Na AND K DISTRIBUTION IN LEAF AND FRUIT COMPONENTS OF *THESPESIA POPULNEA* (L.) SOL. EX. CORR. UNDER SALINE CONDITIONS AND RELATIONSHIP OF THEIR FOLIAR CONCENRATIONS WITH MONO-UNSATURATED ACIDS IN THE SEED-OIL

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ABSTRACT

The plant material (fresh and older leaves and mature capsules) from variously aged trees of *T. populnea* growing in and around Karachi (Hawkes Bay, Boat Basin areas, Karsaz and North Nazimabad) in differentially saline soils were collected. In these samples, Na and K were extracted by acid digestion method of dry young and older leaves, and fruit components such as pericarp, seed coat and embryo. Cations, Na and K, were estimated using Petra court PFP-Flame photometer. Over a range of ECe - 7.7 to 64.7dS.m^{-1}, Na content increased in fresh and older leaves and pericarp significantly but didn't vary in concentration in seed coat and embryo. K contents in leaves and pericarp declined with salinity but didn't change in seed coat and embryo. There was large accumulation of Na in older leaves. K increased in pericarp. Na / K ratio didn't vary in seed components but it was very high (8.2-folds) in older leaves. A significantly positive relationship between monounsaturated fatty acids concentration and foliar concentration of Na in fresh and old leaves was found.

Key Words: Thespesia populnea (L.) Sol Ex. Corr., Na and K Distribution, soil Salinity, monounsaturated fatty acids.

INTRODUCTION

The distribution of various ions among different organs of the plants is suggested to form the basis of a successful strategy for survival of plants under saline conditions. This salt allocation strategy guarantees the establishment and completion of the life history of its offspring successfully in the native halo-catena. Under saline water fertigation, Grava *et al.*, (2001) have reported Ca to be 30-fold higher in leaves than fruits. Cl and Na were found to be 3-4 folds higher in leaves than fruits although K remained more or less same in concentration in leaves and fruits. Rahnama *et al.* (2011) reported sequestration of Na in root and flag leaf sheath and maintained lower Na concentration with higher K / Na ratio in flag leaf blade of salt tolerant genotypes of bread wheat. This phenomenon was considered by the authors to improve the salt tolerance of the genotype. Compartmentalization or sequestration of toxic ions like Na and Cl in pericarp and seed coat has been demonstrated by several workers (Khan *et al.*, 1985, 1986; Khan and Ahmad, 1998;, Atia *et al.*, 2010 a and b).

Thespesia populnea (L.) Sol. Ex. Corr. is a coastal plant and a mangrove associate in vegetation of Samartan mangroves forests, Sarawak, Malaysia (Ashton and Macintosh, 2002). It is highly salt tolerant plant (Aronson, 1989). In Pakistan, it is confined to coastal regions particularly Karachi – cultivated in parks or along roadside as ornamental or a shade plant (Abedin, 1979). Here, in this paper we undertake to investigate distribution of Na and K in leaf, fruit and seed of this species to develop better understanding of its salt tolerance in halo-xeric environment of Karachi (Zubenok, 1977) and relationship of ionic concentration in its various tissues with the quality of its seed-oil.

MATERIALS AND METHODS

The plant material (fresh and older leaves and mature capsules) from seven variously aged trees of *T. populnea* growing in and around Karachi (Hawkes Bay, Boat Basin areas, Karsaz and North Nazimabad), in saline soils were collected and stored for laboratory analysis. In these samples, Na and K were extracted by acid digestion of dry plant parts (young and old leaves, pericarp, testa and embryo) following the method of Toth *et al.* (1948). Cations, Na and K, were estimated using Petra court PFP-Flame photometer. Three replicates were used for each sample in each sample tree. The surface and subsurface (0-30 and 30-60 cm deep, respectively) soil samples collected from underneath the sample plants were analyzed for their salinity status, pH and cationic content (Na and K) as per standard methods of USDA (1956). The seeds of the same trees were employed for determination of oil content and fatty acid composition of the seed oil as per standard methods of AOCS (2003). This data is being published in the current issue of IJBB (Gohar *et al.*, 2011).

Table 1. Soil characteristics associated with the *Thespesia populnea* trees growing in the saline-arid environment of Karachi. ECe, pH and cations are based on saturated soil extract.

Soil Parameters	SAMPLE TREES							
	HM	N1	B1	B2	B5	K1	K2	N3
ECe: dS.m-1								
A**	166.7	11.9	47.00	34.0	8.90	26.70	72.50	7.7
B	24.0	10.5	16.60	27.2	13.50	31.70	28.50	-
C	4.0	8.10	13.50	9.4	11.20	26.90	30.80	-
Mean	64.7	10.20	25.7	23.53	11.20	28.7	43.93	7.7
pH								
A**	8.55	6.25	8.75	8.75	8.55	8.4	7.65	-
B	8.45	7.4	8.6	8.5	8.45	8.25	7.6	-
C	8.1	8.25	8.8	8.7	8.35	7.95	8.05	-
Mean	8.37	7.3	8.7	8.65	8.45	8.2	7.8	8.05
Cations (meq / l) (0 – 60 cm profile)								
Na	-	54.35	304.3	391.3	391.3	336.96	380.43	141.3
K	-	8.33	5.13	30.13	89.7	14.8	-	31.4
Soil Texture (0-60 cm profile)								
0-60 cm profile	SL	L	SL - CLL	SL - CLL	STL-CLL	L	L	L

*, Locality - HM, Hawkes Bay sample (near mosque), N1 and N3, North Nazimabad sample, B1,B2, B5, Boat Basin samples and K1-K2, Karsaz samples. **, A, Surface sample; B, 30 cm deep; C, 60 cm deep. ***, L, Loam; SL, Sandy Loam; STL, Silt Loam; CLL, Clay Loam.

RESULTS

Na and K Contents in young and older Leaves, and fruit Components viz. pericarp, seed coat and embryo Fruit and seed components

Na and K contents and Na / K ratio in leaves and fruit components in seven *T. populnea* trees associated with soils of different salinity levels are given in Table 2. Over a range of ECe - 7.7 to 64.7dS.m^{-1}, Na content increased in fresh and older leaves and pericarp significantly but didn’t vary in concentration in seed coat and embryo. K contents in leaves and pericarp declined with salinity but didn’t change in seed coat and embryo. There was large accumulation of Na in older leaves. K increased in pericarp. Na / K ratio didn’t vary in seed components but it was very high in older leaves. The analysis of variance indicated that in magnitude of Na, K and Na / K ratio all were of, course, significantly affected with the salinity of the soil associated with these trees but more so by the tissue peculiarities of the plants and the interaction between the two was highly significant (Table 3-5). There was significant accumulation of Na in older leaves. Na content was significantly lower in fresh leaves. It further declined progressively in pericarp and testa with the result that Na was declined by a factor of 87.14% in the embryo. With age the leaves progressively became thicker and fleshier. K migrated from the leaves with senescence and there was substantial accumulation of K in pericarp. In embryo, the K contents were, on an average, 47% of the K contents of fresh leaves. Na / K ratio remained almost unchanged in all components tested except older leaves where Na concentration was around 8.2-folds higher than that of K. Average concentration of Na, K in various components of the samples is portrayed in Fig. 1. Na/K ratio maintained in pericarp, testa and embryo was around 0.4-0.45 which is even much lower than that in fresh young leaves. Na accumulation took place in larger amounts in older leaves – more than eight times to that of K.

Table 2. Na and K contents (meq /l) and Na / K ratio in various morphological parts of *Thespesia populnea* trees growing in differentially salinity affected areas of Karachi.

Plant Parts	Na (meq/L)						
	Sample plant Locality						
	HM EC: 64.7	B1 (EC: 24.7)	B2 (EC: 23.53)	B5 (EC:11.2)	N3 (EC:7.7)	K1(EC: 28.7)	K2 (EC: 43.93)
Fresh Leaves	* 10.28 ± 1.01 **17.07	8.57 ± 0.277 5.60	6.190 ± 0.493 13.80	6.233 ± 0.385 10.70	4.580 ± 0.161 6.09	4.160 ± 0.247 10.27	4.343 ± 0.219 8.69
Old Leaves	16.73 ± 0.54 5.63	11.736 ± 0.67 9.82	8.33 ± 1.828 38.01	13.216 ± 1.039 13.61	5.390 ± 0.132 4.240	4.723 ± 0.447 16.40	4.757 ± 0.646 23.53
Pericarp	3.69 ± 0.54 25.70	2.577 ± 0.233 15.67	4.013 ± 0.424 18.32	3.086 ± 0.633 35.52	2.506 ± 0.137 9.48	2.606 ± 0.251 16.69	2.693 ± 0.303 19.51
Testa	1.42 ± 0.058 7.12	1.58 ± 0.126 13.79	1.378 ± 0.175 22.02	1.490 ± 0.219 25.51	1.803 ± 0.674 64.78	1.433 ± 0.0677 8.18	1.130 ± 0.330 50.15
Embryo	0.79 ± 0.064 13.95	0.896 ± 0.239 46.21	1.040 ± 0.064 10.64	0.812 ± 0.029 6.19	0.71 ± 0.030 7.32	0.903 ± 0.0889 17.02	0.827 ± 0.0491 10.289
	K (meq / L)						
Fresh Leaves	* 2.733 ± 0.599 **37.97	3.720 ± 1.093 50.89	7.290 ± 0.970 23.05	6.591 ± 0.189 4.96	4.186 ± 0.372 15.39	3.070 ± 0.129 7.29	4.516 ± 0.726 27.83
Old Leaves	1.070 ± 0.290 46.90	1.680 ± 0.347 35.78	0.72 ± 0.134 32.25	2.213 ± 0.577 45.13	2.800 ± 0.399 24.72	1.205 ± 0.369 53.02	1.043 ± 0.047 7.75
Pericarp	8.170 ± 1.170 24.82	8.973 ± 1.040 20.33	6.210 ± 0.156 4.35	7.462 ± 1.629 37.82	7.263 ± 0.986 23.52	5.916 ± 0.017 0.49	7.846 ± 1.240 27.39
Testa	2.993 ± 0.279 16.16	3.470 ± 0.0208 1.09	4.237 ± 0.389 15.94	3.110 ± 0.278 15.50	2.827 ± 0.252 15.43	2.766 ± 0.232 14.54	3.387 ± 0.182 9.27
Embryo	1.633 ± 0.224 23.76	2.390 ± 0.083 6.04	2.383 ± 0.043 3.15	2.063 ± 0.296 2.49	3.017 ± 1.061 60.93	1.896 ± 0.179 16.34	2.430 ± 0.060 4.28
	Na /K Ratio						
Fresh Leaves	* 2.740 ± 0.599 **37.87	2.690 ± 0.678 43.67	0.893 ± 0.162 31.40	0.947 ± 0.084 15.28	1.113 ± 0.129 20.10	1.350 ± 0.025 3.23	1.021 ± 0.194 32.93
Old Leaves	19.753 ± 7.612 66.75	7.563 ± 1.438 32.94	12.030 ±2.750 39.57	7.13 ± 2.329 56.61	1.960 ± 0.199 17.65	4.260 ± 0.180 7.33	4.627 ± 0.082 30.22
Pericarp	0.458 ± 0.042 15.98	0.293 ± 0.035 20.57	0.650 ± 0.079 21.15	0.416 ± 0.007 2.77	0.350 ± 0.025 12.45	0.443 ± 0.043 16.94	0.346 ± 0.020 8.86
Testa	0.476 ± 0.034 12.31	0.450 ± 0.037 14.29	0.327 ± 0.042 22.37	0.477 ± 0.038 13.97	0.600 ± 0.170 48.76	0.527 ± 0.064 20.92	0.323 ± 0.082 43.99
Embryo	0.492 ± 0.029 10.19	0.373 ± 0.099 45.99	0.387 ± 0.035 15.58	0.435 ± 0.037 14.74	0.283 ± 0.078 47.98	0.480 ± 0.046 16.54	0.340 ± 0.020 10.19

*, mean ± SE; **, % CV.

Table 3. ANOVA for concentration of Na in leaf and fruit and seed components of *T. populnea*.

Source	SS	df	MS	F	p
Salinity	190.479	6	31.747	37.587	0.001
Plant parts	1074.086	4	268.522	317.92	0.001
(salinity X plant parts)	320.726	24	13.364	15.822	0.001
Error	59.123	70	0.8446	-	-
Total	1644.415	104	-	-	-

DMRT and LSD calculation

Salinity ($dS.m^{-1}$)			Plant Parts		
Rank	Treatment	Mean	Rank	Plant Parts	Mean
1	64.7	6.581 a	*1*	Older Leaves	9.2596 a
2	24.7	5.071 b	*2*	Fresh Leaves	6.5161 b
3	11.2	4.967 b	3	Pericarp	3.0252 c
4	23.5	4.419 b	4	Testa	1.4615 d
5	7.7	2.987 c	5	Embryo	0.83761e
6	28.7	2.764 c	$LSD_{0.05}$ = 0.565660		
7	43.9	2.750 c			
$LSD_{0.05}$ = 0.6693					

Table 4. ANOVA for concentration of K in leaf and fruit and seed components of *T. populnea*.

Source	SS	df	MS	F	p
Salinity	18.653	6	3.1089	2.7173	0.0198
Plant parts	457.769	4	114.4118	100.0289	0.001
(salinity X plant parts)	64.380	24	2.6825	2.3447	0.001
Error	80.0861	70	1.14441	-	-
Total	620.889	104	-	-	-

DMRT and LSD calculation

Salinity ($dS.m^{-1}$)			Plant Parts		
Rank	Treatment	Mean	Rank	Plant Parts	Mean
1	11.2	4.288 a	*1*	Pericarp	7.4062 a
2	23.5	4.167 a	*2*	Fresh Leaves	4.7753 b
3	24.7	4.068 a	3	Testa	3.2719 c
4	7.7	4.0 16 a	4	Embryo	2.2590 d
5	43.7	3.848 a	5	Older Leaves	1.5197 e
6	64.7	3.584 ab	$LSD_{0.05}$ = 0.65835		
7	28.7	2.953 b			
$LSD_{0.05}$ = 0.77897					

Relationship of Monounsaturated Fatty Acids Contents with Na^+, And K^+ Concentrations in Leaf and Pericarp

The succulence and fleshiness of the leaves of *T. populnea* under salinity led us to investigate the relationship of saturated, mono-unsaturated, poly-unsaturated and total unsaturated fatty acids with foliar concentrations of Na and Na / K ratio. There were five (N1, B1, B2, B5 and K2) such plants whose ionic content and seed oil content was precisely known. Correlation and regression analyses were thus between oil contents and Na and K contents in various body tissue undertaken for any possible relationships. No significant correlations were found for any of the fatty acid types except that a significantly positive relationship between monounsaturated fatty acid concentration and foliar concentration of Na in fresh and old leaves (Fig. 3 and 4). Na concentration in pericarp had no such effect on mono-unsaturated fatty acids. Over a range of Na; 4 - 8.5 meq/L in fresh leaves and 4 -13.0 meq/L in older leaves, slope value of monounsaturated fatty acids increment for unit increase of Na in foliar milieu was 2.19 times higher in fresh leaves over to that in older leaves. It may be related with the much faster metabolic rate in fresh leaves than senescing leaves being used as sink for excessive salts - a halophytic way to behave against salinity.

Table 5. ANOVA for concentration of Na / K ratio in leaf and fruit and seed components of *Thespesia*.

Source	SS	df	MS	F	p
Salinity	155.789	6	25.965	4.0554	0.0015
Plant parts	961.303	4	240.326	37.536	0.001
(salinity X plant parts)	507.578	24	21.149	3.3030	0.001
Error	448.766	70	6.402	-	-
Total	2072.177	104	-	-	-

DMRT and LSD calculation

Salinity (dS.m^{-1})			Plant Parts		
Rank	Treatment	Mean	Rank	Plant Parts	Mean
1	64.7	4.784 a	*1*	Older Leaves	8.1885 a
2	23.5	2.8574 b	*2*	Fresh Leaves	1.5359 b
3	24.7	2.2733 b	3	Testa	0.4543 b
4	11.2	1.8721b	4	Pericarp	0.4226 b
5	28.7	1.412 b	5	Embryo	0.3929 b
6	43.7	1.332 b	LSD$_{0.05}$ = 0.1.5574		
7	7.7	0.862 b			
LSD$_{0.05}$ = 0.1.8427					

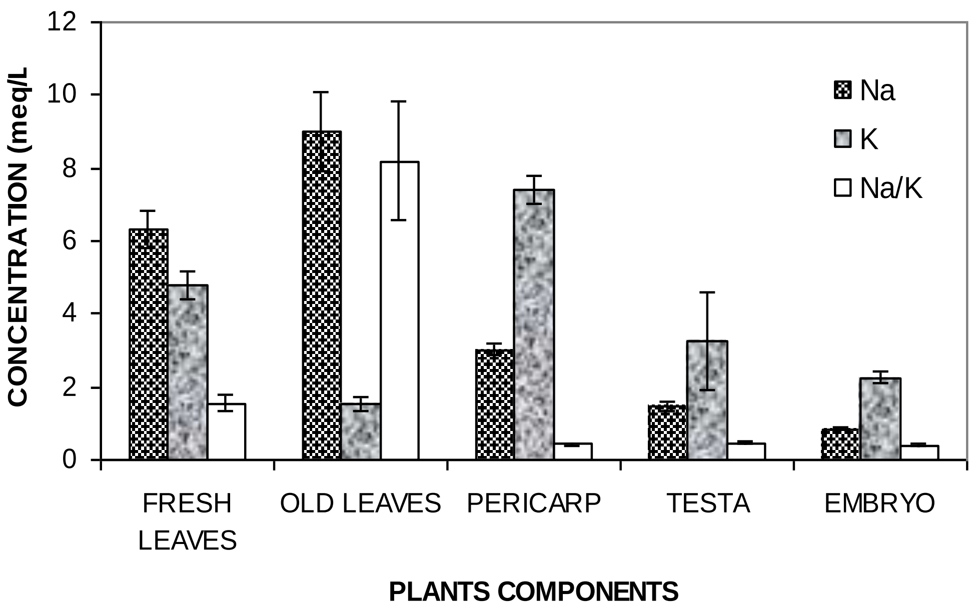


Fig. 1. Average Na and K contents and Na / K ratio in various parts of a mean tree of *T. populnea* growing in saline-arid environment of Karachi.

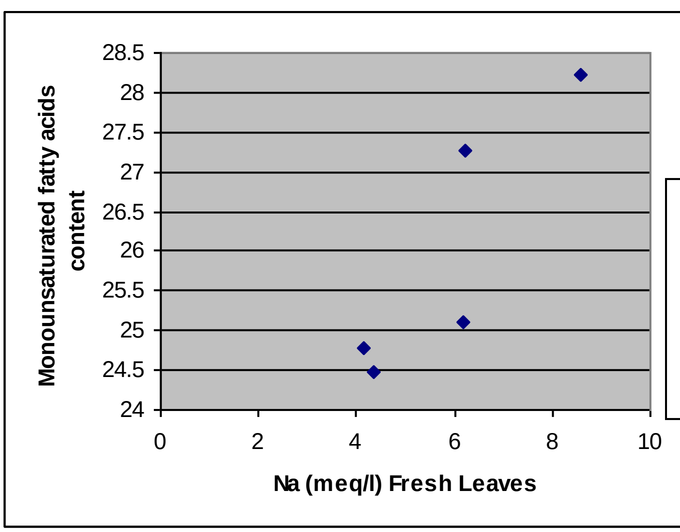
Table 6. Relationship of Na level (meq/l) in fresh leaves, older yellow leaves and Pericarp of fruit with saturated or unsaturated fatty acids contents (g / 100 g fatty acids) and total seed oil content in terms of linear correlation coefficients.

Dependent variable	Independent Variables		
	Na (Fresh leaves)	Na (Old Leaves)	Na (Pericarp)
Saturated fatty acids	- 0.7138 NS	- 0.7346 NS	- 0.6305 NS
Monounsaturated fatty acids	+ 0.8863 ***	+ 0.9089 ***	- 0.1895 NS
Polyunsaturated fatty acids	- 0.6839 NS	- 0.8132 NS	+ 0.4210 NS
Total Unsaturated fatty acids	+0.8578 NS	+ 0.6050 NS	+ 0.4921 NS
Total seed oil content.	+ 0.5785 NS	+ 0.9444 ***	+ 0.1184 NS

***, P < 0.001 (df =3); NS, Non-significant.

Table 7. Relationship of Na / K ratio in fresh leaves, older yellow leaves and Pericarp of fruit with saturated or unsaturated fatty acids contents (g / 100 g fatty acids) and total seed oil content in terms of linear correlation coefficients.

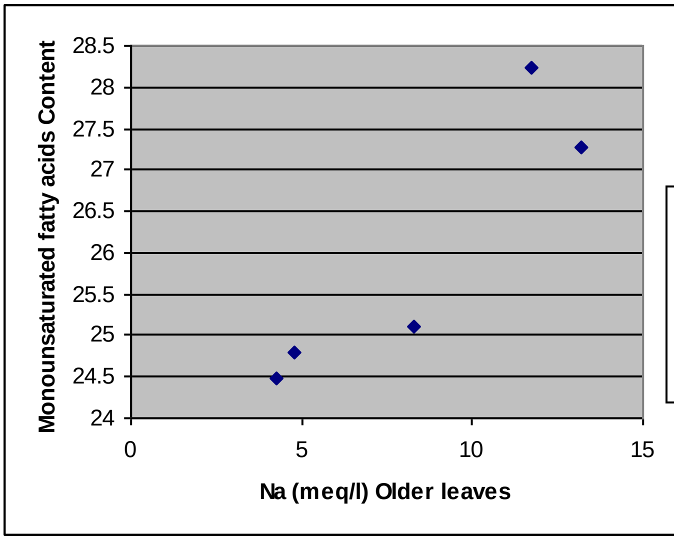
Dependent variable	Independent Variables		
	Na / K ratio (Fresh leaves)	Na / K ratio (Old Leaves)	Na /K ratio (Pericarp)
Saturated fatty acids	- 0.1868 NS	- 0.8253 NS	- 0.4821 NS
Monounsaturated fatty acids	+ 0.7297 NS	+ 0.1909 NS	- 0.4796 NS
Polyunsaturated fatty acids	- 0.6406 NS	+ 0.1112 NS	+ 0.6896 NS
Total Unsaturated fatty acids	+ 0.5157 NS	+ 0.8229 NS	+ 0.3211 NS
Total seed oil content.	+ 0.0764 NS	+ 0.2787 NS	- 0.1781 NS



$Y = 21.0968 + 0.82784\ Na \pm 0.8928$
t = 13.82 t = 3.31
p < 0.001 p< 0.045

r = 0.8863
$R^2 = 0.7854$, Adj. $R^2 = 0.7139$, F = 10.98
p < 0.045

Fig. 3. Relationship of monounsaturated fatty acids contents (g /100g fatty acids) of seed oil with Na contents (meq/l) in fresh leaves of *Thespesia populnea*.



Y = 22.794 + 0.37740 X ± 0.8039
t = 24.84 t = 3.77
$p < 0.001$ $p < 0.033$

r = 0.9089
$R^2 = 0.8261$, Adj. $R^2 = 0.7681$, F = 14.25
$p < 0.033$

Fig.4. Relationship of monounsaturated fatty acids contents (g /100g fatty acids) of seed oil with Na contents (meq/l) in older leaves of *Thespesia populnea*.

DISCUSSION

Na and K contents and Na / K ratio in leaves and fruit components in seven *T. populnea* trees associated with differential salinity levels were investigated. Over a range of ECe - 7.7 to 64.7dS.m^{-1}, Na content increased in fresh and older leaves and pericarp significantly but didn't vary in concentration in seed coat and embryo. K contents in leaves and pericarp declined with salinity but didn't change in seed coat and embryo. There was large accumulation of Na in older leaves. K increased in pericarp. Na / K ratio didn't vary in seed components but it was very high in older leaves. The analysis of variance indicated that the magnitude of Na, K and Na / K ratio all were of, course, significantly affected with the salinity of the soil associated with these trees but more so by the tissue peculiarities of the plants and the interaction between the two was highly significant. There was significant accumulation of Na in older leaves. Na content was significantly lower in fresh leaves. It further declined progressively in pericarp and testa with the result that Na was declined by a factor of 87.14% in the embryo. With age the leaves progressively became thicker and fleshier. K migrated from the leaves with senescence and there was substantial accumulation of K in pericarp. In embryo, the K contents were, on an average, 47% of the K contents of fresh leaves. Na / K ratio remained almost unchanged in all components tested except older leaves where Na concentration was around 8.2-fold higher than that of K. The translocation of Na and K from fruit wall, which is a direct continuum of vegetative parts, to the seeds a resultant structure formed after crossing the barriers of gametogenesis, pollination and subsequent fertilization, appears to be selective in *T. populnea*. The results confirm the idea of selective movement of ions between the phases which restrict the movement of ions into seeds from fruit wall. More or less similar effects of selective uptake have been reported in *Sesbania sesban* and *Leucaena leacocephala* (Ahmad, R. *et. al.*, 1984, 1985), cotton (Abdullah (1986), *Salicornia pacifera* var. *utahensis* (Khan *et. al*, 1985) and Indigofera *oblongifolia* (Khan and Ahmad, 1998). Compartmentalization or sequestration of Cl in seed coat is known in *Salicornia pacifica* ssp. *Utahensis* (Khan et al., 1985) through scanning of seed by X-rays signaling. Na and Cl accumulation in spongy seed coat is reported to be 8- and 11-folds to that of in seed, respectively, in *Suaeda physophora* and *Haloxylon ammodendron* (Song *et al*., 2007). Furthermore, X-ray microanalysis has also revealed that seed coat of *Crithmum maritimum* contains essentially Cl and Na while endosperm and embryo accumulates mg, K, and P (Atia *et al.*, 2010a and b). The mericarp of *C. maritimum* is considered to exert a protective role to the seed from the toxicity of Cl and Na. This phenomenon has important ecological implications in the establishment of this plant in the native halo-catena (Atia *et al.*, 2010b).

T. populnea appeared to be a sodiophillic plant with greater Na accumulation in leaves which are used as sink for excessive Na and shed on maturity. As regard to this typical halophytic behaviour it resembles to *Lupinus luteus*, a legume of limited agricultural significance in Australia (van Steveninck, *et. al.*, 1982) and *Indigofera oblongifolia*, a desert legume of Pakistan (Khan and Ahmad, 1998). The seeds of halophytes are also reported not to accumulate salts any more than the glycophytes (O'Leary, *et. al.*, 1985). The non-accumulation of salts in seeds (Embryo) of *T.*

populnea is not only of biological significance and plant survival under saline conditions but also of economic importance from the viewpoint of the seed oil quality. The increase of monounsaturated fatty acids under saline conditions definitely an added benefit if *Thespesia* seed-oil is considered to be included in human diet. To our knowledge no such report of salinity effects on monounsaturated fatty acids synthesis has so far been reported in the literature. The contents of ω3 fatty acid are, however, reported to be significantly increased under saline irrigation (EC_{iw}: ≤ 6.0 dS.m-1) (Heuer *et al.*, 2005). The concentration of ω3 fatty acid in *Thespesia* seed oil , represented by cis-4,7,10,13,16,19 – docosahexanoic acid (C22:6), is very low and ranged from 0.12 to 0.31g per 100g fatty acids in the seeds (mean = 0.167 ± 0.0273 %) (Gohar *et al.*, 2011).Cucci *et al.* (2010) has reported increase in oleic / linoleic acid ratio in sunflower with increase in salinity. No such increase in oleic / Linoleic acid ratio in *T. populnea* seeds was observed with the rise of salinity in *T. populnea* seed-oil.

Thespesia populnea has great prospect as saline-water-irrigated seed crop. It is, however, apparent from our unpublished results under the influence of high Na concentration in fresh and older leaves and the pericarp, may significantly be reduce the number of seeds per capsule and thus the oil yield.

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(*Accepted for publication March 2011*)