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Ultrasonic (US) – induced growth and yield potentials of fenugreek (*Trigonella foenum-graecum* L.) plants under SO_2 fumigation

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Abstract
The fenugreek plants raised from seeds preconditioned with 2 MHZ ultrasound were found better suited to mild doses of SO_2 applied under fumigation experiments. The leaf dry matter was promoted to the extent of 66.3percent at $T_4 – S_1$ and leaf area upto 42.2 percent at the same treatment. The seed yield was favoured to higher degree with 69.7 percent enhancement at $T_2 – S_1$ and 67.0 percent at $T_3 – S_1$.

Keywords: fenugreek, ultrasound, leaf area, leaf dry matter, seed yield

Introduction
Ultrasound is a sound with its frequencies above the range of human hearing. It has higher energy than other audible sound. Ultrasound, therefore, has heating effects on plants and other objects it comes across. Ultrasound operates upon the intercellular gas – filled channels present in plant tissues of roots, stems and leaves. Exposure to ultrasound causes a resonant pulsation of gas – channels which is similar to the ultrasonic cavitation. The cavitation phenomenon explored as early as 1928 by Harvey and Loomis, is caused under sufficiently high ultrasonic intensity above the cavitation threshold and that the effects on plants were primarily non-thermal in origin and moreover they didn't appear to be due to the same form of cavitation that occurs in liquids.

Effect of ultrasound on plants has been reviewed by Miller (1983) [7]. The gas – channel pulsation in plant cells create very low level acoustic perturbations which are found to disrupt their metabolic activities. Responses of different plants to ultrasound is found to differ to much extent due to variations in intensity, exposure timings and experimental conditions.

Selman (1952) [8] found chromosomal abnormalities in meristematic root-tips of *Allium cepa* that showed anaphase and telophase bridges 90 minutes after ultrasound treatment. But these changes got subsequently recovered. Bleaney and Oliver (1972) [1] recorded reduction in growth rate of *Vicia faba* roots to about 40% of controls that was produced by 1.5 MHz ultrasound, but the growth attained its normal pace after about 10 days. In another experiment with *Vicia faba*, Bleaney and Oliver (1972A) [2] investigated pulsed 1.5 MHz ultrasound of intermittently low and high (1 m sec on, 6 m sec off) exposures that was found more effective than continuous exposures, compared on the basis of time – averaged intensity. It was also found to cause curlings in *Vicia faba* roots. Recently, Maury and Singh (2017) [6] also reported curlings in roots of castor (*Ricinus communis*) exposed to 2 MHz ultrasound. The radicle length of treated seedlings enhanced to 126.3% over the control after 10 days of exposure. The pace of radicle growth was slowed down to 46.1 percent at 16 days and 36.5 percent at 20 days, showing recovery to much extent from the destructive effects of ultrasound.

The ultrasonic waves with high energy levels are able to catalysing reactions in plants. It is environment – friendly and clean device and therefore, now being conveniently used in many industrial processes. The present piece of work deals with the effects of 2 MHz ultrasound – irradiated seeds of fenugreek plants exposed to SO_2 under fumigation chamber. There is paucity of literature dealing with ultrasonic – induced effects on growth and yield of plants under polluted environment.

Material and Methods
Overnight water – soaked fenugreek seed, treated with ultrasonic waves for ½ (T_1), 1 (T_2), 2 (T_3) and 3 (T_4) minutes by a crystal of 2 MHz in a double – walled vessel, were raised in pots

of sufficiently big size, filled with approximately 5 kg of loam soil. The plants grown were fumigated with SO_2 doses: S_1 (0.25 ppm), S_2 (0.5 ppm) and S_3 (1.0 ppm) generated by chemical reaction of Na_2SO_3 with dilute H_2SO_4 under fumigation chambers.

Closed – top fumigation chambers of thick, transparent polythene sheet were prepared of 100 x 100 x 200 cm size. A slieve of appropriate size was stitched on one side. The stitchings were applied with grease to avoid leakage of SO_2 from the chambers. On the metallic frames of suitable thickness, the polythene sheets were mounted.

Thirty days old plants were considered for fumigation. Ten fumigations, each at three days intervals were made, each fumigation lasted for 2 hrs. duration. Three types of control plants consisted of: (1) treated with US alone, (2) exposed to SO_2 alone, (3) plants not exposed to US and SO_2 both, but had uniform conditions of soil and water regime. Data on various parameters were collected at the flowering and fruiting of plants. Seeds were collected when their ripening took place. The statistical analysis of data was made by the Anova.

Result and Discussion

Growth and yield performance of fenugreek plants raised from overnight water – soaked seeds preconditioned with ultrasound was studied. The various parameters considered fall under two categories:

(1) Plant vigour and vegetative growth,
(2) Seed yield.

(1) Plant Vigour and Vegetative Growth

The shoot height, leaf number, leaf area, leaf drymatter and shoot biomass per plant at the flowering and fruiting were recorded.

The shoot height ranging from 30 cm to 41 cm of plants raised from ultrasonicated – seeds at $T_1 – T_4$ alone (control plants) were longer than those of the dual treatments, US – SO_2. This showed induction of shoot length by US alone. The pure control plants grown without US and SO_2, attaining 32.5 cm long shoots were also surpassed by T_4 control plants (40 cm) of US alone.

Leaf number was significantly higher in plants from $T_2 – S_1$ (167 leaf per plant, P<1) to $T_4 – S_1$ (186 leaf per plant, P<1), compared to 145 and 130 leaf per plant respectively of their control.

The leaf area and drymatter also had the similar pattern of growth with significantly increase in their values at US – S_1, attaining peaks at $T_4 – S_1$. Enhancement in leaf biomass, on dry weight basis, was to the magnitude of 66.3 percent at $T_4 – S_1$ (Fig. 3), compared to 42.2 percent of leaf area at the same treatment (Fig. 2). The higher extent of drymatter build up in leaf than its foliar spread or leaf area is probably due to higher assimilation and accumulation of nutrient materials in the leaf tissues induced by the ultrasound, causing higher leaf drymatter.

The shoot drymatter of plants exposed to US – SO_2, took a lead over the control plants grown under US alone from $T_1 – T_4$. The shoot biomass harvested to the limit of 12.93 g/plant (P<1) at $T_3 – S_1$, got gradually lowered to 10.15 g/plant (P<5) at $T_3 – S_2$ and 7.95 g/plant at $T_3 – S_3$ (Table 1) that had not significant increase over T_3 alone of the control (4.95 g/plant). The ultrasonic irradiation impacts persisting for longer duration throughout the life cycle of the fenugreek had unique potential for building up of the entire plant (leaf number, leaf area, leaf drymatter, stem and total aboveground biomass).

(2) Seed Yield

The fenugreek plants had significantly higher seed yield, 9.67 g/plant (P<1) at $T_3 – S_1$ and 8.56 g/plant (P<1) at $T_3 – S_2$ with respect to 5.79 g/plant of T3 alone (control). US – S_3 was found entirely toxic, bringing non-significantly higher seed yield at all the treatments (Table 2). Maury *et al*. (2009) [5] recorded 4- fold increase in seed yield of yellow mustard (*Brassica campestris* L) raised from water – soaked seeds irradiated with 2 MHz ultrasound for 5 minutes. In the other category of plants grown from seeds soaked under $ZnSO_4$ – a widely used agro- fertilizer, about 80 percent increase in the seed yield at 4 minutes of seed ultra – sonication was recorded. Ultrasound, therefore, is a safe and clean methodology that can be exploited for boosting yield of economic plants, without use of any fertilizer.

The enhancement in growth and yield of the fenugreek plants fumigated with SO_2 is definitely attributed to ultrasonic (US) – mediated absorption and utilization of enough SO_2 by the fumigated plants. SO_2, after entering the leaf tissues through stomata, is converted into sulphite form (SO_3). Sulphite, although toxic to plants, is soon oxidised to sulphate (SO_4) which serves as an essential mineral nutrient for plants (Levitt, 1980) [4]. Exploration of mechanism found helpful in accelerating growth and seed yield of the fenugreek is underway.

Table 1: Shoot drymatter (g/plant) of the fenugreek under US – SO_2 treatments

SO_2 Doses (ppm)	Shoot drymatter (g/plant) under US – SO_2				SO_2 Alone
	T_1	T_2	T_3	T_4	
S_1	10.3^{NS}	10.7**	12.93**	9.7*	8.66*
S_2	8.35^{NS}	8.43**	10.15*	8.59*	6.88^{NS}
S_3	7.65^{NS}	7.45**	7.95^{NS}	6.7^{NS}	5.59^{NS}
S_0 (Cont.) (US alone)	5.02	5.53	4.95	4.25	3.66 (without US & SO_2)
SE (m)	1.87	0.36	0.88	1.00	0.88
CD at 5%	7.61	1.46	3.58	4.07	3.60
CD at 1%	14.19	2.73	6.67	7.59	6.72

NS – Non-significant, * significant at 5%, ** Significant at 1%

Table 2: Seed yield (g/plant) of the fenugreek under US – SO_2 treatments

SO_2 Doses (ppm)	Seed yield (g/plant) under US – SO_2				SO_2 Alone
	T_1	T_2	T_3	T_4	
S_1	6.42*	7.18*	9.67*	6.51*	6.10*
S_2	6.18*	6.11*	8.56*	5.49*	4.75^{NS}
S_3	5.12*	5.36*	6.61*	4.49*	3.76^{NS}
S_0 (Cont.) (US alone)	3.96	4.23	5.79	4.41	3.15

SE (m)	0.365	0.37	0.36	0.408	0.424
CD at 5%	1.48	1.50	1.48	1.66	1.725
CD at 1%	2.77	2.80	2.77	3.096	3.128

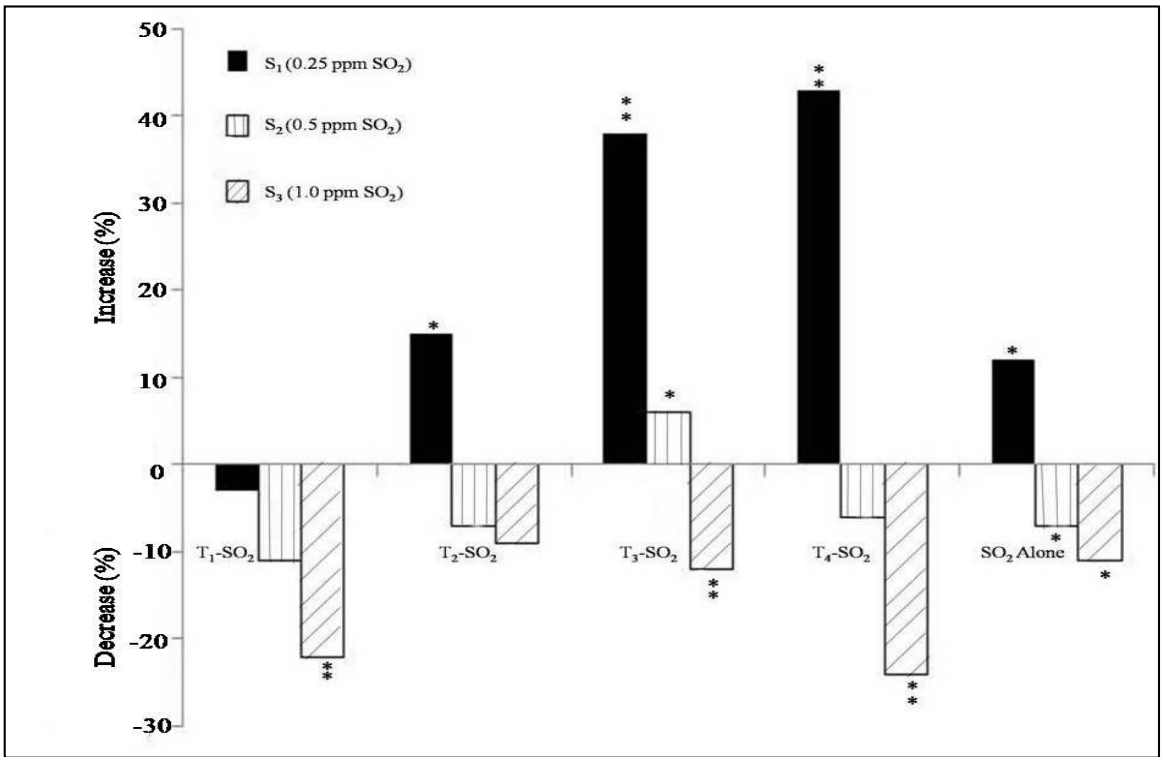

Fig 1: Percent increase /decrease in leaf number per plant of the fenugreek under US-SO_2 and SO_2 alone at the flowering & fruiting. T_1, T_2, T_3, & T_4 are the ultrasonic irradiation of seedlings to ½, 1, 2 & 3 minutes respectively (* = P<5, ** = P<1)

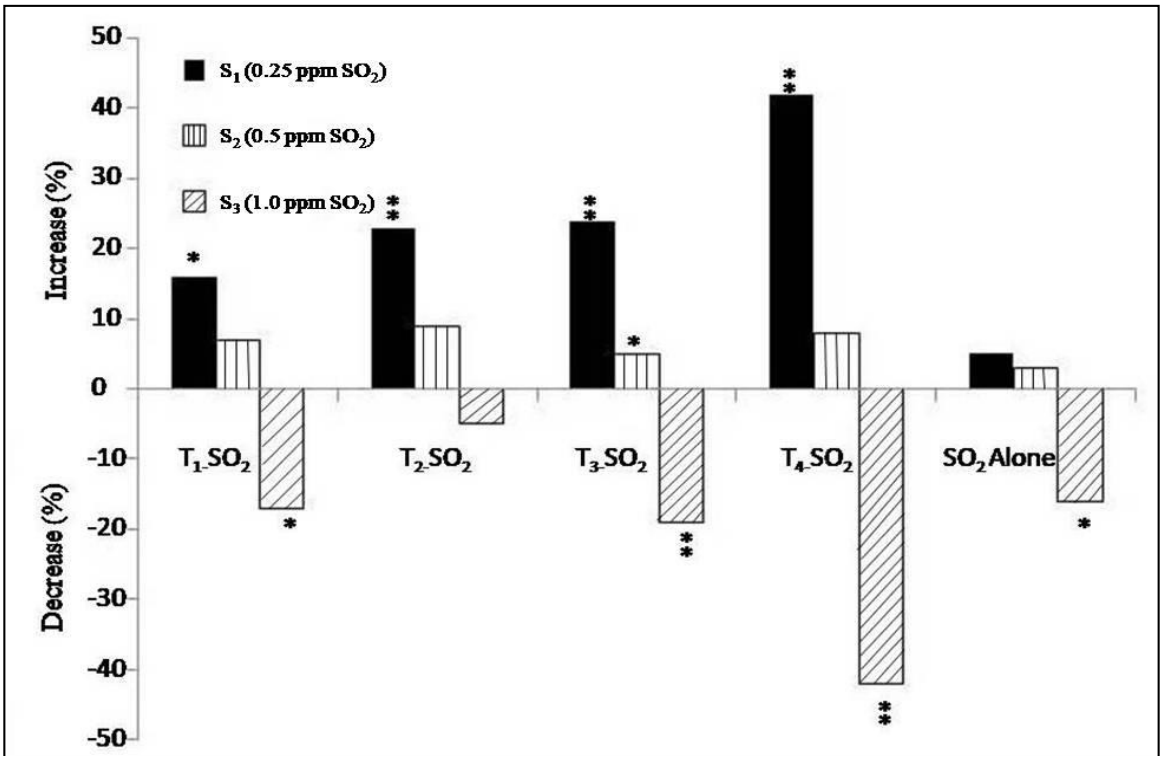

Fig 2: Percent increase /decrease in leaf area of the fenugreek under US-SO_2 and SO_2 alone at the flowering & fruiting (* = P<5, ** = P<1).

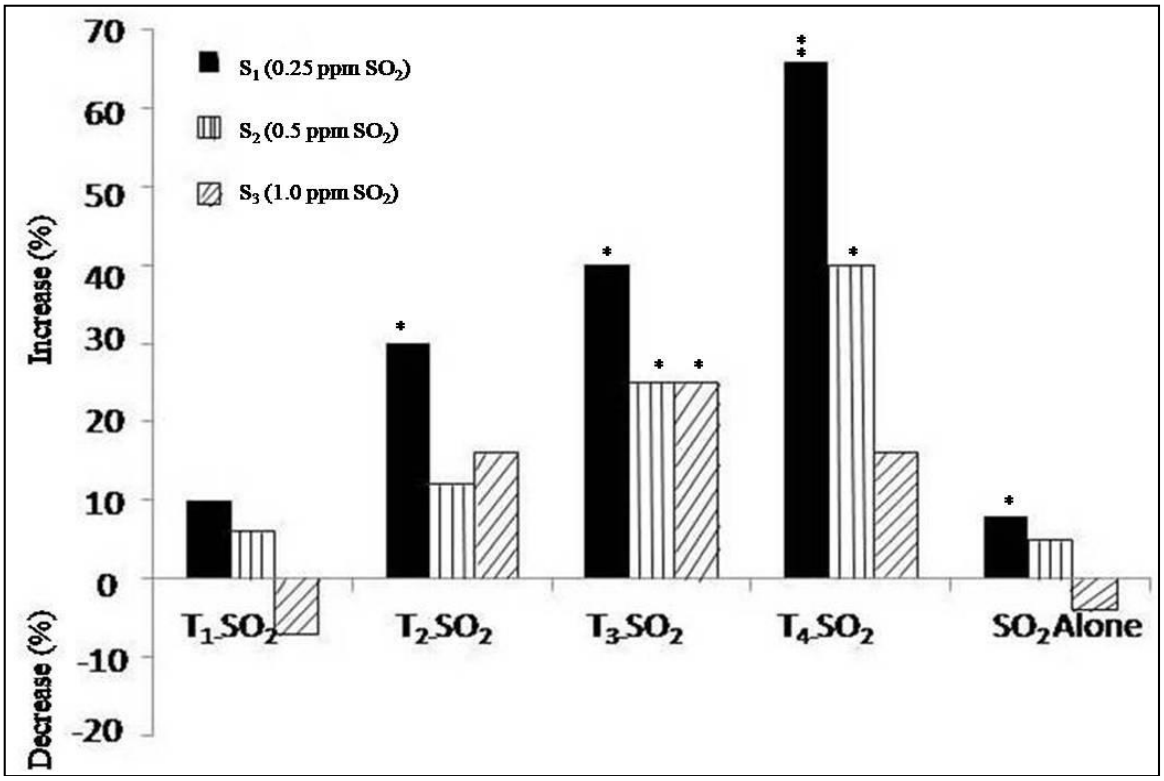

Fig 3: Percent increase /decrease in leaf drymatter of the fenugreek under US-SO_2 and SO_2 alone at the flowering & fruiting.

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