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Response of sweet basil to jasmonic acid application in relation to different water supplies.

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Pot experiments were carried out at the Faculty of Agriculture, Minoufiya University in 2008 and 2009 seasons, to investigate the effect of water stress levels 100 (w_0), 60 (w_1) and 40 (w_2) % of field capacity (FC) and the foliar application of jasmonic acid (JA) at the rates of 0.0 (JA_0) as a control, 2.4 (JA_1) and 4.8 (JA_2) mM on growth, physiological and biochemical aspects, as well as anatomical structure in sweet basil plants. Results showed that water stress caused a significant reduction in height, Number of inflorescence branches, leaf area and dry weight of leaves and stems, relative water content and chlorophyll concentration, while the reverse effect was obtained in osmotic potential and membrane integrity compared to the control. Peroxidase and catalase activity was decreased under water stress condition. 40% FC decreased total sugars but increased total amino acids and proline concentration. Moreover, 40% FC caused a highly significant reduction in the ground tissue, total stem area and conductive tissues. Essential oil % and major component (Menthol) were decreased under 40% FC compared with the control. The reverse results were true with JA application at the rate of 4.8 mM under water stress condition. From the obtained results, it could be recommended that, spraying JA at 4.8 mM under 40% FC resulted in high yield of oil with good quantity and quality in both seasons.

Key words: Water stress, Sweet basil, Jasmonic acid, Peroxidase, Catalase, Essential oil, Anatomical structure

Sweet basil (*Ocimum basilicum* L.) (Fam. Labitaceae) is considered an important medicinal and aromatic plants. Its importance is mainly due to its several uses in pharmaceutical and food industries, which its leaves contain essential oils used for medicinal purposes (widely used in traditional medicines) (Ntezurubanza *et al.,* 1986).

Water stress is one of the main environmental factors that adversely affect plant growth, productivity and survival. Drought causes visible injury to leaves and stomatal closure, leaf rolling and physiological disorders. Water stress usually induces the accumulation of reactive oxygen species (ROS), which cause oxidative damage to plants (Apel and Hirt 2004).

Water stress also accelerates the decline of chlorophyll and protein content, negatively alter both the structure and function of membranes (Basisak *et al.,* 1994 and Wang, 1999). Plant can protect itself against oxidative damage by antioxidant system including antioxidative enzymes and nonenzymatic compounds (Mittler, 2002).

Jasmonic acid and its volatile methyl ester, methyl Jasmonate, collectively termed Jasmonates, are regarded as endogenous regulators that play important roles in regulating stress responses, plant growth and development (Creelman *et al.,* 1997), level of endogenous Jasmonates increases during stress conditions such as wounding, ozone stress and water stress (Koda, 1992; Parthier *et al.,* 1992; Creelman *et al.,* 1997 and Kumari *et al.,* 2003).

Therefore, the current work was carried out to find out the convenient rate of Jasmonic acid application under the drought conditions for optimum yield and quality of sweet basil plant.

MATERIALS AND METHODS

Two pot experiments were performed at

the Faculty of Agriculture, Minoufiya University, Shibin El-Kom during the two growing successive seasons of 2008 and 2009, to investigate the effect of water stress and Jasmonic acid on growth, some physiological and biochemical aspects, as well as anatomical structure in sweet basil plants.

Sweet basil seeds (*Ocimum basiliacum*) were obtained from The Agricultural Research center of Giza Egypt. The seeds were sown in the nursery at the end of March of both seasons. The uniform seedlings were transplanted at the end of May in pots (25 cm inner diameter) containing 8 kg of clay loam soil each.

After transplanting, nitrogen fertilization in the form of ammonium sulphate, at the rate of 220 kg / fed. (1.76g/pot) and potassium sulphate (48% K_2O) at the rate of 100kg/fed.(0.8g/pot) were added at two portions, the first one was added after one month from transplanting and the second after 15 days from the first application. Phosphorus in the form of calcium super phosphate (15.5% P_2O_5) at the rate of 100 kg / fed. (0.8) g/pot were added before transplanting.

After transplanting, the pots were watered immediately and pest control as well as the other agriculture practices was used as necessary.

Jasmonic acid was kindly obtained from Prof. Miersch at the Dept. of Plant Biochemistry, Martin Luther Univ., Germany. JA was sprayed at rates of 2.4 and 4.8 mM on the plant foliage to run off the plants. Tween 20 (0.1%) was used as a surfactant and water sprayed as control treatment. JA was sprayed at two times, after 21 and 36 days from transplanting.

The experiment was conducted under three levels of soil moisture (40, 60 and 100% of field capacity) with 6 replicates for each level. The pots were weighed daily and water was added to bring back the soil moisture content to field capacity. The experiment layout in split-plot design with 6 replication included two factors, the main plot consisted of three levels of water supplies [100, 60 and 40% F.C.] and the second factor (subplot) JA [0.00, 2.4 mM and 4.8 mM].

Plant samples ware taken through the growth period at 75 days from transplanting, three plants were taken randomly from each treatment and separated to different plant parts and the following data were recorded:

Plant growth characters: plant height (cm), leaf area (cm^2), plant dry weight of leaves and stem (g) / plant.

Water relations: Relative water content (RWC %) was determined according to the method of Barrs and Weatherley (1962) using the following equation:

$$RWC\ \% = \frac{F.\ wt - Dr.\ wt}{T.\ wt - Dr.\ wt} \times 100$$

Where
F. wt: fresh weight of leaves.
T. wt: weight of turgid.
Dr. wt: dry weight of leaves.
Osmotic pressure was measured as described by Gosev (1960).

Chemical analysis:
Photosynthetic pigments were extracted and determined as mg / g Dr.wt as described by Witham *et al.* (1971).

Membrane leakage was determined following the method of Leopold *et al.* (1981).

Antioxidant enzymes activity, peroxidase and catalase activity was measured in fresh leaves using the methods described by Fehrman and Dimond (1967) and Samantary (2002), respectively.

Total soluble sugars were determined by the method of Dubois *et al.* (1956).

Proline concentration in fresh leaves was determined following the method of Bates *et al.* (1973).

Total amino acids were determined by the method of Rosen (1957).

Oil yield: essential oil % and volatile oil % were determined following the method of British Pharma Copea (1963) and essential oils were analysed using GLC by the method of Teranish (1971).

Anatomical studies: samples were taken for each treatment from the middle internode of the stem (5^{th} internode from the top) at 75 days after transplanting and treated with FAA and embedded in paraffin (O'Brien and MeCully, 1981). Sections were microtomed at 15 microns and different areas were estimated in mm^2.

Statistical analysis: The collected data for both years were statistically analysed using Co-stat Software (1985) and L.S.D. Test was calculated according to Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

Plant growth characters: It was well noticed from Table (1) that all water stress levels significantly reduced plant height of sweet basil plants. Water stress negatively affected the leaf area and dry weight of leaves and stem, the lowest values were recorded fewer than 40% FC which reduced by 30.7% and 33.3 %, respectively in the Second season compared to the control plants. These results are in harmony with those reported by Ali *et al.* (1998) on rose plants and Faried *et al.* (1999) on roselle plants.

Data in Table (1) showed clearly that JA application generally decreased plant height and leaf area, whereas increased the number of inflorescence branches and dry weight of leaves and stems. The highest JA concentration was more effective in this respect.

The induction in the number of inflorescence branches and dry weight were recorded by 100% and 25.6%, respectively when the plant treated by JA_2+W_2 compared to the W_2 treated plants. The same trend was observed by Ueda *et al.* (1995).

It can be clearly noticed that application JA_2 fewer than 40% FC recorded a better results compared to untreated plants under the same level of water stress.

The reduction of growth as a result of decreasing available soil moisture content may be attributed to the inhibiting enlargement of cell division. In addition Abd El-Razik (1996) reported that water stress depressed plant growth could ascribe to the reduction of photosynthetic rate which directly reduced dry matter accumulation. In addition, the physiological activities of root decreased under water stress condition which affects aeration consequently both minerals and water absorption (Hammad, 2000 and Gendy & Sorial, 2002). Moreover, Hassanein *et al.* (2009) reported that the inhibition of plant growth due to water stress appeared to be the results of the inhibition of auxins and gibberellins biosynthesis.

Water relations: Data recorded in Table (2) showed a gradual decrease in RWC and osmotic potential (more negative) with increasing drought stress. Jasmonic acid application generally increased RWC and osmotic potential compared with untreated plants. Jasmonic acid at 4.8 mM. was more effective than JA at 2.4mM. Todorove *et al.* (1998) reported that plants treated with JA showed improving water balance as they had a higher water potential than untreated plants. It is possible that this effect was caused by increasing root to shoot ratio and decrease root resistance to water flow (Pedranzani *et al.,* 2007).

Chemical analysis:

Photosynthetic pigments: Drought stress significantly increased photosynthetic pigments (Table 2) compared with the control plants. The highest chlorophyll pigments concentration was obtained under 60% FC In this regard; Longstreth *et al.* (1984) reported that stressed leaves became thicker than normal leaves leading to increase chloroplasts per unit area.

Jasmonic acid treatments recorded an increase in chlorophyll concentration compared with untreated plants. Jasmonic acid application under water stress (60% FC) recorded the highest chlorophyll concentration. Kovac and Ravnikar (1994) reported that JA treatment resulted in an increase of active cytokinin concentration which enhances chlorophyll pigments.

Enzymes activity and membrane leakage: Regarding to the activity of peroxidase and catalase activity (Fig. 1), W_2 decreased the activity of previous enzymes. Significant increase in the activity of these enzymes was observed by JA treatment over the control or drought stress. Generally (W_1JA_2) treatment recorded the highest values followed by (W_1JA_1) treatment in peroxidase activity. Respecting to the catalase activity, the highest value was detected with the application of JA at the rate of 4.8 mM (JA_2)

Exogenous JA application led to increases in antioxidant capacity (Bandurska *et al.,* 2003). Evidence has showed that the expression of jasmonate responsive genes is altered under stress conditions and thus contributes to stress tolerance in plants (Chang and Zong, 2010).

Table : 1. Interaction effects of water stress and JA application on plant height, number of inflorescence branches, leaf area, dry matter of plant [leaves + stem] of sweet basil plants in 2008 and 2009 seasons.

JA / WS	First season				Second season			
	JA_0	JA_1	JA_2	Mean	JA_0	JA_1	JA_2	Mean
				Plant height (cm)				
W_0	45^{a}	44^{a}	42^{b}	$43.^{0}$	43^{a}	43^{a}	39^{c}	41.3^{A}
W_1	42^{b}	41^{b}	39^{c}	40.7^{B}	39^{b}	39^{b}	$36c^{d}$	37.7^{B}
W_2	38^{d}	39^{c}	37^{d}	38.0^{C}	36^{d}	36^{d}	$33d^{f}$	34.7^{C}
Mean	41.7^{A}	41.3^{A}	39.3^{B}		39.3^{A}	38.3^{B}	36.0^{C}	
				Number of inflorescence branches / plant				
W_0	5.0^{c}	7.0^{b}	8.0^{a}	6.7^{A}	7.0^{c}	8.0^{b}	9.0^{a}	8.0^{A}
W_1	3.0^{d}	4.0^{c}	4.0^{c}	3.7^{B}	3.0^{d}	5.0^{c}	7.0^{b}	5.0^{B}
W_2	1.0^{e}	3.0^{d}	2.0^{de}	2.0^{C}	2.0^{ef}	$4.0d^{e}$	5.0^{d}	3.0^{C}
Mean	3.00^{B}	4.7^{A}	4.7^{A}		4.0^{C}	5.7^{B}	7.0^{A}	
				Leaf area (cm^2 / plant)				
W_0	285^{a}	253^{b}	218^{c}	252^{A}	212^{a}	197^{b}	193^{c}	201^{A}
W_1	266^{b}	207^{c}	198^{d}	224^{B}	185^{b}	168^{c}	163^{d}	172^{B}
W_2	255^{d}	196^{d}	176^{df}	209^{C}	147^{c}	144^{d}	138^{de}	143^{C}
Mean	269^{A}	219^{B}	197^{C}		181^{A}	170^{B}	165^{C}	
				Dry weight (leaves + stem) (g / plant)				
W_0	5.7^{b}	6.1^{a}	6.2^{a}	6.0^{A}	5.4^{b}	6.0^{a}	6.3^{a}	5.9^{A}
W_1	4.9^{c}	5.3^{b}	5.4^{b}	5.2^{B}	4.3^{d}	4.6^{c}	4.7^{c}	4.5^{B}
W_2	3.9^{e}	4.6^{d}	4.9^{c}	4.4^{C}	3.6^{f}	3.7^{e}	3.9^{e}	3.7^{C}
Mean	4.8^{B}	5.3^{A}	5.5^{A}		4.4^{B}	4.8^{A}	4.9^{A}	

W_0 = 100% f.c JA_o = 00.0 mM
W_1 = 60 % f.c JA_1 = 2.40 mM
W_2 = 40 % f.c JA_2 = 4.80 mM

Table: 2. Interaction effects of water stress and JA application on relative water content, osmotic potential, chlorophyll concentration (a + b) and carotenoids, total sugars, total amino acids and proline concentration of sweet basil plants in 2009 season.

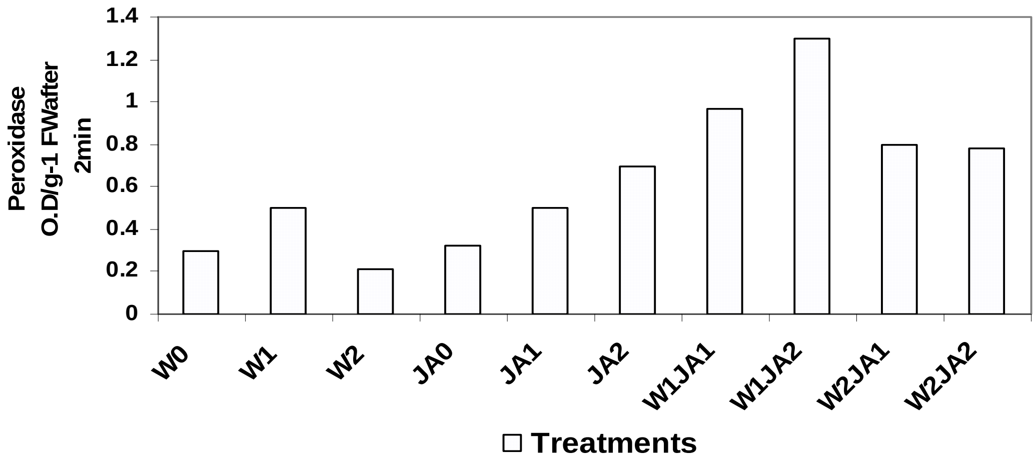
JA / WS	JA_0	JA_1	JA_2	Mean	JA_0	JA_1	JA_2	Mean
		Relative water content (%)				Osmotic potential (-Mpa)		
W_0	74.6^{c}	75.2^{b}	76.1^{a}	75.3^{A}	5.96^{de}	6.86^{d}	8.06^{c}	6.96^{C}
W_1	55.3^{d}	56.4^{c}	57.4^{b}	56.3^{B}	7.82^{d}	8.69^{c}	9.01^{b}	8.69^{A}
W_2	44.8^{de}	45.9^{d}	46.5^{c}	45.7^{C}	9.65^{c}	9.77^{b}	9.96^{b}	9.79^{A}
Mean	58.30^{C}	59.17^{B}	59.95^{A}		7.81^{C}	8.44^{B}	9.01^{A}	
		Chlorophyll (a + b) conc. (mg / g Dr.wt.)				Carotenoids conc. (mg / g Dr.wt.)		
W_0	4.849^{dc}	4.967^{d}	6.404c	5.407^{C}	3.336^{dc}	3.620^{d}	3.770^{c}	3.539^{C}
W_1	9.661^{c}	10.044^{b}	10.650^{a}	10.118^{A}	7.256^{c}	3.670^{b}	7.824^{a}	7.586^{A}
W_2	7.676^{d}	7.903^{c}	9.159^{b}	8.246^{B}	5.248^{d}	5.525^{c}	5.675^{b}	5.483^{B}
Mean	7.395^{C}	9.638^{B}	8.738^{A}		5.246^{C}	4.272^{B}	5.756^{A}	
		Total sugars (mg / g Dr.wt.)				Total amino acids (mg / g Dr.wt.)		
W_0	231.17^{c}	208.4^{d}	202.6^{e}	214.1^{C}	9.97^{dc}	17.38^{d}	18.3^{d}	14.55^{C}
W_1	261.3^{a}	256.8^{b}	240.29^{c}	253.8^{A}	14.49^{d}	27.14^{b}	24.78^{c}	22.14^{B}
W_2	243.1^{b}	237.3^{c}	235.3^{d}	238.6^{B}	19.69^{b}	32.41^{a}	33.42^{a}	28.50^{A}
Mean	245.19^{A}	234.2^{B}	226.1^{C}		14.05^{B}	25.6^{A}	25.5^{A}	
		Proline concentration (mg / gm Dr.wt.)						
W_0	40^{d}	64^{d}	93^{c}	65.7^{C}				
W_1	66^{d}	84^{c}	100^{b}	83.3^{B}				
W_2	101^{c}	131^{b}	143^{a}	125.0^{A}				
Mean	69.0^{C}	93.0^{B}	112.0^{A}					

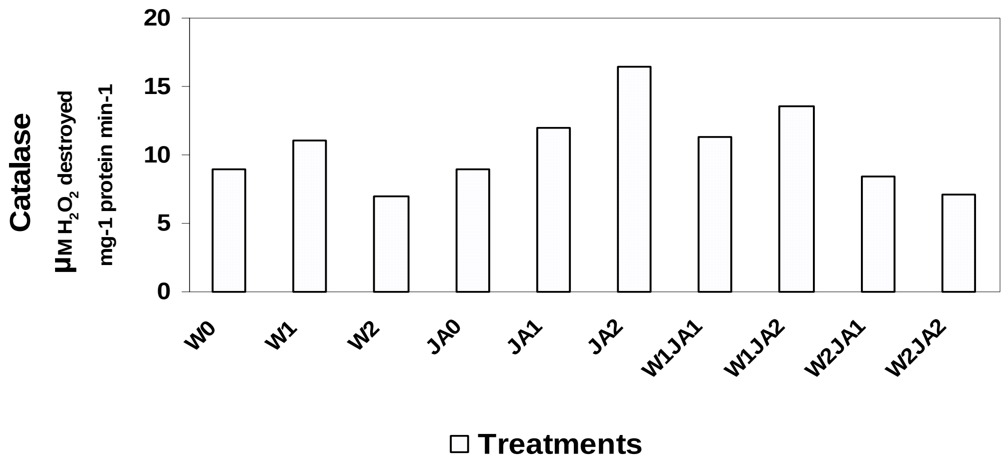
W_0 = 100% f.c JA_o = 00.0 mM
W_1 = 60 % f.c JA_1 = 2.40 mM
W_2 = 40 % f.c JA_2 = 4.80 mM

Moreover, Bandurska *et al.* (2003) and Mittler (2002) proved that JA exogenously applied increased antioxidative ability of plants under water stress. Regarding to membrane leakage, it can be noticed that membrane damage increased with increasing drought stress (Fig. 1), whereas 40% FC was more effective in increasing membrane leakage moreover, JA application protect the membranes from damage by reducing membrane leakage (Bandurska *et al.,* 2003).

3.3. Compatible osmolytes (proline, total soluble sugars and total amino acids):

Data presented in Table (2) showed that,





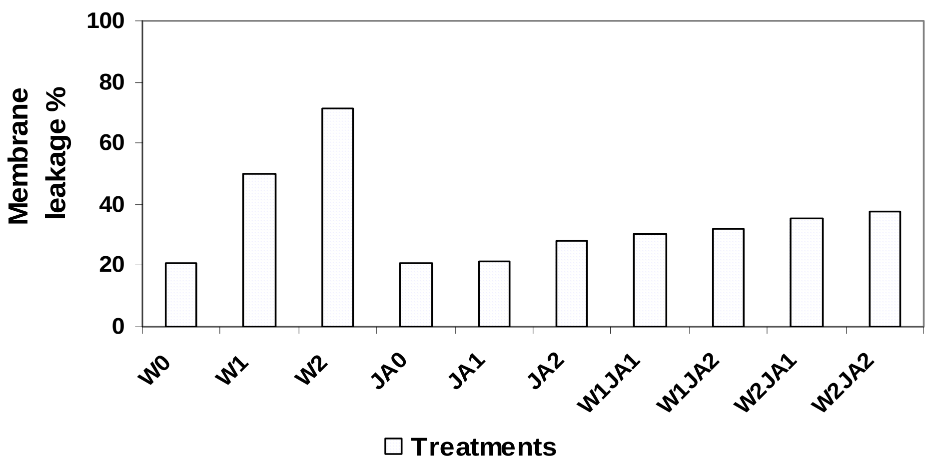


Figure: 1. Effect of jasmonic acid on enzymes activity and membrane leakage of sweet basil plants grown under water stress conditions (2009).

W_0 = 100% FC JA_o = 00.0 mM
W_1 = 60 % FC JA_1 = 2.40 mM
W_2 = 40 % FC JA_2 = 4.80 mM

Table: 4. Interaction effects of water stress and JA application on anatomical structure (nm^2) of sweet basil plants in 2009 season.

JA / WS	JA_0	JA_1	JA_2	Mean	JA_0	JA_1	JA_2	Mean
		Cortex				Pith		
W_0	12.9^{c}	13.4^{b}	14.2^{a}	13.5^{C}	10.9^{c}	11.1^{b}	12.1^{a}	11.4^{A}
W_1	9.5^{d}	10.5^{c}	11.7^{b}	11.9^{B}	7.4^{d}	8.4^{c}	9.0^{b}	8.3^{B}
W_2	5.2^{e}	6.5^{d}	6.7^{c}	6.1^{C}	4.7^{e}	5.4^{d}	6.5^{c}	5.5^{C}
Mean	9.2^{C}	10.1^{B}	10.9^{A}		7.6^{C}	8.3^{B}	9.2^{A}	
		Ground tissue				Phloem tissue		
W_0	23.7^{c}	24.5^{b}	26.3^{a}	24.8^{A}	2.56^{c}	2.88^{b}	3.14^{a}	2.86^{A}
W_1	16.9^{d}	19.0^{c}	20.7^{b}	18.9^{B}	2.18^{d}	2.03^{c}	2.49^{b}	2.33^{B}
W_2	9.9^{e}	11.9^{d}	13.2^{e}	11.7^{C}	1.34^{e}	1.47^{d}	1.73^{e}	1.52^{C}
Mean	16.8^{C}	18.5^{B}	20.1^{A}		2.03^{C}	2.22^{B}	2.45^{A}	
		Xylem tissue				Conductive tissue		
W_0	15.37^{c}	15.87^{b}	16.70^{a}	16.0^{A}	17.93^{c}	18.75^{b}	19.84^{a}	18.84^{A}
W_1	10.40^{d}	10.86^{c}	11.36^{b}	11.04^{B}	12.58^{d}	13.16^{c}	14.36^{b}	13.37^{B}
W_2	6.35^{e}	7.85^{d}	8.69^{c}	7.63^{C}	7.69^{e}	9.32^{d}	10.42^{c}	9.15^{C}
Mean	10.71^{C}	11.53^{B}	12.42^{A}		12.73^{C}	13.75^{B}	14.87^{A}	
		Total area				X. V diameter		
W_0	41.64^{c}	43.26^{b}	46.10^{a}	43.67^{A}	0.046^{c}	0.049^{b}	0.053^{a}	0.049^{A}
W_1	29.45^{d}	32.13^{c}	35.09^{b}	32.22^{B}	0.038^{d}	0.041^{c}	0.042^{b}	0.040^{B}
W_2	17.55^{e}	21.27^{d}	23.61^{c}	20.81^{C}	0.024^{c}	0.026^{d}	0.027^{c}	0.026^{C}
Mean	29.55^{C}	32.22^{B}	34.93^{A}		0.036^{C}	0.038^{B}	0.041^{A}	

W_0 = 100% f.c JA_o = 00.0 mM
W_1 = 60 % f.c JA_1 = 2.40 mM
W_2 = 40 % f.c JA_2 = 4.80 mM

with increasing drought stress, the concentrations of total sugars, proline and total amino acids was increased. The highest values were recorded under 40% FC Moreover, JA application generally decreased total soluble sugars compared to untreated plants and JA_1 was more effective in increasing total soluble sugars than JA_2. Whereas, JA increased proline and total amino acids concentrations especially JA_2. These results are in agreement with Walters *et al.* (2002) and Lee *et al.* (2001).

Jasmonic acid application can promote the biosynthesis of some substances (putrascine and proline) related to environmental stresses (Chen & Kao, 1993 and Gao *et al.,* 2004).

Oil yield and main volatile oil (linalool): Generally there was a gradual decrease in essential and linalool oil percentage with increasing drought stress. Jasmonic acid application at high concentration was more effective in increasing it. These increases were about 42.7% and 9.2% compared with the control plants, respectively in the second season (Table 3). These data are in harmony with those obtained with Ichimura *et al.* (1995) and Kim *et al.* (2009) who proved that methyl jasmonate application under drought stress prevents grain yield loss.

Anatomy:

High water stress caused a significant reduction in stem total area (57.9%) compared with the control (Table 4 and Figure 2). W_2 reduced the ground tissue (58.2%), xylem tissue (58.7%) and phloem tissue (47.7%). Whereas, JA_2 application generally increased the pith area (19.5 %), cortex area (18.5 %), xylem vessel (13.9 %), phloem (20.7%) and ground tissue (19.6%) compared with the control plants. There was an important observation which recorded a maximum increase in previous parameters with JA_2 under 60 and 40% FC (Figure 2).

Table: 3. Interaction affects of water stress and JA application on essential oil yield and linalool percentages of sweet basil plants in 2009 season.

JA / WS	JA_0	JA_1	JA_2	Mean
		Essential oil yield (%)		
W_0	0.09^{c}	0.10^{b}	0.115^{a}	0.102^{A}
W_1	0.065^{d}	0.085^{c}	0.100^{b}	0.080^{B}
W_2	$0.05d^{c}$	0.065^{d}	0.075^{c}	0.060^{C}
Mean	0.068^{B}	0.083^{A}	0.097^{A}	
		Main volatile (linalool) oil (%)		
W_0	48.1^{c}	50.2^{b}	9.1^{a}	49.8^{A}
W_1	15.1^{d}	16.5^{c}	17.1^{b}	16.23^{B}
W_2	8.6^{de}	9.9^{d}	10.2^{c}	9.57^{C}
Mean	23.93^{C}	25.53^{B}	26.13^{A}	

W_0 = 100% f.c JA_o = 00.0 mM
W_1 = 60 % f.c JA_1 = 2.40 mM
W_2 = 40 % f.c JA_2 = 4.80 mM

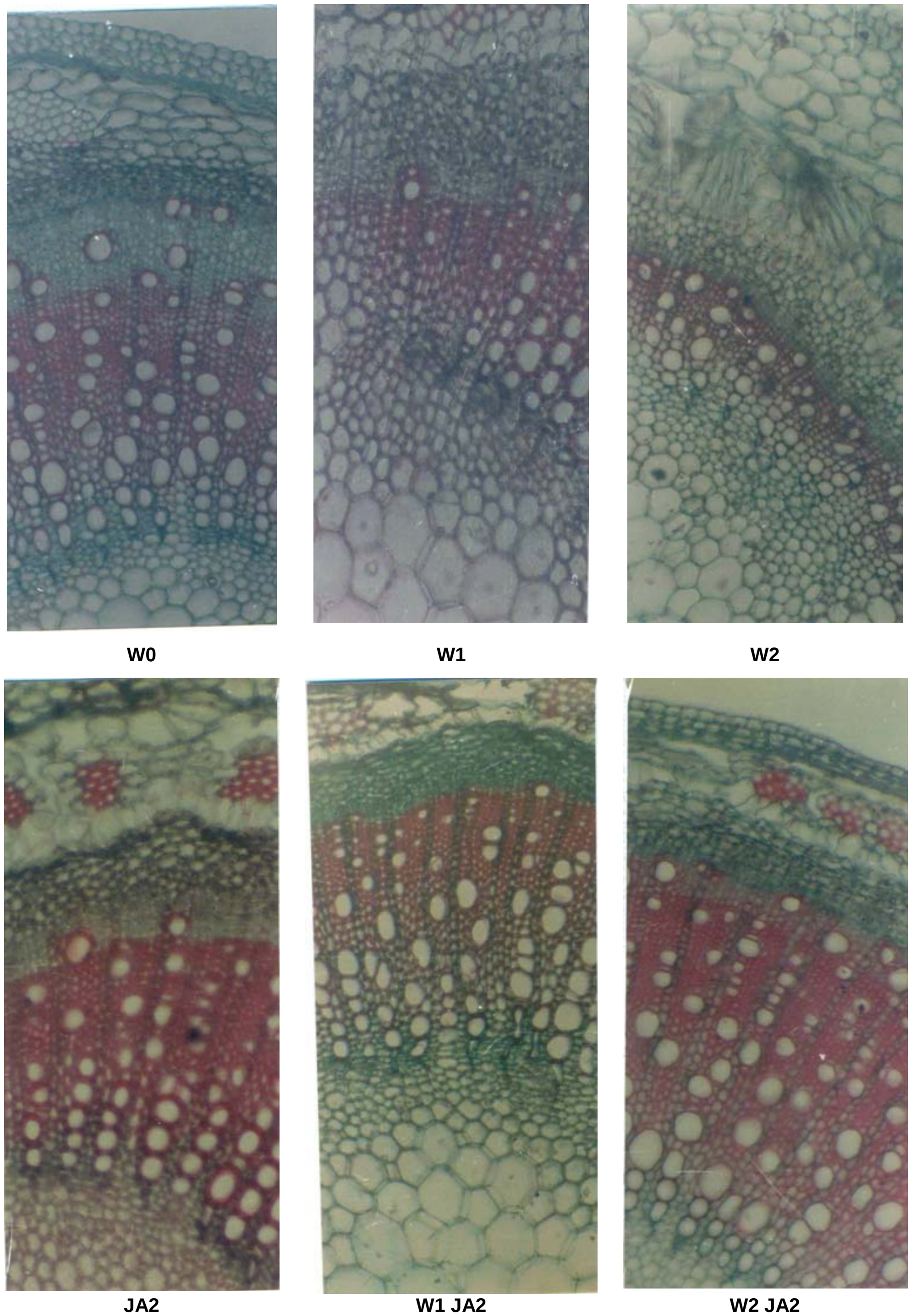


Figure: 2. Cross sections of sweet basil plants, 75 days after treatment with Jasmonic acid grown under water stress condition in 2009 season.

Generally, it can be concluded that application of JA showed a better performance of sweet basil plants under severe drought 40% FC This may be due to the maintenance of dry mass, higher relative water content which reflected higher oil yield and best anatomical structure than untreated plants. Exposure plants to stress are often followed by growth retardation and reduced fresh weight of fruit production. Jasmonic acid is among plant hormones, which mediate in certain types of stress responses and action results in decreasing the deleterious effect of drought on plant growth and chemical constituents as well as tissue structures which reflect higher yield.

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