CHANGES IN OIL AND FATTY ACID COMPOSITION OF DEVELOPING SEEDS OF TARAMIRA (*ERUCA SATIVA* MILL.) AS INFLUENCED BY SULPHUR AND NITROGEN NUTRITION

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Received on 10 Feb., 2005, Revised on 8 Dec., 2005

SUMMARY

Randomized field experiments were conducted to study the interactive effect of sulphur (S) and nitrogen (N) on oil, protein, sugar and fatty acid composition in developing seeds of taramira (*Eruca sativa* Mill.). Two combinations of S and N (in kg ha^{-1}): $S_0 + N_{100}$ (-S+N) and $S_{40} + N_{100}$ (+S+N) were used. In +S+N treatments, S was applied either as a single basal dose (T_2) or in two (T_3) or three splits (T_4). A rapid increase in total oil content commenced at 20 days after flowering (DAF) and continued to 40 DAF; the dry matter however, continued to accumulate until maturity. A gradual rise was found thereafter and finally the oil accumulated as storage oil. Combined application of S and N resulted in increased oil accumulation in the seeds right from the initial stage. The maximum increase was observed, when S and N were applied in three splits. There was a strong positive correlation between S and oil content in the seeds. Soluble protein content increased from 10 to 30 DAF, followed by a decline until maturity. Among the +S+N treatments, i.e. T_2, T_3 and T_4, the treatment T_4 resulted in maximum increase in soluble protein content of developing seeds. Total sugar content decreased from the initial stage of seed development till harvest. The fatty acid composition of the oil changed substantially during seed development. S and N application in three splits (T_4) resulted in the increased oleic acid (18:1) content, while decreased erucic acid (22:1) content over other treatments. Reduction in 22:1/18:1 fatty acid ratio in the oil resulted in improved quality of oil.

***Key words*: *Eruca sativa*, fatty acids, N:S ratio, oil, protein, sulphur.**

INTRODUCTION

Mineral elements play an important role in plant growth and development by acting as co-factors for a number of enzymes (Nason *et al*. 1951) and their deficiency affects the overall yield and quality of the oil in oilseed crops (Appelqvist 1977, Abdin *et al*. 2003). Very little information however, is available on the influence of mineral nutrients on oil accumulation and its composition in oilseeds (Munshi *et al*. 1990, Fazli *et al*. 2005). The oil-synthesizing capacity of oilseed plants is significantly affected by different kinds of stresses viz. moisture (Munshi *et al*. 1986), nutrient (Sukhija *et al*. 1987, Abdin *et al*. 2003) or insecticides (Munshi *et al*. 1987).

Sulphur is an essential nutrient for all plants and animals, as it is a constituent of cysteine, methionine, several coenzymes (e.g. biotin, coenzyme A, thiamine pyrophosphate and lipoic acid), thioredoxins and sulpholipids. It is required along with nitrogen in the synthesis of proteins and enzymes (Schnug and

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Hanneklaus 2000, Abdin *et al*. 2003). Oilseed crops have a high demand of S; approximately 16 kg of S is required to produce one ton of seeds containing 91% dry matter (McGrath and Zhao 1996). Consequently, they respond not only to applied S, but their requirement for S is also the highest among crop plants, indicating a role of the nutrient in oil biosynthesis (Abdin *et al*. 2003, Fazli *et al*. 2005). In our earlier studies, we have shown that the combined and balanced application of S and N resulted into the increased oil accumulation and reduced ratio of erucic to oleic acid in rapeseed-mustard (Ahmad and Abdin 2000, 2000a). In this communication we report the interactive effect of these nutrients on the pattern of oil accumulation and its composition in the developing seeds of taramira, an oilseed crop with low oil content. An attempt has also been made to correlate the physiochemical changes occurring in seeds of taramira during various developmental stages with oil content and its fatty acid profile under the combined application of S and N.

MATERIALS AND METHODS

The seeds of *Eruca sativa* Mill. were obtained from National Research Centre on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi, India. The crop was raised in the experimental field of Hamdard University, New Delhi, during 2003-2004 according to the procedure described by Ahmad *et al*. (1999). The soil was a sandy loam, with pH 7.2, and deficient in S (0.001%) and N (0.07%). All plots received 40 kg ha^{-1} of phosphorous and potassium at the time of sowing. S was applied at the rate of 40 kg ha^{-1} as $CaSO_4$ either as a single basal dose or in two or three splits. Nitrogen was applied as urea at the rate of 100 kg ha^{-1} in two or three splits. The first dose of S and N was given at the time of sowing (basal), the second at 35 days after sowing (before flowering) and the third at 55 days after flowering (after flowering). The control treatment was made without S. Thus, there were four treatments: T_1 (S_0 N_{50+50}), T_2 (S_{40} N_{50+50}), T_3 (S_{20+20} N_{50+50}) and T_4 ($S_{20+10+10}$ $N_{50+25+25}$). These treatments were chosen on the basis of earlier results in which significant increase was observed in the seed and oil yield of rapeseed-mustard with the application of these treatments (Ahmad *et al*. 1999). The experiments were conducted using a randomized block design with three replicates of each treatment. The plot size was 9 m^2 (3 m x 3 m) with 7 rows and row to row distance of 30 cm. Four irrigations were given at different time intervals during the growth period of the crop. Weeding was done frequently. Two weeks after sowing, seedlings were thinned to keep an intra-row spacing of 15 cm.

Samples were collected at an interval of ten days after flowering (10 DAF) till harvest (HR, 60 DAF). The pods were removed from the plants, packed in polyethylene bags, and brought to the laboratory. Seeds were separated from the pods and analyzed. Each sample was collected in five replications and analyzed statistically (Nageswar Rao 1983). The values are the mean of five replications and the bars indicate ± standard error (S.E.).

Seeds were oven-dried at 60^{O}C until constant weight for dry weight determination. The oil content was estimated by rapid gravimetric determination described by Kartha and Sethi (1957). Nitrogen concentration in dried seed samples was determined following digestion by the method of Linder (1944). Sulphur content was estimated by the method of Chesnin and Yein (1950).

The oil was extracted with petroleum ether using soxhlet (A.O.A.C. 1960). The methyl esters of fatty acids present in the oil were prepared by esterification according to the method of Morrison and Smith (1964) with slight modification. The fatty acid methyl esters were determined by gas chromatography (Model Nucon) equipped with a flame ionization detector (FID). A 2 m long stainless steel packed column containing 3% OV-17 on chromosorb (WHP 100-200 mesh) was used. Helium was the carrier gas. Injector and detector temperatures were 240^{O}C and the oven temperature was maintained at 215^{O}C for 30 minutes. The carrier gas flow rate was 30 ml min^{-1}. Five fatty acids namely palmitic acid, oleic acid, linoleic acid, linolenic acid and erucic acid were estimated. Before running the samples, standards of the above mentioned fatty acids procured from Sigma-Aldrich Co., USA were run on GLC and the retention time as well as peak area of individual fatty acids were noted. After running the samples, individual fatty acids were identified on the basis of the retention time and their amount was calculated by extrapolating their peak area on the peak area of standard fatty acids. Each fatty acid was thereafter, expressed as the percentage of total fatty acid content.

RESULTS

Irrespective of treatments, the increase in dry matter accumulation started from 10 DAF and continued till 40 DAF, thereafter no further increase was observed (Fig. 1). Combined application of S and N (T_2, T_3 and T_4) increased the dry weight of the seed over -S treatment (T_1) right from the initial stage of seed development until maturity. The largest accumulation of dry matter in the seeds was observed for the T_4 treatment. The per cent increase in the dry matter content with treatment T_4 were 207, 70, 58, 17 and 16%, respectively, at 10, 20, 30, 40 DAF and at harvest, as compared to the treatment T_1.

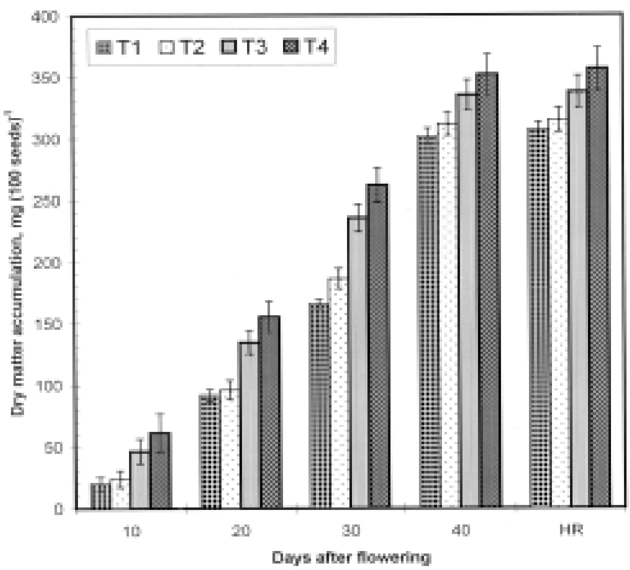

Fig. 1. Dry matter accumulation in the developing seeds of taramira as influenced by sulphur and nitrogen nutrition
(Treatments T_1, T_2, T_3 and T_4 details given in the text)

The oil accumulation increased from 10 DAF and continued till 40 DAF, beyond which no significant increase was observed (Fig. 2). Combined application of S and N (T_2, T_3 and T_4) increased the oil accumulation at all stages of seed development compared to -S treatment (T_1). Among the +S+N treatments (T_2, T_3 and T_4), the amount of oil accumulated was significantly higher when S and N were applied in three splits (T_4),

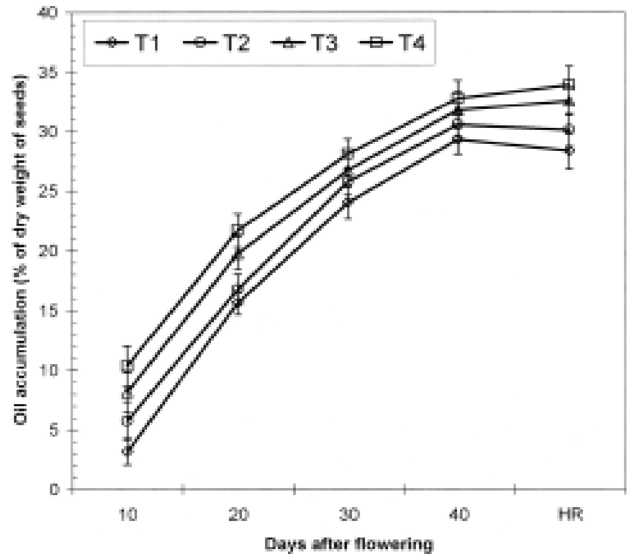

Fig. 2. Oil accumulation in the developing seeds of taramira as influenced by sulphur and nitrogen nutrition
(Treatments T_1, T_2, T_3 and T_4 details given in the text)

compared to the application of S and N in two splits (T_3) or S as a single basal dose and N in two splits (T_2). The per cent increase in the oil content with treatment T_4 were 227, 39, 17, 12 and 20% respectively at 10, 20, 30, 40 DAF and at harvest, when compared to treatment T_1.

Five species of fatty acids, palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3) and erucic acid (22:1) are described here, because these fatty acids comprised over 90% of the total oil in developing seeds of taramira.

Palmitic acid (16:0) content was 12.70, 13.30, 15.18 and 16.53 per cent at 10 DAF in the treatments T_1, T_2, T_3 and T_4, respectively, which declined gradually at subsequent stages of seed development, and was only 3.26, 3.13, 3.39 and 3.51 percent, respectively, in the oil of mature seeds (Table 1). This indicated that +S+N treatments (T_2, T_3 and T_4) increased the palmitic acid content by 5-30% at 10 DAF and 4-8% at maturity of the seeds over -S+N treatment (T_1).

Oleic acid (18:1) was the major fatty acid at the initial stages (10-20 DAF) of seed development, accounting for 49.50, 51.88, 43.66 and 35.71 percent of total fatty acids at 10 DAF in the treatments T_1, T_2, T_3 and T_4, respectively (Table 1). Its level declined gradually

Table 1. Changes in fatty acid content (%) in the oil during seed development in taramira

	Treatment	Days after flowering				
		10	20	30	40	Maturity (HR)
Palmitic acid						
(16:0)	T_1	12.7±0.95	9.54±1.02	11.35±0.86	5.59±0.52	3.26±1.11
	T_2	13.3±1.01	9.92±0.85	10.53±0.76	5.9±1.21	3.13±0.47
	T_3	15.18±1.09	10.26±0.75	10.75±0.95	6.38±1.11	3.39±0.58
	T_4	16.53±0.56	11.12±0.29	8.27±0.85	6.03±1.01	3.51±0.76
Oleic acid						
(18:1)	T_1	49.5±3.02	51.14±2.68	35.88±3.43	19.87±1.95	18.27±1.99
	T_2	51.88±1.88	52.28±2.53	36.49±2.01	21.71±1.46	20.12±2.32
	T_3	43.66±2.65	37.89±1.95	29.1±3.01	25.01±3.21	23.36±1.68
	T_4	35.71±2.65	36.99±3.54	30.75±2.95	27.23±4.01	26.35±2.63
Linoleic acid						
(18:2)	T_1	2.5±1.83	4.16±1.65	6.44±1.24	7.03±2.21	7.92±0.95
	T_2	3.18±0.88	4.77±1.21	8.04±1.50	8.36±0.65	8.89±0.39
	T_3	13.77±1.29	17.62±1.68	12.56±1.95	10.5±0.55	9.93±0.68
	T_4	23.09±0.85	18.87±0.95	15.07±1.21	12.17±1.50	10.19±0.55
Linolenic acid						
(18:3)	T_1	3.06±0.35	4.63±0.45	6.05±0.25	7.78±0.5	8.87±0.65
	T_2	3.26±0.15	4.94±0.29	6.32±0.38	8.04±0.47	8.91±0.59
	T_3	3.69±0.19	5.2±0.28	6.81±0.35	8.68±0.51	9.02±0.68
	T_4	3.97±0.55	5.68±0.6	7.15±0.41	8.96±0.37	9.52±0.52
Erucic acid						
(22:1)	T_1	7.24±1.81	18.77±1.65	36.28±1.98	41.43±2.25	42.14±1.12
	T_2	6.48±1.85	17.76±2.01	35.28±1.65	39.4±1.89	40.89±1.14
	T_3	9.3±1.85	17.17±1.23	30.02±1.10	37.29±1.15	38.16±1.45
	T_4	11.54±2.65	12.89±3.52	25.51±1.89	33.49±2.59	35.85±2.69

Treatments T_1, T_2, T_3 and T_4 details given in the text.

at the subsequent stages of seed development; and it was only 18.27, 20.12, 23.36 and 26.35% in the oil of mature seeds with treatments T_1, T_2, T_3 and T_4, respectively.

Linoleic acid (18:2) content was 2.50, 3.18, 13.77 and 23.09 per cent at 10 DAF and 7.92, 8.89, 9.93 and 10.19 per cent in the mature seeds with the treatments T_1, T_2, T_3 and T_4, respectively (Table 1).

Linolenic acid (18:3) content increased slightly in the oil during seed development. The content was 3.06, 3.26, 3.69 and 3.97 per cent at 10 DAF and 8.87, 8.91, 9.02 and 9.52 per cent in the mature seeds with the treatments T_1, T_2, T_3 and T_4, respectively (Table 1).

Erucic acid (22:1) content increased gradually until the seeds matured (Table 1). Combined application of S and N greatly decreased its content in the oil of mature seeds. In the treatment T_1, the content was 7.24 per cent at 10 DAF and reached 42.14 per cent in the oil of mature seeds. In the treatments T_2, T_3 and T_4, the initial content was 6.48, 9.30 and 11.54 per cent, respectively, while the final content was 40.89, 38.16 and 35.85 percent, respectively.

Sulphur content in the developing seeds increased until 40 DAF, and thereafter became constant till harvest. Combined application of S and N (T_2, T_3 and T_4) increased S content in the developing seeds over control (T_1). The highest seed-S was found with T_4 (Fig. 3). The N: S ratio in the developing seeds decreased considerably with the combined application of S and N (T_2, T_3 and T_4) over control (T_1). The maximum decrease was observed with the T_4 treatment.

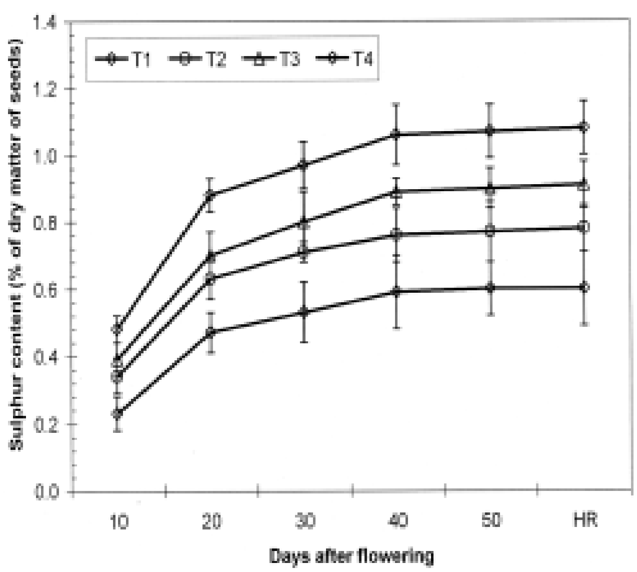

Fig. 3. Sulphur content in the developing seeds of taramira as influenced by sulphur and nitrogen nutrition
(Treatments T_1, T_2, T_3 and T_4 details given in the text)

N: S ratio was 8, 5.96, 5.43 and 4.73 at 10 DAF and 4.94, 4.01, 3.56, 3.14 in the mature seeds with the treatments T_1, T_2, T_3 and T_4, respectively.

DISCUSSION

The dry matter accumulation in taramira seeds continued from 10 DAF to 40 DAF. Application of sulphur along with nitrogen in three splits resulted in largest accumulation of dry matter in the seeds. The ability of the plant to produce dry matter depends on the size, efficiency and duration of the photosynthetic organs, which is measured in terms of leaf area index (LAI) and leaf area duration (LAD). Increase in the dry matter of seeds with the application of split doses of S and N may be attributed to improvement in LAI and LAD, compared with the application of S as a single basal dose. It has already been reported in rapeseed-mustard that split doses of S and N significantly improved the LAI and LAD, compared with the application of S as a single basal dose (Ahmad *et al.* 1999). Application of S in a single basal dose and N in two splits (T_2) may cause an imbalance in S and N supply to the crop that makes the genotype unable to cope with the increased demand of S and N during various growth stages. Among the split doses of S and N (T_3 and T_4), T_4 proved to be the best in increasing dry matter accumulation in the seeds, because in taramira flowering takes place just after the application of third dose of S and N (55 DAS). Inadequate supply of these nutrients at flowering time not only decreased the photosynthetic area and its duration but also the photosynthetic rate as reported by Ahmad *et al.* (1999) in rapeseed-mustard. Thus, reduced availability of photosynthates may restrict the development of floral buds as well as development of newly formed pods and seeds as reported earlier (Ahmad *et al.* 1999).

The period of rapid oil filling fell between 10 and 30 DAF, i.e. in the middle of the developmental span of the seed. These observations confirm previous findings on other oilseed crops (Munshi *et al.* 1983, 1990, Sukhija *et al.* 1983, Ahmad and Abdin 2000a, Fazli *et al.* 2005). The low rate of oil accumulation at early stages has been attributed to reduced availability of metabolites like ATP, NADPH and glycerol for oil biosynthesis (Simcox *et al.*

1979, Munshi *et al*. 1983). A reduction in total yield and oil content due to S deficiency was earlier reported in *Brassica* seeds and attributed to less availibility (Ahmad and Abdin 2000, 2000a, Ahmad *et al*. 1999; Fazli *et al*. 2005) and slower translocation of photosynthates in the developing seeds (Archer 1974). Sulphur is required for the synthesis of proteins (Ahmad *et al*. 2000, Fazli *et al*. 2005) including acyl-carrier proteins (Ohlrogge *et al*. 1979) and mobilizes metabolites (Abdin *et al*. 2003, Archer 1974) for the enhanced biosynthesis of oil in oilseeds (Sukhija *et al*. 1987). It is therefore, expected that the application of S would improve the quantity of S-containing amino acids (Bhatty *et al*. 1977) and proteins (Higgins 1984) and, influences oil biosynthesis.

The high proportion of 16:0, 18:1 and 18:2 fatty acids at initial stages (10 DAF) form an integral part of membrane lipids (Donaldson and Beevers 1977). The application of S along with N (T_2, T_3 and T_4) improved the quality of oil by increasing the linoleic acid (18:2) and oleic acid (18:1) content and reducing the erucic acid (22:1) content, when compared with the application of N alone (T_1). Among the +S+N treatments (T_2, T_3 and T_4), the application of T_4 caused the largest decrease in the 22:1/18:1 ratio at 20 DAF onwards (Fig. 4) and thus, improving the quality of oil. Similar findings were reported by Ahmad and Abdin (2000, 2000a) and Munshi *et al*. (1990) in rapeseed-mustard.

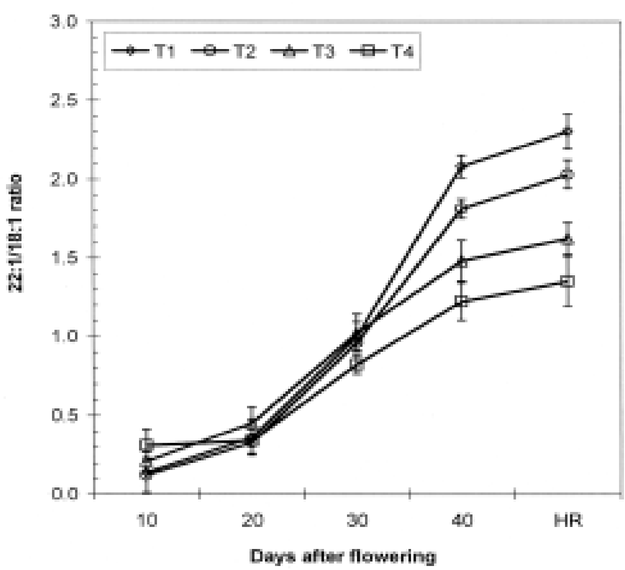

Fig. 4. 22:1/18:1 ratio in the oil of developing seeds of taramira as influenced by sulphur and nitrogen nutrition
(Treatments T_1, T_2, T_3 and T_4 details given in the text)

Sulphur content in the developing seeds increased until 40 DAF, and thereafter became constant till harvest. It is interesting to note that the accumulation of S in the developing seeds followed the same trend as that of oil accumulation and that S content strongly correlated (r^2 = 0.851) with oil content in the seeds (Fig. 5). This indicates that application of S along with N plays a significant role in oil accumulation and its composition in taramira. The higher yield and better quality of oil in this crop could be achieved by the application of these nutrients in three splits.

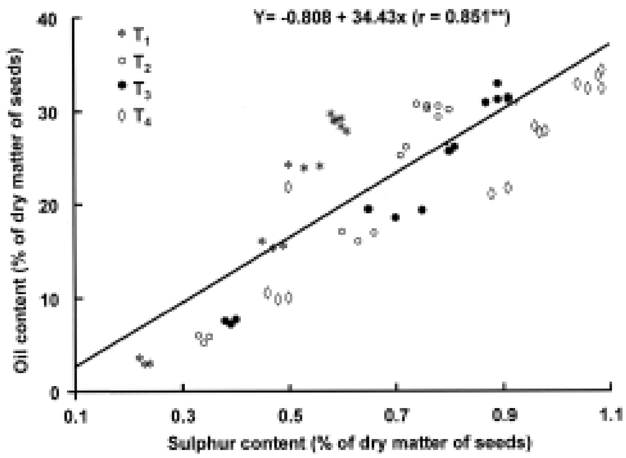

Fig. 5. Relationship between S and oil content in the developing seeds of taramira
(Treatments T_1, T_2, T_3 and T_4 details given in the text)

The study suggested that S and N should be applied in three split doses to optimize oil yield and its quality in taramira (*Eruca sativa* Mill.). Three split application of S and N will provide adequate amounts of these nutrients to the crop during the entire period of its growth and development. The oil accumulation and its composition in the seeds of oilseed crops with low oil content can be improved using this treatment (T_4) as achieved in case of taramira, a low-oil accumulating genotype.

ACKNOWLEDGEMENTS

This study was supported by a research grant to Dr. M.Z. Abdin from TMOP&M/CSIR, Ministry of Agriculture and Cooperation, Govt. of India. Dr. Inayat

Fazli is highly grateful to TMOP&M/CSIR for granting a senior research fellowship. The authors wish to thank Dr. Mohd Israr, Dr. Shaista Qadir, Dr. Mohd. Irfan Qureshi and Mrs. Samina Aquil for their generous help during this study.

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