.287) when the means of air temperatures °C (max, min and mean) were (25.83), (15.00) and (20.64) respectively and relative humidity %s (max, min and mean) were (81), (35) and (58) respectively.

Table 3: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Gharbia governorate during seasons of 2009.

Seasons	Alkaloids %	Air temperature °C			Relative humidity %		
		Max	Min	Mean	Max	Min	Mean
Winter	0.18	19.80	9.80	14.80	82	39	60
Spring	0.31	28.10	16.80	22.50	84	27	56
Summer	0.40	33.50	21.60	27.60	81	36	58
Autumn	0.24	23.80	13.70	18.70	79	32	55
Mean	0.283	26.30	15.48	20.90	81	35	57

Table 4: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Gharbia governorate during seasons of 2010.

Seasons	Alkaloids %	Air temperature °C			Relative humidity %		
		Max	Min	Mean	Max	Min	Mean
Winter	0.22	20.10	9.70	15.80	79	36	57
Spring	0.32	28.30	17.10	22.70	82	29	56
Summer	0.37	32.20	20.70	26.45	82	39	60
Autumn	0.24	22.70	12.50	17.60	80	35	57
Mean	0.287	25.83	15.00	20.64	81	35	58

3- Alkaloids content of the dry herb of *C. roseus* L under different meteorological conditions in Giza governorate.

Tables (5&6) shows alkaloids contents (%) in the dry herb of *C. roseus* L during seasons of two successive years (2009 and 2010), air temperatures and relative humidity %s (max, min, and mean) in Giza governorate. It is clear that, in the first year (2009), winter alkaloids % was (0.25) when air temperatures °C (max , min and mean) were (22.70), (9.30) and (16.00) respectively while relative humidity %s (max, min and mean) were (77), (27) and (52) respectively. In spring, it was (0.34) when air temperatures °C (max, min and mean) were (31.2), (18.0) and (24.6) respectively and relative humidity %s (max, min and mean) were (75), (21) and (48) respectively. In summer, alkaloids % was (0.37) when air temperatures °C (max, min and mean) were (35.0), (22.0) and (28.5) respectively while relative humidity %s (max, min and mean) were (77), (23) and (50) respectively. In autumn, alkaloids % was (0.24) when air temperature °C (max, min and mean) were (24.3), (11.5) and (17.9) respectively and relative humidity %s (max, min and mean) were (77), (30) and (53) respectively. The mean of alkaloids % was (0.30) when the means of air temperatures °C (max, min and mean) were (28.30), (15.20) and (21.75) respectively and relative humidity %s (max, min and mean) were (76), (25) and (51) respectively.

In the second year (2010), winter alkaloids % was (0.21) when air temperatures °C (max, min and mean) were (21.1), (7.8) and (14.5) respectively and relative humidity %s (max, min and mean) were (76), (26), and

(51) respectively. Spring alkaloids % was (0.36) when air temperatures °C (max, min and mean) were (32.3), (17.2) and (24.8) respectively and relative humidity %s (max, min and mean) were (75), (20) and (48), respectively. In summer, it was (0.33) when air temperatures °C (max, min and mean) were (36.4), (22.3) and (29.3) respectively and relative humidity %s (max, min and mean) were (77), (24) and (50), respectively. Autumn alkaloids % was (0.25) when air temperatures °C (max, min and mean) were (25.9), (13.6) and (19.8) respectively and relative humidity %s (max, min and mean) were (77), (29) and (53) respectively. The mean of alkaloids % was (0.29) when the means of air temperatures °C (max, min and mean) were (28.93), (15.23) and (22.10) respectively and relative humidity %s (max, min and mean) were (76), (25), and (50) respectively.

Table 5: Alkaloids % in dry herb of *C. roseus* L under different meteorological conditions in Giza governorate during seasons of 2009.

Seasons	Alkaloids %	Air temperature °C			Relative humidity %		
		Max	Min	Mean	Max	Min	Mean
Winter	0.25	22.70	9.30	16.00	77	27	52
Spring	0.34	31.20	18.00	24.60	75	21	48
Summer	0.37	35.00	22.00	28.50	77	23	50
Autumn	0.24	24.30	11.50	17.90	77	30	53
Mean	0.300	28.30	15.20	21.75	76	25	51

Table 6: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Giza governorate during seasons of 2010.

Seasons	Alkaloid %	Air temperature °C			Relative humidity %		
		Max	Min	Mean	Max	Min	Mean
Winter	0.21	21.10	7.80	14.50	76	26	51
Spring	0.36	32.30	17.20	24.80	75	20	48
Summer	0.33	36.40	22.30	29.30	77	24	50
Autumn	0.25	25.90	13.60	19.80	77	29	53
Mean	0.287	28.93	15.23	22.10	76	25	50

4- Alkaloids content of the dry herb of *C. roseus* L under different meteorological conditions in Minia governorate.

Tables (7&8) shows alkaloids contents (%) in the dry herb of *C. roseus* L during seasons of two successive years (2009 and 2010), air temperatures (max, min and mean °C) and relative humidity %s (max, min, and mean) in Minia governorate. It is clear that, in the first year (2009), winter alkaloids % was (0.21) when air temperatures °C (max , min and mean) were (21.4), (7.3) and (14.3) respectively, while relative humidity %s (max, min and mean) were (78), (36), and (57) respectively. In spring, it was (0.35) when air temperatures °C (max, min and mean) were (32.7), (16.3) and (24.5) respectively and relative humidity %s (max, min and mean) were (70), (21), and (45) respectively. In summer, alkaloids % was (0.40) when air temperatures °C (max, min and mean) were (35.7), (20.3) and (28.0) respectively while relative humidity %s (max, min and mean) were (70), (23) and (47) respectively. In autumn, alkaloids % was (0.26) when air temperatures °C (max, min and mean) were (24.3), (10.6) and (17.5) respectively and relative humidity %s (max, min and mean) were (74), (32) and (53) respectively. The mean of alkaloids% was (0.31) when the means of air temperatures °C (max, min and mean) were (28.53), (13.63) and (21.08) respectively and relative humidity %s (max, min and mean) were (73), (28) and (50) respectively.

Table 7: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Minia governorate during seasons of 2009.

Seasons	Alkaloids %	Air temperature °C			Relative humidity %		
		Max	Min	Mean	Max	Min	Mean
Winter	0.21	21.40	7.30	14.30	78	36	57
Spring	0.35	32.70	16.30	24.50	70	21	45
Summer	0.40	35.70	20.30	28.00	70	23	47
Autumn	0.26	24.30	10.60	17.50	74	32	53
Mean	0.31	28.53	13.63	21.08	73	28	50

In the second year (2010), alkaloids % was (0.18) in winter when air temperatures °C (max, min and mean) were (20.3), (6.3) and (13.3) respectively and relative humidity %s (max, min and mean) were (75), (31) and (53) respectively. Spring alkaloids % was (0.36) when air temperatures °C (max, min and mean) were (33.2), (17.4) and (25.3) respectively and relative humidity %s (max, min and mean) were (72), (22), and (47) respectively. In summer, it was (0.37) when air temperatures °C (max, min and mean) were (35.8), (21.0) and (28.4), respectively and relative humidity %s (max, min and mean) were (71), (23) and (47) respectively. Autumn alkaloids % was (0.21) when air temperature °C (max, min and mean) were (22.2), (7.4) and (14.8) respectively and relative humidity %s (max, min and mean) were (75), (31) and (53), respectively. The mean of

alkaloids % was (0.28) when the means of air temperatures °C (max, min and mean) were (27.88), (13.03) and (20.50) respectively and relative humidity %s (max, min and mean) were (73), (27) and (50) respectively.

Table 8: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Minia governorate during seasons of 2010.

Seasons	Alkaloids %	Air temperature °C			Relative humidity %		
		Max	Min	Mean	Max	Min	Mean
Winter	0.18	20.30	6.30	13.30	75	31	53
Spring	0.36	33.20	17.40	25.30	72	22	47
Summer	0.37	35.80	21.00	28.40	71	23	47
Autumn	0.21	22.20	7.40	14.80	75	31	53
Mean	0.28	27.88	1673	20.45	73	27	50

5-Alkaloids content of the dry herb of *C. roseus* L under different meteorological conditions in Wady Gadead governorate.

Tables (9&10) shows alkaloids contents (%) in the dry herb of *C. roseus* L during seasons of two successive years (2009 and 2010), air temperatures (max, min and mean °C) and relative humidity %s (max, min and mean) in Wady Gadead. It is clear that, in the first year (2009), winter alkaloids % was (0.27) when air temperature °C (max, min and mean) were (28.4), (9.8) and (19.1) respectively, while relative humidity %s (max, min and mean) were (70), (37) and (53) respectively. In spring, it was (0.34) when air temperatures °C (max, min and mean were (39.0), (19.7) and (29.4) respectively and relative humidity %s (max, min and mean were (53), (31) and (42) respectively. In summer, alkaloids % was (0.21) when air temperature °C (max, min and mean) were (42.2), (23.4) and (32.8) respectively while relative humidity %s (max, min and mean) were (56), (35) and (46) respectively. In autumn, alkaloids % was (0.28) when air temperatures °C (max, min and mean) were (31.2), (13.3) and (22.3) respectively and relative humidity %s (max, min and mean) were (77), (42) and (60) respectively. The mean alkaloids % was (0.275) when the means of air temperature °C (max, min, and mean) were (35.20), (16.55) and (25.90) respectively and relative humidity %s (max, min and mean) were (64), (36) and (50) respectively.

In the second year (2010), alkaloids % recorded (0.26) in winter when air temperatures °C (max, min and mean) were (27.5), (8.6) and (18.1) respectively and relative humidity %s (max, min and mean) were (74), (41) and (58) respectively. Spring alkaloids % was (0.33) when air temperatures °C (max, min, and mean) were (39.8), (19.1) and (29.5) respectively and relative humidity %s (max, min and mean) were (54), (33) and (43), respectively. In summer, it was (0.17) when air temperatures °C (max, min and mean) were (43.5), (23.6) and (33.6) respectively and relative humidity %s (max, min and mean) were (55), (32), and (44), respectively. Autumn alkaloids % was (0.26) when air temperatures °C (max, min and mean) were (31.8), (13.9) and (22.9) respectively and relative humidity %s (max, min, and mean) were (77), (41)and (59), respectively. The mean alkaloids % was (0.255) when the means of air temperatures °C (max, min and mean) were (35.65), (16.30) and (26.03) respectively and relative humidity %s (max, min and mean) were (65), (37) and (51) respectively.

Table 9: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Wady Gadead governorate during seasons of 2009.

Seasons	Alkaloids %	Air temperature °C			Relative humidity%		
		Max	Min	Mean	Max	Min	Mean
Winter	0.27	28.40	9.80	19.10	70	37	53
Spring	0.34	39.00	19.70	29.40	53	31	42
Summer	0.21	42.20	23.40	32.80	56	35	46
Autumn	0.28	31.20	13.30	22.30	77	42	60
Mean	0.275	32.70	16.55	25.90	64	36	50

Table 10: Alkaloids % in the dry herb of *C. roseus* L under different meteorological conditions in Wady Gadead governorate during seasons of 2010.

Seasons	Alkaloids %	Air temperature °C			Relative humidity%		
		Max	Min	Mean	Max	Min	Mean
Winter	0.26	27.50	8.60	18.10	74	41	58
Spring	0.33	39.80	19.10	29.50	54	33	43
Summer	0.17	43.50	23.60	33.60	55	32	44
Autumn	0.26	31.80	13.90	22.90	77	41	59
Mean	0.2	35.65	16.30	26.03	65	37	51

6- Effect of different meteorological conditions and four different sowing dates in five governates of Egypt on alkaloids content of the dry herb of *C.roseus* L:

Tables 11&12 elucidate that, in the winter season of 2009, Gharbia, Minia and Alexandria governorates which recorded the lowest average temperatures (14.30. 14.80, 14.90 °C) recorded the lowest percentage of alkaloids (0.18, 0.21 and 0.22), while Giza recorded high percentage (0.25) with a higher average temperature

(16°C). The highest percentage of alkaloids (0.27) was in Wady Gadead governorate, where the highest average temperature (19.10 °C).

In the winter season of 2010, Minia governorate recorded the lowest proportion of alkaloids (0.18) where average temperature was the lowest (13.30), followed by Giza, Gharbia and Alexandria governorates (0.21, 0.22 and 0.23) and the increase in alkaloids content was associated with increased average temperature (14.50, 14.80 and 15.20). Wady Gadead governorate recorded the highest proportion of alkaloids (0.26) where average temperature was the highest (18.10°C).

Table 11: Effect of different meteorological conditions and four different sowing dates in five governates of Egypt on alkaloids % of the dry herb of *C. roseus* L during seasons of 2009.

Governorate	Alkaloid % and Meteorological conditions	Seasons				
		Winter	spring	Summer	Autumn	Mean alkaloids%
Alexandria	Alkaloids %	0.22	0.33	0.41	0.28	0.310
	Average temperature °C.	14.9	22.0	27.20	18.60	20.68
	Relative humidity %	82	79	61	80	75
Gharbia	Alkaloids %	0.18	0.31	0.40	0.24	0.282
	Average temperature °C.	14.30	22.50	28.40	18.60	20.90
	Relative humidity %	60	56	47	80	57
Giza	Alkaloids %	0.25	0.34	0.37	0.24	0.300
	Average temperature °C.	16.00	24.6	28.50	17.90	21.75
	Relative humidity %	53	48	50	53	51
Minia	Alkaloids %	0.21	0.35	0.40	0.26	0.305
	Average temperature °C.	14.80	24.50	28.40	17.50	21.08
	Relative humidity %	57	45	47	53	50
Wady Gadead	Alkaloids %	0.27	0.34	0.21	0.28	0.275
	Average temperature °C.	19.10	29.50	32.80	25.90	25.90
	Relative humidity %	52	43	46	50	50
Mean of alkaloids %		0.226	0. 334	0.358	0.260	0.294
L.S.D at 5 %	Sowing date	0.001				
	Location	0.003				

Table 12: Effect of different meteorological conditions and four different sowing dates in five governates of Egypt on alkaloids % of the dry herb of *C. roseus* L during seasons of 2010.

Governorate	Alkaloid % and Meteorological conditions	Seasons				
		Winter	spring	Summer	Autumn	Mean alkaloids%
Alexandria	Alkaloids %	0.23	0.31	0.38	0.26	0.295
	Average temperature °C.	15.20	22.10	28.30	14.80	21.50
	Relative humidity %	79	80	71	53	76
Gharbia	Alkaloids %	0.22	0.32	0.37	0.24	0.287
	Average temperature °C.	14.80	22.70	26.45	17.60	20.64
	Relative humidity %	57	56	60	57	58
Giza	Alkaloids %	0.21	0.36	0.33	0.21	0.277
	Average temperature °C.	14.50	24.80	29.30	19.80	22.10
	Relative humidity %	51	48	50	53	50
Minia	Alkaloids %	0.18	0.36	0.37	0.25	0.290
	Average temperature °C.	13.30	25.30	28.40	14.80	20.45
	Relative humidity %	53	47	47	53	50
Wady Gadead	Alkaloids %	0.26	0.33	0.17	0.260	0.255
	Average temperature °C.	18.10	29.50	33.60	22.90	26.03
	Relative humidity %	58	43	44	59	51
Mean of alkaloids %		0.22	0.336	0.324	0.236	0 .279
L.S.D at 5 %	Sowing date	0.002				
	Location	0.004				

In the spring season of 2009, Minia governorate recorded the highest rate in alkaloids accumulation (0.35 %) followed by Wady Gadead, Giza, Alexandria and Gharbia governorates (0.34, 0.34, 0.33 and 0.31%). The highest accumulation alkaloids was at an average temperature of 24.50°C.

In the spring season of 2010, the highest alkaloids accumulation rate was in Minia and Giza governorates (0.36) at average temperature 24.60– 25.30 °C followed by Wady Gadead (0.33%) at an average temperature of 29.5 °C and the lowest alkaloids rates were in Alexandria and Gharbia (0.32 and 0.31) at average temperatures between 22.10-22.70 °C. The accumulation of alkaloids at an average temperature of 25 °C was higher than that at temperature of 29.5 °C.

In the summer season of 2009, Alexandria recorded the highest rate of alkaloids (0.41) at an average temperature of 27.2 ^{0}C, followed by Gharbia, Minia and Giza governorates (0.40, 0.40 and 0.37%) respectively

while there was a clear decrease of alkaloids percentage (0.21%) in Wady Gadead when the average temperature is raised to 32.8 °C.

In the summer season of 2010, the results also indicate increasing the proportion of alkaloids at an average temperatures of 26-29 ^{0}C with a clear decrease of alkaloids percentage at an average temperature more than 30 °C. The most shortage of alkaloids percentage was in Wady Gadead (0.17%) when the average temperature was raised to 33.6 ^{0}C.

In the autumn season 2009, Wady Gadead and Alexandria recorded the highest proportion of alkaloids (0.28) followed by Minia (0.26) and Giza and Gharbia governorates (0.24).

In the autumn season of 2010, Wady Gadead and Alexandria governorates also recorded the highest proportion of alkaloids accumulation (0.26) followed by Giza (0.25) and Gharbia and Minia governorates (0.24 and 0.21).

Thus, our data may lead to conclude that, The high percentages of alkaloids were associated with the increase of average temperature and the best average temperature for alkaloids accumulation was at 25 -29°C. The increase of average temperature led to an acute decrease of alkaloids content and increasing alkaloids shortages was at temperature exceeds 30°C.

Discussion

According to results, there are significant differences in the content of alkaloids as secondary metabolites in *C. roseus* grown under varying temperature and humidity conditions. These results are in agreement with Erik (2006) who reported that, the temperature is the primary and most important factor controls growth and alkaloids content of *C. roseus* and the warmer temperature gives faster growth and higher alkaloids contents of *C. roseus*. Moreover, Julius (2009) reported a high content of alkaloids in *Lupinus angustifolius* seeds harvested in growing season characterized by high ambient temperatures and in a second green house experiment at different temperatures, higher temperatures resulted in the production of seeds of higher alkaloids content.

Also, The results confirmed significant effects of sowing dates and locations on alkaloids content of *C.roseus* in the five governorates of Egypt. These results are in consistency with Trease and Evans (1978), Gabidzashvili (1974) Balbaa (1976), Heinze (1976), Harridy (1986), Bannister (1986), Jeong and Hong (1987), Narkiewicz (1991), Toivonen *et al.,* (1992), Pietsch *et al.,* (1995), Erick (2006) and Paul and jean (2008). Heinze (1976) pointed out that, the chief factor affecting growth and alkaloids content of *C. roseus* was temperature; growth was best at 25 °C for 66 days after pricking out and at 20 °C thereafter. Suspension cultures of *C. roseus* cells grown at a range of temperatures (16-40 °C) greatly increased with a temperature increase from 16 to 27°C (Courtois and Guern, 1980). Likq, *et al.* (1984) reported that, dry weight and alkaloids content of *Fritillaria cirrhosa* bulb increase with temperatures decrease where higher temperatures are not suitable for the growth of *F. cirrhosa*. Narkiewicz, (1991) stated that heated and good illuminated glasshouse gave a good alkaloid yield of seeds of *C. roseus.* Toivonen *et al.,*(1992) reported that, the amount of crude alkaloids fraction/cell and the specific content of serpentine and ajmalicine increased with an increase in temperature from 12 to 27 °C. The growth of hairy root cultures of *C. roseus* was fastest at the highest temperature 32°C.

In our results, the effect of temperature on alkaloids contents of *C. roseus* L. grown under different temperature conditions may be attributed to the impact of temperature on rates of respiration and photosynthesis processes. Respiration and photosynthesis are vital processes catalyzed by enzymes which are very sensitive to temperature variations.

Generally, alkaloids are organic nitrogen containing compounds, mainly derived from amino acids. Temperature range which enhances photosynthesis may lead to adequate amino acids for both protein synthesis and alkaloids production. On the other hand, lowering photosynthetic rate by a temperature range may drastically affect synthesis of protein and alkaloids (Transeau.,1994). Also, activation of respiration rates with a definite temperature range may be associated with liberation of certain amino acids from storage proteins which may be are intermediates in alkaloids biosynthesis and cannot be used in protein synthesis, therefore these amino acids may be incorporated into alkaloids production. George and Edmund (1978) pointed out that, at very higher temperatures, alkaloids content may be negatively affected, due to breaking down of some alkaloids to furnish nitrogen and carbon for fresh amino acids biosynthesis for accommodation to higher temperatures.

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