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Effect of physical seed pretreatments on morphology and yield of *Ocimum basilicum* L.

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Abstract
The effect of physical seed pretreatments on morphology and yield of *Ocimum basilicum* L. was investigated in the study. The seeds were treated in different physical treatments *viz*., scarification using sand paper, overnight water soaking, 65 ºC hot water for 10 min and concentrated H_2SO_4 for 1 min. Seeds after pretreatments were sown in protrays to study their effect on seedling parameters. After 30 days, seedlings were transplanted to growbags to study their effect on plant morphology and yield. All the treatments except concentrated sulphuric acid recorded on par germination with untreated seeds, which exhibited the highest germination of 58.67 per cent. The seeds exposed to water soaked seeds exhibited highest (18.27) seedling vigour index. At harvest stage, the morphological parameters and yield parameters did not exhibit any significant difference among the pretreatments tried. Hence it is observed that the physical seed pretreatments tried did not have any significant effect on morphological and yield parameters in *O. basilicum*.

Keywords: Physical seed pretreatments, *Ocimum basilicum*, seedling vigour index, herbage yield

Introduction
The *Ocimum*, one of the largest genera of the family Lamiaceae encompasses aromatic annual or perennial herbs and shrubs native to the tropical and subtropical regions of the world. *Ocimum basilicum* L. (family Lamiaceae) is a commonly and widely cultivated for herbal spice and sweet aroma. The above ground portion of the part is used for the extraction of essential oils. It is also used in in the treatment of stomach spasms, loss of appetite, intestinal faltulence, kidney disorder, colds, warts, worm infections and insect bites (Joshi, 2014) [6]. Methyl chavicol (estragole), one of the major *O. basilicum* oil constituents, is extensively used as flavoring, antimicrobial, antioxidant, anti-edematogenic, and anti-inflammatory agents (Kumar *et al.*, 2016) [8]. Seed pretreatment is any pre-sowing treatment to which seeds are exposed to for improving germination, survival and seedling stand (Shahrousvand, 2010) [11]. Seed priming. It curtails the time interval between sowing and seedling establishment. It also enable the seed to counteract the effect of stresses that the seeds may be exposed to after the sowing (Ansari *et al.*, 2012) [1]. The study has been carried out to analyse the effect of physical pretreatments on the seedling parameters, plant growth and herbage yield.

Materials and Methods
The seeds were subjected to different physical treatments *viz*., scarification using sand paper (SC), overnight water soaking (WS), 65 ºC hot water for 10 min (HW) and concentrated H_2SO_4 for 1 min (CSA). The untreated seeds were taken as the control (CTL).

Germination per cent
The germination per cent was calculated by the following equation,

$$\text{Germination per cent} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds initially sown}} \times 100$$

Mean Germination Time (MGT)
Mean germination time (MGT) is a measure of the rate and time-spread of germination. MGT was computed using the formula described by Schelin *et al.* (2003).

$$\text{Mean germination time (MGT)} = \frac{\Sigma f_i n_i}{\Sigma N}$$

fi = Day during germination period
ni = Number of germinated seeds on fi
N = Total number of germinated seeds

Seedling Shoot Length (SSL)
Three seedlings were randomly selected from each replication and using a ruler or measuring tape**,** length was measured from the base to the highest point of the plant at 30 days after sowing.

Seedling Root Length (SRL)
Three seedlings were randomly selected per replication and uprooted carefully. The root length was calculated from the base of the plant to the tip of primary roots at 30 days after sowing.

Allometric Index (AI)
The shoot length and root length recorded at 30 days after sowing were used to calculate the allometric index using the formula described by Hosseini *et al*. (2013) [4].

$$\text{Allometric Index} = \frac{\text{Root length}}{\text{Shoot length}}$$

Seedling Vigour Index (SVI)
The seedling vigour index was estimated using the following formula suggested by Vashisth and Nagarajan (2010) [11].
Seedling vigour index = Germination per cent × (Shoot length + Root length)

Statistical Analysis
The experiments in the first phase of the study were laid out in completely randomized design (Panse and Shukhatme, 1985). The data generated from the experiments were subjected to analysis of variance (ANOVA).

Morphological Parameters
Three plants from each replication of each treatment were tagged as observational plants. The morphological observations were recorded at 30 DAS (at transplanting), 60 DAS and 90 DAS (at harvest).

Plant Height
The height of the plant was measured from the base to the tip of the plant. The mean values were recorded and expressed in centimeter (cm).

Number of Branches
The total number of branches arising from the main branch of each observational plants were counted and the mean values were recorded.

Basal Stem Girth
The girth of the stem was recorded by measuring the circumference at the collar region using a thread and ruler. The mean girth was calculated and expressed in cm.

Number of Nodes
The nodes are the points on a stem where the leaves and branches originate. The total number of nodes per plant were counted and mean value recorded.

Phenological Parameters
Days to Flower Initiation
The days to flower initiation was recorded by counting the number of days taken from sowing to the initiation of the first flower.

Days to Fruit set
The days to fruit set was recorded by counting the number of days taken from initiation of the first flower to fruit set.

Days to Fruit Maturity
The days to fruit maturity was recorded by counting the number of days taken from the fruit set to fruit maturity. The fruit maturity is indicated by the blackening of the nutlets.

Yield Parameters
The yield parameters were recorded at the time of harvest, at 90 days after sowing.

Total Leaf Biomass
The fresh weight of all the leaves (including the petiole) present in the plant at the time of harvest was recorded. The samples were dried to a constant weight in a hot air oven at temperature of 70 ± 5 ºC. The fresh and dry weights were expressed in g plant^{-1}.

Total Stem Biomass
The fresh weight of the stem present in the plant at the time of harvest was recorded. The samples were dried to a constant weight in a hot air oven at temperature of 70 ± 5 ºC. The fresh and dry weights were expressed in g plant^{-1}.

Total Shoot Biomass
The shoot biomass is indicative of the yield of the plant, as the herbage, including leaves and stem. The fresh weight of the above ground portion of the plant at the time of harvest was recorded. The samples were dried to a constant weight in a hot air oven at temperature of 70 ± 5 ºC. The fresh and dry weights were expressed in g plant^{-1}.

Results
The seedling parameters as influenced by physical seed pretreatments are presented in Table 1 and plate 1. The germination was observed to highest (58.67 per cent) in the control treatment, which was observed to be on par with scarified, water soaked and hotwater treated seeds. The sulphuric acid treated seeds recorded the lowest germination of 32 per cent. The physical treatments tried had significant variation with respect to mean germination time. Water soaking recorded the least mean germination time (4.73 days). This was on par with hot water and control. Scarification treatment was observed to give the highest mean germination time (6.50 days) and this was on par with the treatment Conc. H_2SO_4. Water soaking treatment recorded the highest seedling shoot length of 19.23 cm which was on par with scarification, hot water and Conc. H_2SO_4. The lowest (15.27 cm) shoot length was observed in the control treatment. The data indicated that physical treatments did not show any significant variation in root length. No significant variation was observed in allometric index among the various physical treatments tried. Water soaking treatment recorded the highest vigour index of 18.27 which was on par with scarification, hot water and control. The lowest (10.53) vigour index was observed in the conc. H_2SO_4 treatment.
The effect of physical seed treatment on plant height (cm) of *O. basilicum* were recorded at 30 DAS, 60 DAS and 90DAS (at harvest) and are presented in Table 2. At 30 DAS**,** maximum plant height (19.23 cm) was recorded in plants obtained water soaked seeds and it was on par with

scarification, hot water and Conc. H_2SO_4. The lowest plant height (15.27 cm) was recorded in the control. At 60 DAS, the highest plant height was recorded in control and it was statistically on par with T_4 and T_1. A significantly lower plant height (24.20 cm) was observed in water soaking and it was on par with hot water. At 90 DAS, physical seed treatments had no significance influence on plant height.

The effect of physical seed treatments on the number of branches at 60 DAS and 90 DAS are given in Table 2. At 30 DAS, no branching from the main shoot was observed. No significant variation was observed on number of branches due to the physical seed treatments imposed on the plants either during 60 DAS or 90DAS.

Table 3 shows the effect of physical pretreatments on basal stem girth at 30, 60 and 90 DAS. At 30 DAS and 60 DAS, physical seed treatments did not have any significant influence on stem girth. At 90 DAS, hot water registered a significantly higher basal stem girth (4.00 cm). The lowest stem girth (2.6 cm) was recorded in the plants derived from the treatment control. This was on par with scarification and water soaking.

The result of number of nodes at 3 different stages, 30, 60 and 90 DAS. There was no significant difference in the number of nodes due to the different physical seed pretreatments applied in the study.

The result of the effect of various seed pretreatments on phenological parameters *viz.*, days to flower initiation, days to fruit set and days to fruit maturity, of transplanted *O. basilicum* is presented in Table 4. The parameter, days to flower initiation alone showed significant variation among the physical pretreatments tried. Among the treatments scarification recorded the least number of days (58.00 days) for flower initiation which was found to be on par with water soaking and hot water. However, Con.H_2SO_4 treated seeds took maximum number of days (73.00 days) to flower initiation, which was observed to be par with water soaking and control. The parameters days to fruit set and fruit maturity, did not show any significant variation among the physical seed pretreatments.

The data on the effect of physical seed pretreatments on yield parameters in transplanted *O. basilicum* are presented in Table 5. The data indicates that both the leaf biomass did not exhibit any significant difference among the physical treatments tried. The treatments tried did not show any significant variation with respect to stem biomass. The data shows that the physical seed pretreatments had no effect on the shoot biomass in transplanted *O. basilicum.*

Discussion

In the study, it was observed that none of the treatments performed over the control treatment with respect to germination percent. The scarified, water soaked and hotwater treated seeds gave on par germination per cent with that of control. The sulphuric acid treated seeds gave a very low germination. Jin *et al.* (2006) [5] reported that Conc. H_2SO_4 treatment could damage the seeds, resulting in poor germination and emergence. In the study, water soaking resulted in faster germination. In contrast to the study, Sedaghat and Rahemi (2011) [14] indicated that mean germination time of pistachio was enhanced by water soaking. The seedling obtained from water soaked seeds recorded maximum shoot length and seedling vigor index. This is in agreement with the study of Khan *et al.* (1999) [7] which demonstrated that water soaking for 48 h duration produced long pistachio seedlings. Farahani and Maroufi (2011) [2] reported higher seedling vigour index in the seeds of *O. basilicum* and *Helianthus annus*, seeds exposed to watersoaking for 12 h. According to Mahmoodi *et al.* (2011) [9, 10], watersoaking of seeds could ensure optimum plant stand in maize. Seedling vigour index have profound influence on the establishment and yield of crops (Tabrizian & Osareh, 2007) [16]. Vigorous plant could be produced from rapid emergence of seedlings (Ghassemi-Golezani *et al*., 2008) [3]. The lowest seedling vigour index was observed in the Conc. H_2SO_4 treatment. The earliest flower initiation was observed in plants from scarified, water soaked and hotwater treated seeds compared to the control treatment. And in plants from concentrated sulphuric acid seeds, the flower initiation was significantly late. The low vigour index of plants from sulphuric acid treated seeds might have resulted in late initiation of flowering. Though seedling vigour index showed significant variation, no variation was observed in morphological (except for basal stem girth) and yield parameters among the treatments at harvest.

Table 1: Effect of physical seed pretreatments on germination and seedling parameters of *O. basilicum*

T. No	Physical SP	Germination (%)	MGT (Days)	SSL (cm)	SRL (cm)	AI	SVI
T_1	SC	49.33±2.19	6.50±0.67	17.83±0.26	13.77±0.67	0.77±0.17	15.57±1.18
T_2	WS	55.33±3.11	4.73±0.38	19.23±1.08	14.60±1.41	0.75±0.24	18.27±1.61
T_3	HW	52.67±2.09	5.10±0.00	18.50±0.61	15.47±0.64	0.84±0.1	17.95±1.36
T_4	CSA	32.00±3.05	6.03±0.17	17.60±0.44	15.30±0.44	0.86±0.1	10.53±1.19
T_5	CTL	58.67±2.16	5.20±0.31	15.27±0.50	13.03±0.58	0.85±0.14	16.56±1.03
SEm(±)		3.186	0.222	0.574	0.945	0.003	1.425
C.D. (0.05)		9.56	0.707	1.882	NS	NS	4.718

T. No. – Treatment Number; SC-Scarification; WS-Water soaking; HW-Hot water; CSA-Conc. Sulphuric Acid; CTL – Control; DAS- Days after sowing. Each figure represents mean (±SD) of three replications

Table 2: Effect of physical seed pretreatments on plant height & number of branches in transplanted *O. basilicum*

T. No	Physical SP	Plant height (cm)			Number of branches		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	SC	17.83 ± 0.26	25.77 ± 0.26	60.10 ± 1.63	-	13.77 ± 1.16	14.67 ± 1.54
T_2	WS	19.23 ± 1.08	24.20 ± 0.8	57.27 ± 1.17	-	13.33 ± 0.81	15.33 ± 1.32
T_3	HW	18.50 ± 0.61	24.90 ± 0.51	56.83 ± 1.45	-	13.53 ± 0.88	13.33 ± 1.54
T_4	CSA	17.60 ± 0.44	26.97 ± 1.17	57.83 ± 1.86	-	12.87 ± 0.65	16.00 ± 1.07
T_5	CTL	15.27 ± 0.50	27.50 ± 0.56	52.40 ±1. 22	-	12.20 ± 0.64	12.63 ± 1.23
SEm(±)		0.574	0.707	2.356	-	0.810	1.915
C.D. (0.05)		1.882	2.258	NS	-	NS	NS

T. No. – Treatment Number; SC-Scarification; WS-Water soaking; HW-Hot water; CSA-Conc. Sulphuric Acid; CTL – Control; DAS- Days after sowing. Each figure represents mean (±SD) of three replications

Table 3: Effect of physical seed treatments on basal stem girth and number of nodes in transplanted *O. basilicum*

T. No.	Physical SP	Basal stem girth (cm)			Number of nodes		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T_1	SC	0.80 ± 0.24	1.40 ± 0.24	3.10 ± 0.38	14.00 ± 1.07	51.30 ± 1.07	174.00 ± 2.82
T_2	WS	0.77 ± 0.17	1.27 ± 0.3	3.10 ± 0.41	11.33 ± 1.15	45.60 ± 1.44	160.00 ± 2.03
T_3	HW	0.73 ± 0.17	1.43 ± 0.26	4.00 ± 0.51	11.33 ± 1.15	49.07 ± 1.25	168.67 ± 2.74
T_4	CSA	0.73 ± 0.17	1.23 ± 0.17	3.23 ± 0.44	10.00 ± 1.41	46.40 ± 1.15	158.00 ± 1.51
T_5	CTL	0.80 ± 0.24	1.47 ± 0.26	2.60 ± 0.38	7.33 ± 0.81	53.67 ± 1.09	173.97 ± 3.11
SEm(±)		0.045	0.065	0.194	1.366	1.508	6.906
C.D. (0.05)		NS	NS	0.619	NS	NS	NS

T. No. – Treatment Number; SC-Scarification; WS-Water soaking; HW-Hot water; CSA-Concentrated Sulphuric Acid; CTL – Control; DAS-Days after sowing. Each figure represents mean (±SD) of three replication

Table 4: Effect of physical seed pretreatments on phenological parameters in transplanted *O. basilicum*

T. No.	Physical SP	Days to flower initiation (Days)	Days to fruit set (Days)	Days to fruit maturity (Days)
T_1	SC	58.00 ± 1.31	1.33 ± 0.57	8.00 ± 0.76
T_2	WS	65.00 ± 1.7	1.33 ± 0.57	9.33 ± 0.57
T_3	HW	63.33 ± 1.65	1.00 ± 0.00	8.00 ± 0.76
T_4	CSA	73.00 ± 2.00	1.33 ± 0.57	8.33 ± 0.57
T_5	CTL	67.67 ± 1.61	1.67 ± 0.57	9.33 ± 1.09
SEm(±)		2.894	0.298	0.683
C.D. (0.05)		9.238	NS	NS

T. No. – Treatment Number; SC-Scarification; WS-Water soaking; HW-Hot water; CSA-Conc. Sulphuric Acid; CTL – Control; Each figure represents mean (±SD) of three replications

Table 5: Effect of physical seed pretreatments on yield parameters in transplanted *O. basilicum* at harvest (90 DAS)

T. No.	Physical SP	Leaf biomass (g plant^{-1})	Stem biomass (g plant^{-1})	Shoot biomass (g plant^{-1})
T_1	SC	58.97 ± 1.60	28.63 ± 1.96	87.60 ± 2.39
T_2	WS	55.07 ± 1.63	26.13 ± 1.77	81.20 ± 2.21
T_3	HW	50.60 ± 3.09	44.63 ± 2.50	95.23 ± 2.88
T_4	CSA	59.40 ± 2.72	39.70 ± 3.33	99.10 ± 4.21
T_5	CTL	54.17 ± 1.67	24.7 ± 0.74	78.86 ± 1.51
SEm(±)		5.794	6.150	9.459
C.D. (0.05)		NS	NS	NS

T. No. – Treatment Number; SC-Scarification; WS-Water soaking; HW- Hotwater; CSA-Conc. Sulphuric Acid; CTL – Control; Each figure represents mean (±SD) of three replications

Conclusion

In the study it was observed that higher seedling vigour index did not reflect higher herbage yield. This indicates that further studies with respect to the seed pretreatments are needed to be carried out to improve the performance of the *O. basilicum* with respect to seedling growth, plant development and yield responses.

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