

An assessment of seed germination under the effect of natural dyes

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Abstract

Natural dyes are extracted from leaves, flowers, fruits etc. These substances are natural, hence after the extraction of natural dyes from them they are disposed off in the soil. Even the natural dyes after being used up are disposed off in the soil. Some experiments were conducted in order to show that these dyes during their production and after their use disposed in the soil, they do not spoil the soil quality. On the other hand the waste left after the extraction of dyes serves as manure, hence enriches the soil, Gram & Wheat were taken for experimental purpose, and their morphological characters like shoot length, root length, fresh weight & dry weight were studied by growing them in solutions containing different concentration of natural dyes. These characters were then compared with seed grown in deionised water. Analysis of variance was applied to know whether the difference was significant or insignificant.

Key words : *Natural dyes, Shoot length, Root length, Fresh weight, Dry weight.*

Introduction

The use of natural dye is enjoying a revival more importantly due to the non hazardous effects on skin and environment (Agarwal et al., 1993; Gulati and Turner, 1929; Kumar and Bharti, 1998; Lakhande and Naik, 1997). They do not release harmful chemicals during their production and subsequent use (Encyclopedia Britannica, 1943; Everyman's 1967; Rafai 1989; Rameshwar 2000; Shenai, 1987).

Experiments were conducted using gram and wheat seeds to evaluate the effect of natural dyes on the growth of plants during seed germination. This study has revealed that natural dyes are not harmful to the seed germination and it does not spoils the quality of the soil when it enters into it. The changes and response magnitude of the seedling in the dye solution was compared with each other as well as with the control seedlings.

Analysis of variance was applied to the above experiment, to see the effect of concentration of the dye as well as the time period on the growth of the plant seedling. F coefficient for variance between columns and rows is worked out. F coefficients are compared with their corresponding table values. If it is found that calculated value of coefficient for variance between samples is greater than its table value then difference between values is considered to be significant. If it is found that calculated value of coefficient for variance between samples is lesser than its table value than differ significant.

Materials and Methods

In the present study Gram grains (*Cicer arietinum*) & Wheat grains (*Triticum aestivum*) were taken for experimental analysis. The seeds were collected from Agriculture College, Indore and natural dyes from Rohini Herbals Pvt. Ltd., Indore. The four natural dyes used for the experimental purpose are namely - Tesu (*Butea monosperma*), Anar (*Punica granatum*), Majeeth (*Rubia tinctoria*) and Heena (*Lawsonia alba*). Seeds were sterilized in 0.01% mercuric chloride (HgCl_2) solution for 10 minutes. The seeds were washed 2-3 times with deionised water. The seeds were soaked in a petridish and allowed to germinate under dark condition. Further germination of the seeds has been conducted on wet filter paper in petridishes having various concentrations of dye solution 0.2%, 0.4%, 0.6% and 1%. Simultaneously made a control with deionised water. Seeds growth in these concentration were sampled and analyzed for 24 hours, 48 hours and 72 hours respectively. Triplicate sets were arranged for each parameter and triplicate readings were taken into consideration and their average values were tabulated accordingly. The changes and responses magnitude of the seedling in the dye solution was compared with each other as well as with the control seedlings.

Results

The average root length, shoot length, fresh weight and dry weight of the wheat and gram seed were examined under morphological parameters as follows :

Shoot length of Wheat

The shoot length increases after every 24 hours. When the dye 'Tesu' is used the shoot length was about 1.3 cm in deionised water, which decreased to 0.98 cm. in 1% concentration of the dye, in 24 hours. After 48 hours the shoot length was 2.8 cm. in deionised water which reduced to 2.6 cm. In 1% dye concentration. After 72 hours it was 5.2 cm in deionised water which changed to 4.2 cm in 1% dye concentration. The calculated value for F coefficient for row works out to be 3.57, and for column it works out to be 506.06 (Table 1). When the dye Majeeth is used the shoot length which was 1.3 cm in deionised water decreased to 1.21 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.43, and for column it works out to be 1051.09.

When the dye Anar is used the shoot length which was 1.5 cm in deionised water decreased to 1.24 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.06, and for column it works out to be 221.86. When the dye Heena is used the shoot length which was 1.6 cm in deionised water decreased to 0.85 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 1.34 and for column it works out to be 63.37.

Shoot length of Gram

When the dye 'Tesu' is used the shoot length was about 1.1 cm in deionised water, which decreased to 0.78 cm. in 1% concentration of the dye, in 24 hours. After 48 hours the shoot length was 2.6 cm. in deionised water which reduced to 2.45 cm. in 1% dye concentration. After 72 hours it was 5.0 cm in deionised water which changed to 4.0 cm in 1% dye concentration. The calculated value for F coefficient for row works out to be 3.37, and for column it works out to be 487.46. (Table. 1).

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When the dye *Majeeth* is used the shoot length which was 0.93 cm in deionised water decreased to 0.72 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.10, and for column it works out to be 3576.50. When the dye *Anar* is used the shoot length which was 1.26 cm in deionised water decreased to 0.98 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.29, and for column it works out to be 341.50. When the dye *Heena* is used the shoot length which was 1.23 cm in deionised water decreased to 0.98 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.26 and for column it works out to be 351.89.

Root length of Wheat

When the dye '*Tesu*' is used the root length was about 1.8 cm in deionised water, which decreased to 1.38cm. in 1% concentration of the dye, in 24 hours. After 48 hours the root length was 2.9 cm. In deionised water which reduced to 2.22 cm. in 1% dye concentration. After 72 hours it was 4.3 cm in deionised water which changed to 3.19 cm in 1% dye concentration. The calculated value for F coefficient for row works out to be 2.16, and for column it works out to be 94.34. (Table 1). When the dye *Majeeth* is used the root length which was 2.2cm in deionised water decreased to 2.06 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.43, and for column it works out to be 830.20.

When the dye *Anar* is used the root length which was 2.2 cm in deionised water decreased to 1.98 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.43, and for column it works out to be 46.61. When the dye *Heena* is used the root length which was 2.26 cm in deionised water decreased to 2.00 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.37 and for column it works out to be 335.46.

Root length of Gram

When the dye '*Tesu*' is used the root length was about 2.17 cm in deionised water, which decreased to 2.06 cm. in 1% concentration of the dye, in 24 hours. After 48 hours the root length was 2.83 cm. in deionised water which reduced to 2.00 cm. in 1% dye concentration. After 72 hours it was 5.14 cm in deionised water which changed to 5.00 cm in 1% dye concentration. The calculated value for F coefficient for row works out to be 3.38, and for column it works out to be 508.88 (Table 1).

When the dye *Majeeth* is used the root length which was 2.16 cm in deionised water decreased to 2.00 cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.26, and for column it works out to be 619.12. When the dye *Anar* is used the root length which was 2.17 cm in deionised water decreased to 2.06cm in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.42, and for column it works out to be 460.97.

When the dye *Heena* is used the root length which was 2.20 cm in deionised water decreased to 2.06 cm in 1% concentration of the dye in 24 hours. The same correlation

was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.43 and for column it works out to be 830.20.

Fresh weight of Wheat

In each case the average fresh weight decreased but to a very small extent with the increasing dye concentration. When the dye '*Tesu*' is used the fresh weight was about 0.058 gm. in deionised water which decreased to 0.045 gm. in 1% concentration of the dye, in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 2.16, and for column it works out to be 94.34. (Table 2). When the dye '*Majeeth*' is used the fresh weight, which was 0.054 gm. in deionised water, decreased to 0.047 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.43, and for column it works out to be 830.20. When the dye '*Anar*' is used the fresh weight which was 0.072 gm. in deionised water decreased to 0.059 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.43, and for column it works out to be 46.61. When the dye '*Heena*' is used the fresh weight, which was 0.073 gm. in deionised water, decreased to 0.068 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.37, and for column it works out to be 335.46.

Fresh weight of Gram

When the dye '*Tesu*' is used the fresh weight was about 0.32 gm. in deionised water which decreased to 0.26 gm. in 1% concentration of the dye, in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 2.78, and for column it works out to be 625.53. (Table 2). When the dye '*Majeeth*' is used the fresh weight, which was 0.36 gm. in deionised water, decreased to 0.25 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.23, and for column it works out to be 6.92.

When the dye '*Anar*' is used the fresh weight which was 0.42 gm. in deionised water decreased to 0.33 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.15, and for column it works out to be 10.46. When the dye '*Heena*' is used the fresh weight, which was 0.40 gm. in deionised water, decreased to 0.32 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.43, and for column it works out to be 830.20.

Dry weight of Wheat

When the dye '*Tesu*' is used the dry weight was about 0.03 gm. in deionised water, which decreased to 0.21 gm. in 1% concentration of the dye, in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 1.69, and for column it works out to be 170.06. (Table 2). When the dye '*Majeeth*' is used the dry weight, which was 0.031 gm. in deionised water, decreased to 0.025 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 1.79, and for column it works out to be 191.94.

When the dye *Anar* is used the dry weight of root which was 0.042 gm. in deionised water decreased to 0.031 gm. in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 3.36, and for column it works out to be 26.74. When the dye *Heena* is used the dry weight, which was 0.052 gm. in deionised water, decreased to 0.047 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 1.98, and for column it works out to be 789.86.

Dry weight of Gram

When the dye '*Tesu*' is used the dry weight was about 0.46 gm. in deionised water, which decreased to 0.03 gm. in 1% concentration of the dye, in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 2.28, and for column it works out to be 82.59. (Table 2). When the dye *Majeeth* is used the dry weight, which was 0.048 gm. in deionised water, decreased to 0.037 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 2.12, and for column it works out to be 248.70. When the dye *Anar* is used the dry weight which was 0.049 gm. in deionised water decreased to 0.038 gm. in 1% concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 2.11, and for column it works out to be 412.07. When the dye *Heena* is used the dry weight, which was 0.051 gm. in deionised water, decreased to 0.045 gm. in 1 % concentration of the dye in 24 hours. The same correlation was observed after 48 hours and 72 hours. The calculated value for F coefficient for row works out to be 1.83, and for column it works out to be 173.83.

Conclusion

It was found that as the time increases, i.e., 24 to 48 hours to 72 hours, there is an increase in all the morphological characters. But as the concentration of the dye solution is increased there is a slight decrease in the growth characteristics, which shows that the increased dye concentration do effect the growth. But the effect is too small or negligible as compared to that of synthetic dyes.

When analysis of variance was applied to the above experiments, to see the effect of concentration of dye as well as time on growth of plants, it was found out that F ratio concerning variance between columns, i.e., concerning growth at different time interval works out to be greater than the table value of F which shows that the difference is significant. The F ratio concerning variance between rows i.e. concerning the growth at different concentration of the dye work out is less than the table value in all cases, which shows that the difference is significant.

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Name of the Dye	% Conc.	Shoot Lengths of Wheat (cms.)			Shoot Lengths of Gram (cms.)			Root Lengths of Wheat (cms.)			Root Lengths of Gram (cms.)		
		24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
TESU	D.W.	1.3	2.8	5.2	1.1	2.6	5	1.8	2.9	4.3	2.17	2.83	5.14
	0.20	1.22	2.77	4.94	1.02	2.57	4.74	1.76	2.82	4.07	2.16	2.75	5.12
	0.40	1.15	2.72	4.69	0.95	5.52	4.49	1.63	2.61	3.76	2.1	2.61	5.02
	0.60	1.05	2.65	4.45	0.85	2.5	4.25	1.5	2.41	3.47	2.09	2.32	5
	1.00	0.98	2.6	4.2	0.78	2.45	4	1.38	2.22	3.19	2.06	2	4.85
MAJEETH	D.W.	1.43	2.12	3.25	0.93	2.74	4.65	2.2	2.9	5.41	2.16	2.73	5.12
	0.20	1.42	2.11	3.23	0.9	2.72	4.63	2.17	2.85	5.4	2.15	2.65	5.1
	0.40	1.39	2.08	3.16	0.86	2.69	4.56	2.11	2.77	5.35	2.08	2.51	5.09
	0.60	1.35	2.01	3.11	0.81	2.63	4.5	2.09	2.43	5.3	2.05	2.22	5
	1.00	1.3	1.98	2.66	0.72	2.54	4.41	2.06	2.22	5.21	2	2	4.85
ANAR	D.W.	1.5	3.05	5.6	1.26	2.38	4.48	2.2	3.2	4.5	2.17	2.88	5.15
	0.20	1.42	3	5.4	1.25	2.36	4.45	2.16	3	4.48	2.16	2.75	5.15
	0.40	1.36	2.84	4.95	1.1	2.25	4.34	2.1	2.3	4.4	2.09	2.67	5.03
	0.60	1.3	2.79	4.55	1	2.2	4.31	2.04	2.2	4.3	2.08	2.33	4.99
	1.00	1.24	2.7	4.2	0.98	1.35	4.22	1.98	2.09	4.18	2.06	2	4.48
HEENA	D.W.	1.6	2.74	3.66	1.23	2.35	4.43	2.26	3.38	5.48	2.2	2.9	5.41
	0.20	1.2	2.72	3.6	1.22	2.34	4.43	2.25	3.36	5.45	2.17	2.85	5.4
	0.40	1	2.67	3.52	1.2	2.23	4.32	2.1	3.25	5.35	2.11	2.77	5.35
	0.60	0.96	2.6	3.44	1.1	2.2	4.25	2	3.25	5.31	2.09	2.43	5.3
	1.00	0.85	2.55	3.79	0.98	1.35	4.2	1.98	2.35	5.19	2.06	2.22	5.21

An assessment of Seed Germination

TABLE 2													
Effect of Natural Dyes on Fresh & Dry Weight of Wheat & Gram at Different Intervals of Time													
Name of the Dye	% Conc.	Fresh Weight of Wheat (cms.)			Fresh Weight of Gram (cms.)			Dry Weight of Wheat (cms.)			Dry Weight of Gram (cms.)		
		24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.
TESU	D. W.	0.058	0.217	0.47	0.32	0.45	0.53	0.03	0.049	0.19	0.046	0.065	0.19
	0.20	0.05	0.195	0.038	0.31	0.44	0.51	0.027	0.047	0.17	0.041	0.064	0.17
	0.40	0.048	0.164	0.33	0.29	0.43	0.5	0.026	0.046	0.16	0.038	0.062	0.16
	0.60	0.047	0.15	0.28	0.28	0.42	0.48	0.023	0.044	0.14	0.033	0.06	0.14
	1.00	0.045	0.136	0.22	0.26	0.39	0.47	0.021	0.042	0.13	0.03	0.058	0.11
MAJEETH	D. W.	0.054	0.066	0.22	0.36	0.46	0.76	0.042	0.052	0.086	0.048	0.084	0.26
	0.20	0.051	0.065	0.21	0.35	0.45	0.76	0.041	0.05	0.085	0.046	0.083	0.24
	0.40	0.05	0.064	0.19	0.29	0.41	0.62	0.04	0.048	0.08	0.043	0.081	0.22
	0.60	0.048	0.062	0.18	0.28	0.39	0.42	0.034	0.045	0.075	0.04	0.079	0.21
	1.00	0.047	0.06	0.16	0.25	0.32	0.22	0.031	0.042	0.04	0.037	0.077	0.19
ANAR	D. W.	0.072	0.109	0.184	0.42	0.56	0.84	0.072	0.109	0.184	0.049	0.081	0.29
	0.20	0.065	0.1	0.178	0.4	0.55	0.8	0.065	0.1	0.178	0.047	0.079	0.28
	0.40	0.063	0.085	0.17	0.39	0.51	0.75	0.063	0.085	0.17	0.043	0.078	0.26
	0.60	0.061	0.06	0.165	0.35	0.49	0.55	0.061	0.06	0.165	0.041	0.076	0.24
	1.00	0.059	0.031	0.16	0.33	0.43	0.34	0.059	0.031	0.16	0.038	0.075	0.23
HEENA	D. W.	0.073	0.171	0.32	0.4	0.52	0.82	0.053	0.086	0.32	0.051	0.089	0.22
	0.20	0.072	0.169	0.318	0.38	0.5	0.8	0.051	0.084	0.31	0.048	0.086	0.2
	0.40	0.071	0.168	0.315	0.36	0.49	0.76	0.05	0.083	0.29	0.047	0.085	0.19
	0.60	0.069	0.167	0.311	0.35	0.48	0.54	0.048	0.081	0.28	0.047	0.083	0.17
	1.00	0.068	0.166	0.299	0.32	0.42	0.35	0.047	0.079	0.27	0.045	0.081	0.16